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RESEARCH ARTICLE

Quality evaluation of Perillae Folium by HPLC/PDA

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Abstract To establish a standard of quality control for Perillae Folium (Lamiaceae Family), four standard compounds including rosmarinic acid, elemicin, perillaldehyde, and dillapiole were evaluated by high-performance liquid chromatography (HPLC)/photodiode array (PDA). The four standards were analyzed with a Phenomenex Kinetex C18 (250 × 4.6 mm, 5 μm) column by gradient elution using 0.1 % formic acid in water and methanol as the mobile phase. The standards were quantified by HPLC/PDA from Perillae Folium, which included the leaf and twig of *Perilla frutescens* L. Britton var. *acuta* (Thunb.) Kudo or *Perilla frutescens* Britton var. *crispa* Decne. The method was successfully used in the analysis of Perillae Folium, and the linearity, recovery, precision, accuracy, stability, and robustness were satisfactory according to the validation results. In Perillae Folium samples, the average contents (wt%) of rosmarinic acid, elemicin, perillaldehyde, and dillapiole were 0.540, 0.059, 0.050, and 0.056 %, respectively. The results indicate that the established HPLC/PDA method is suitable for the quantitation and quality evaluation of Perillae Folium.

Keywords Perillae Folium · Lamiaceae · Quantitation · HPLC

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Introduction

Perillae Folium (Lamiaceae) is used in East Asia as a traditional herbal medicine for treating depression, infection, inflammation, and allergies (Nitta et al. 2006; Jeoung et al. 2008). This herb is also used in a wide variety of applications including in foods, food coloring, and flavoring (Kang et al. 1992). Several studies have reported that the extract of Perilla Folium have anti-oxidant, anti-inflammation, anti-coagulation, anti-ulcer, anti-cancer, anti-allergy, and anti-infection activities (Farina et al. 1998; Jeoung et al. 2008; Son et al. 2010; Song et al. 2001; You et al. 2001).

The Korean Pharmacopoeia (K.P. X) and the Japanese Pharmacopoeia (J.P.) both stipulate that Perillae Folium is the leaf and twig of *Perilla frutescens* Britton var. *acuta* Kudo (PA) or *P. frutescens* Britton var. *crispa* Decaisne (PC). However, it is prescribed to contain more than 0.08 % of perillaldehyde in the J.P. only, but K.P. do not include a quantitative analytical method for marker compounds of Perillae Folium (Korea Food and Drug Administration 2013; The Minister of Health, Labor and welfare of Japan 2008). The Chinese Phamacopoeia (C.P.) stipulates that Perillae Folium is the leaf and twig of *P. frutescens* Britt. only, and is prescribed to contain more than 0.40 % volatile oil. The specific components were not provided in C.P. (The Pharmacopoeia Commission of the PRC 2010). Previously, Jang et al. identified seventeen

volatile components in Perillae Folium using combined gas chromatography (GC) and GC–MS (Jang et al. 1991). Ohk and Chae classified *P. frutescens* collections into four chemotypes using thirty volatile oil compounds by GC–MS (Ohk and Chae 2004). Kim et al. developed a HPLC method for the quantitative analysis of rosmarinic acid and caffeic acid in *Perilla frutescens* var. *japonica* and PA, but the method was not effective due to poor resolution and having a short retention time (about 4 or 14 min) (Kim et al. 2008). Chen et al. developed an analytical method for the assessment of three triterpene acids (tormentic acid, oleanolic acid, and ursolic acid) by HPLC/UV. However, the complicated processing procedure of the sample (refluxed thrice with methanol at 80 °C, for 1 h each) was difficult to apply in the quantitation of multiple samples (Chen et al. 2003). Therefore, it is still necessary to develop a quality evaluation method for Perillae Folium.

The purpose of this study was to establish a stable HPLC method which could quantitatively analyze bioactive components from Perillae Folium, and then to propose the developed method as an official analytical method in the next K.P. revision. In the present study, the contents of four constituents (rosmarinic acid, elemicin, perillaldehyde, and dillapiole) in PA and PC, collected from geographically different areas of China or Korea, were determined and validation of the established method was demonstrated by HPLC/PDA.

Materials and methods

Materials

All of the standard compounds were provided by Prof. Kang Ro Lee, Sungkyunkwan University, Suwon, Korea. Their structures were unambiguously identified as rosmarinic acid, elemicin, perillaldehyde, and dillapiole. Structures of the standard compounds are shown in Fig. 1. Purity of standard compounds was estimated to be higher than 95 % based on HPLC analysis. Internal standard, ethyl *p*-hydroxybenzoate, was purchased from Sigma Chemicals (St. Louis, MO, USA). Methanol was purchased from Merck K GaA (Darmstadt, Germany). All other chemicals used were of analytical grade. Distilled water was prepared using the Milli-Q purification system (Millipore, Bedford, MA, USA). Seventy-four samples cultured in different regions were provided by the National Center for Standardization of Herbal Medicine. This study adopted the following seventy-four samples corresponding to Perillae Folium [forty-eight PA from Korea (PA01–PA48), six PA from China (PA49–PA54), three PC from Korea (PC55–PC57), and seventeen PC from China (PC55–PC74)].

Sample preparation

Sample powder was used to determine the contents of the four marker compounds and pattern recognition analysis of each extraction of Perillae Folium including PA and PC. The powdered sample was sieved through 50 mesh. About 1.0 g of the powder, accurately weighed, was added to 50 mL of 75 % methanol containing an internal standard (I.S.; 50 ppm ethyl *p*-hydroxybenzoate), the weight was accurately measured, and the sample was sonicated for 60 min. The solution was weighed again, and the loss in weight was made up with 75 % methanol containing I.S. The solution was filtered through a 0.21-μm membrane filter and the filtrate was used as the test solution.

HPLC/PDA conditions

The HPLC equipment was a Waters 2695 separations module (Waters, Milford, MA, USA) with Waters 996 photodiode array detector. Phenomenex Kinetex C18 (250 × 4.6 mm, 4 μm), Phenomenex Gemini C18 110A (250 × 4.6 mm, 5 μm) and Shiseido C18 pak (250 × 4.6 mm, 5 μm) columns were tested with the guard column (3.9 × 20 mm XTerra RP18 5 μm) filled with the same stationary phase. An elution system consisting 0.1 % formic acid in water (**A**) and 0.1 % formic acid in methanol (**B**) was used. Gradient elution started with 70 % solvent A, change to 25 % A for 40 min. The analysis was carried out at a flow rate of 1.0 mL/min with UV detection absorbance at 210 nm.

Analytical method validation

The standards (5.0 mg) of rosmarinic acid, elemicin, perillaldehyde, and dillapiole were each accurately weighed and then dissolved in 10 mL of 75 % methanol containing I.S. to produce stock standard solutions of 500 ppm. The calibration curves were generated after diluting the stock solution with 75 % methanol containing I.S. A reference solution of the four marker compounds at concentrations of 6.25–200.0 μg/mL was analyzed by HPLC/PDA. The regression equations were calculated in the form of $y = ax + b$, where y and x correspond to peak ratio (compound peak area/I.S. area) and compound concentration, respectively.

Recovery tests were executed by mixing a powdered sample (1.0 g) of the reference compounds at three control levels (low, medium, and high concentrations from the calibration curves). The mixture was then extracted with sonication in 50 mL of 75 % methanol containing I.S. at room temperature for 60 min. The extract solution was filtered through a 0.21-μm membrane. The HPLC/PDA analysis experiments were performed in triplicate for each control level. The data from the standard solution and the extracted sample were compared. Precision and accuracy

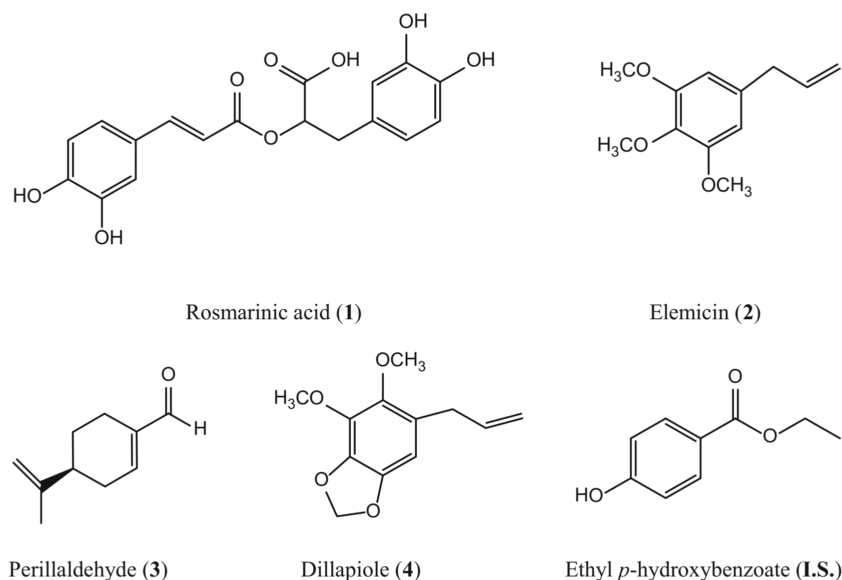

Fig. 1 Structures of standards and an internal standard

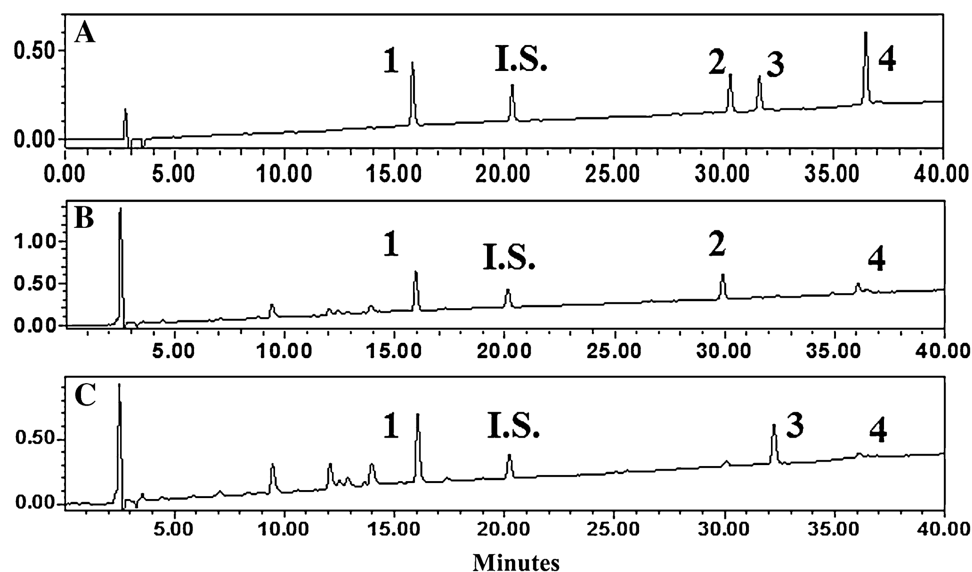

Fig. 2 HPLC chromatograms of standards mixture (**a**), *P. frutescens* Britton var. *acuta* Kudo (**b**; PA01 sample) and *P. frutescens* Britton var. *crispa* Decaisne (**c**; PC56 sample). rosmarinic acid (**1**), elemicin (**2**), perillaldehyde (**3**), and dillapiole (**4**)

were determined by multiple analyses ($n = 5$) of quality control samples spiked at low, medium, and high concentrations in the scope of calibration range.

The robustness of the method was studied by introducing changes in columns [(i.e. Phenomenex Kinetex C18 (250 × 4.6 mm, 4 μm), Phenomenex Gemini C18 110A (250 × 4.6 mm, 5 μm), and Shiseido C18 pack (250 × 4.6 mm, 5 μm)], separation temperatures (i.e. 25, 30, 35, and 40 °C), and three different flow rates (i.e. 0.8, 1.0, and 1.2 mL/min).

Stability was tested with mixed standard solution stored in the dark at 4 °C and room temperature (25 °C). The two samples were analyzed in triplicate at 0, 0.5, 1, 2, 5, 10, 15, and 30 days. The compounds showed stable during the tested period (data not shown).

Statistical analysis

To evaluate the phytochemical equivalency among the seventy-four of Perillae Folium samples for pattern recognition analysis using software of SPSS statistics 19.0. Statistically significant differences for the different extraction efficiency of methods were evaluated with two-way analysis of variance (ANOVA) by Graphpad prism 5.0. The $p < 0.05$ was considered to be significant.

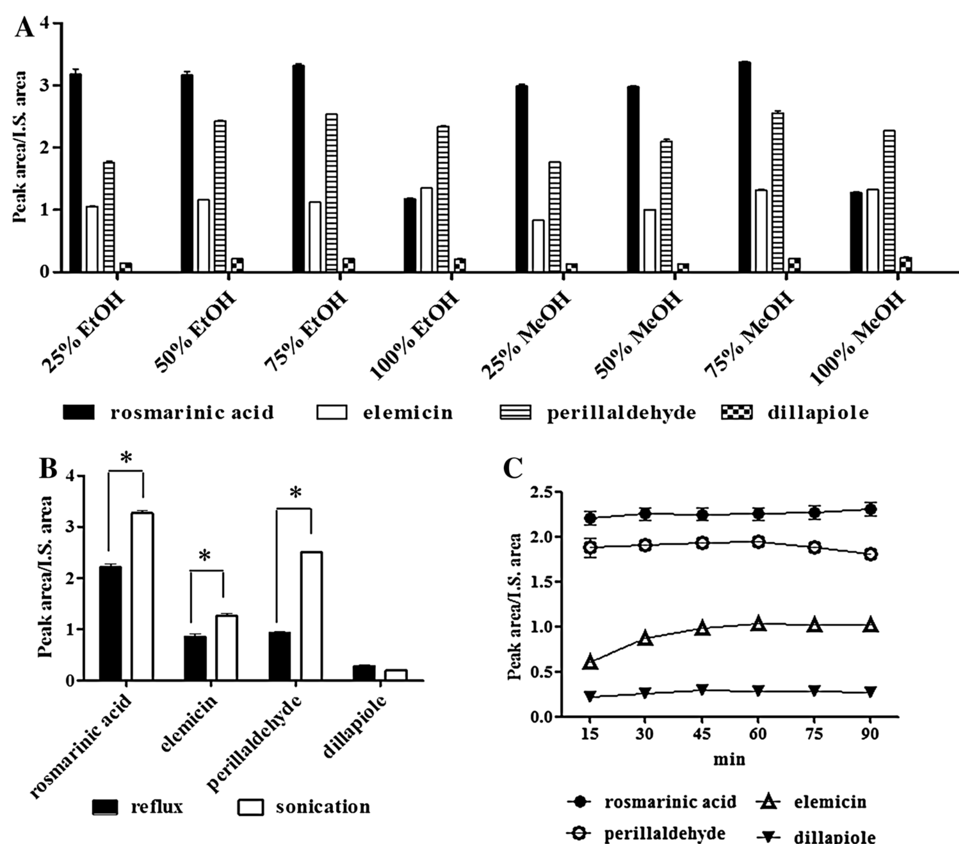

Fig. 3 Comparison of the extraction solvent (**a**), extraction method (**b**), and extraction time (**c**) for extraction efficiencies of marker compounds ($n = 3$). Significantly different ($*p < 0.05$) between two groups (each sonication efficiency vs. reflux efficiency)

Table 1 Linearity, linear ranges, LOD and LOQ ($n = 3$)

Analytes	Linear range (μg/mL)	Slope (a)	Intercept (b)	Correlation coefficient (r^2)	LOD (ng/mL)	LOQ (ng/mL)
Rosmarinic acid (**1**)	6.25–200	0.0138	0.0029	0.9997	75.50	251.65
Elemicin (**2**)	6.25–200	0.0117	0.0058	0.9998	120.83	402.78
Perillaldehyde (**3**)	6.25–200	0.0673	−0.0623	0.9997	55.12	183.72
Dillapiole (**4**)	6.25–200	0.0186	0.0220	0.9996	70.74	235.80

Results and discussion

Optimization of chromatographic conditions

High-performance liquid chromatography (HPLC) conditions were selected according to the requirement for obtaining chromatograms with better resolution of adjacent peaks within a short retention time. The effect of the mobile phase composition on separation was examined to optimize the chromatographic conditions. The use of methanol as an organic modifier significantly improved the separation. We also tested the addition of 0.01, 0.1, and 1 % acid (acetic acid, formic acid, and phosphoric acid) to the mobile phase. The addition of 0.1 % formic acid to the mobile phase resulted in good resolution of all compounds, as well as satisfactory peak symmetry and shape. The typical chromatograms of samples and standards mixtures are shown in Fig. 2. All target compounds as well as an internal standard were completely separated within 40 min. The chromatographic peaks in the sample solution were identified by comparing their retention times with those of the reference standards and were further confirmed by spiking samples with reference compounds. The extract sample was scanned between 200 and 400 nm using a PDA detector to select the detection wavelength. Among the standard compounds, rosmarinic acid, elemicin, and dillapiole had the maximum absorption at a wavelength of 210 nm. The absorbance of perilladehyde was still high at 210 nm, even though the maximum absorbance of **3** was 233 nm. Hence, the chromatographic condition was set at 210 nm for the entire study.

Table 2 Precision and accuracy of analytical results

Analytes	Fortified conc. (μg/mL)	Sample conc. (μg/mL)	Intra-day ($n = 5$)				Sample conc. (μg/mL)	Inter-day ($n = 5$)			
			Observed (μg/mL)	Accuracy (%)	SD[b]	Precision (%)		Observed (μg/mL)	Accuracy (%)	SD	Precision (%)
Rosmarinic acid (**1**)	10	122.97 ± 0.26[a]	133.81 ± 1.24	108.36	1.95	1.80	122.88 ± 0.31	133.77 ± 1.63	108.92	1.59	1.46
	30	122.97 ± 0.26	153.84 ± 0.80	102.91	1.53	1.49	122.88 ± 0.31	152.20 ± 0.22	97.74	0.88	0.90
	50	122.97 ± 0.26	172.10 ± 0.22	98.26	1.24	1.26	122.88 ± 0.31	172.68 ± 1.13	99.61	1.34	1.35
Elemicin (**2**)	30	88.25 ± 0.31	118.41 ± 4.47	100.53	3.89	4.05	85.78 ± 0.77	115.65 ± 2.10	99.56	1.44	1.44
	90	88.25 ± 0.31	176.30 ± 2.27	97.84	2.79	2.86	85.78 ± 0.77	180.68 ± 0.93	105.44	0.85	0.81
	150	88.25 ± 0.31	232.32 ± 0.93	96.05	2.12	2.11	85.78 ± 0.77	233.11 ± 0.54	98.22	0.65	0.67
Perilladehyde (**3**)	10	–	10.45 ± 0.73	104.51	0.73	0.70	–	9.90 ± 5.88	98.97	5.88	5.94
	30	–	30.82 ± 2.34	102.73	2.34	2.28	–	30.33 ± 3.17	101.11	3.17	3.13
	50	–	50.65 ± 1.18	101.31	1.18	1.16	–	50.46 ± 3.63	100.91	3.63	3.60
Dillapiole (**4**)	25	29.93 ± 1.30	54.79 ± 2.40	99.42	1.85	1.86	29.91 ± 1.68	54.36 ± 0.88	97.82	1.28	1.31
	50	29.93 ± 1.30	83.24 ± 1.20	106.61	1.25	1.17	29.91 ± 1.68	83.23 ± 1.29	106.65	1.49	1.39
	100	29.93 ± 1.30	125.02 ± 2.03	95.08	1.67	1.75	29.91 ± 1.68	129.90 ± 2.12	99.99	1.90	1.89

[a] Mean ± SD (standard deviation; $n = 5$)

[b] Standard deviation (SD) of accuracy

Table 3 Average contents (wt%) of rosmarinic acid, elemicin, perillaldehyde, and dillapiole in Perillae Folium

	Mean ± SD[a] (wt%)		
	PA ($n = 54$)	PC ($n = 20$)	Total ($n = 74$)
Rosmarinic acid (**1**)	0.449 ± 0.556	0.786 ± 0.500	0.540 ± 0.557
	(0.007–2.993)[b]	(0.030–1.802)	(0.007–2.993)
Elemicin (**2**)	0.078 ± 0.166	0.009 ± 0.038	0.059 ± 0.146
	(0–0.604)	(0–0.173)	(0–0.878)
Perillaldehyde (**3**)	0.064 ± 1.280	0.015 ± 0.147	0.050 ± 0.113
	(0–0.641)	(0–0.056)	(0–0.641)
Dillapiole (**4**)	0.063 ± 0.101	0.035 ± 0.071	0.056 ± 0.094
	(0–0.643)	(0–0.227)	(0–0.643)

PA P. frutescens Britton var. *acuta*

PC P. frutescens Britton var. *crispa* Decaisne

Total: mean (wt%) of PA + mean (wt%)of PC

[a] The standard deviation (SD)

[b] The content range of each standard

Optimization of sample preparation conditions

To achieve complete extraction of the components studied from Perillae Folium, eight solvent systems consisting of pure methanol, 75 % methanol, 50 % methanol, 25 % methanol, pure ethanol, 75 % ethanol, 50 % ethanol, and 25 % ethanol were used for the test. The extraction efficiencies of all the components from each solvent extraction system were obtained and compared. The results indicated that for all the components, the 75 % methanol solvent systems were the highest efficient (Fig. 3a). In addition, two extraction methods, namely ultra-sonication and reflux using 75 % methanol extraction solvent were compared. The contents of rosmarinic acid, elemicin, and perillaldehde after extraction with sonication were higher than those with reflux procedures ($p < 0.05$) in our work (Fig. 3b). To determine the time needed for complete extraction, samples were extracted for five different lengths of time (15, 30, 45, 60, 75, and 90 min). 75 % methanol was employed in the extraction using the sonication method at room temperature. With an extraction time of 60 min, the contents were similar to those at 75 min for all components. Therefore, when the extraction time was 60 min (Fig. 3c), all compounds were sufficiently extracted.

Validation of the method

Each coefficient of correlation (r^2) was >0.999, as determined by least square analysis, suggesting good linearity between the peak area ratio and the compound concentrations. The limits of detection (LOD) and limits of quantitation (LOQ) were evaluated at signal-to-noise (S/N) ratios of 3 and 10, respectively. The LOD and LOQ under our experimental conditions are listed in Table 1. The values obtained for both LOD and LOQ for these four standards were low enough to detect traces of these compounds in either crude extract or its preparation.

Precision and accuracy were determined by multiple analyses ($n = 5$) of quality control samples prepared with three different concentrations of marker compounds at low, medium and high concentrations in the scope of the calibration range, spiked in a PA01 sample for subsequent extraction and filtration. Three concentrations of 10, 30, and 50 μg/mL for rosmarinic acid and perilladehyde, and 30, 90 and 150 μg/mL for elmicin, and 25, 50, and 100 μg/mL for dillapiole were evaluated. Intra-assay precision and accuracy were determined from the variability of multiple analyses ($n = 5$) of the quality control samples within the same analytical run. The quality control samples had intra-assay precision below 4.05 % and accuracy between 95.08 and 108.36 %. Inter-assay precision and accuracy were evaluated from the variability of multiple analyses ($n = 5$) of quality control samples within a single analytical run for five consecutive days. The quality control samples had an inter-assay precision of less than 5.94 % and accuracy between 97.74 and 108.92 %, indicating that the developed method is highly reproducible. The precision and accuracy data are presented in Table 2.

Robustness was determined to evaluate the reliability of the established HPLC method. The experimental conditions, such as column temperature, column species, and flow rates, were purposely altered. The theoretical plate ($\boldsymbol{N}$), capacity factor ($\boldsymbol{k'}$), separation factor ($\boldsymbol{\alpha}$), and resolution ($\boldsymbol{Rs}$) were evaluated. We attempted to optimize the

Table 4 The contents (wt%) of components (**1**–**4**) in Perillae Folium ($n = 74$)

No.	Mean (wt%)				Origin	No.	Mean (wt%)				Origin
	1	**2**	**3**	**4**			**1**	**2**	**3**	**4**	
PA01	0.681	0.563	0.000	0.123	Korea	PA38	0.355	0.000	0.011	0.124	Korea
PA02	0.572	0.000	0.063	0.000		PA39	0.101	0.000	0.118	0.016	
PA03	0.336	0.000	0.019	0.048		PA40	0.202	0.000	0.007	0.000	
PA04	0.295	0.581	0.015	0.142		PA41	0.202	0.104	0.012	0.082	
PA05	0.426	0.262	0.012	0.061		PA42	2.993	0.000	0.599	0.000	
PA06	0.307	0.676	0.014	0.158		PA43	0.453	0.000	0.264	0.303	
PA07	0.308	0.217	0.018	0.053		PA44	0.541	0.000	0.641	0.000	
PA08	0.608	0.274	0.017	0.061		PA45	0.453	0.000	0.094	0.000	
PA09	0.149	0.052	0.023	0.026		PA46	0.308	0.000	0.080	0.000	
PA10	0.303	0.097	0.018	0.050		PA47	0.132	0.000	0.164	0.000	
PA11	1.073	0.000	0.012	0.021		PA48	0.663	0.000	0.119	0.643	
PA12	0.276	0.000	0.015	0.039		PA49	2.635	0.000	0.391	0.000	China
PA 13	0.007	0.000	0.014	0.067		PA50	0.148	0.000	0.032	0.063	
PA14	0.007	0.000	0.014	0.067		PA51	0.026	0.017	0.026	0.034	
PA15	0.587	0.000	0.018	0.057		PA52	0.907	0.000	0.009	0.000	
PA16	0.481	0.000	0.018	0.134		PA53	0.232	0.000	0.009	0.072	
PA17	0.160	0.000	0.025	0.100		PA54	0.907	0.000	0.007	0.000	
PA18	1.486	0.604	0.024	0.162		PC55	0.958	0.000	0.000	0.042	Korea
PA19	1.148	0.134	0.033	0.178		PC56	0.134	0.000	0.053	0.025	
PA20	0.513	0.163	0.019	0.077		PC57	0.630	0.000	0.014	0.000	
PA21	0.067	0.027	0.021	0.074		PC58	0.909	0.000	0.009	0.000	China
PA22	0.202	0.000	0.048	0.022		PC59	0.518	0.000	0.012	0.000	
PA23	0.055	0.070	0.038	0.050		PC60	1.707	0.000	0.011	0.000	
PA24	0.233	0.024	0.023	0.031		PC61	1.019	0.000	0.014	0.000	
PA25	0.252	0.000	0.030	0.045		PC62	1.366	0.000	0.013	0.000	
PA26	0.297	0.041	0.020	0.000		PC63	0.925	0.000	0.012	0.000	
PA27	0.423	0.055	0.022	0.000		PC64	0.498	0.000	0.013	0.000	
PA28	0.055	0.203	0.022	0.000		PC65	0.302	0.000	0.009	0.000	
PA29	0.073	0.000	0.026	0.000		PC66	1.019	0.000	0.015	0.000	
PA30	0.115	0.000	0.033	0.024		PC67	1.008	0.000	0.007	0.000	
PA31	0.120	0.000	0.026	0.128		PC68	0.649	0.000	0.000	0.000	
PA32	0.255	0.000	0.030	0.031		PC69	0.863	0.000	0.000	0.044	
PA33	0.228	0.000	0.037	0.013		PC70	0.030	0.173	0.006	0.000	
PA34	0.017	0.027	0.023	0.019		PC71	0.205	0.000	0.056	0.000	
PA35	0.185	0.000	0.018	0.007		PC72	0.112	0.000	0.015	0.161	
PA36	0.267	0.000	0.015	0.007		PC73	1.062	0.000	0.018	0.227	
PA37	0.413	0.000	0.011	0.000		PC74	1.802	0.000	0.021	0.198	

PA P. frutescens Britton var. *acuta*, *PC P. frutescens* Britton var. *crispa* Decaisne

1 Rosmarinic acid; **2** elemicin; **3** perillaldehyde; **4** dillapiole

chromatographic parameters, but the four analytical factors did not differ greatly when the conditions were changed (data not shown); therefore these experimental conditions were considered sufficiently robust.

Sample analysis

The HPLC method was applied to analyze seventy-four samples corresponding to the leaves of fifty-four PA and twenty PC samples. The average contents (wt%) of rosmarinic acid, elemicin, perillaldehyde, and dillapiole, and their content ranges are presented in Table 3. The average content of rosmarinic acid (0.786 %; range of 0.030–1.802 %) in the PC samples was higher than that of PA (0.449 %; range of 0.007–2.993 %). In contrast, the average contents of elemicin (0.009 %; range of 0–0.173 %), perillaldehyde (0.015 %; range of 0–0.056 %), and dillapiole (0.035 %; range of 0–0.227 %) in the PC samples were lower than those of elemicin (0.078 %; range of 0–0.604 %), perillaldehyde (0.064 %; range of 0–0.641 %), and dillapiole (0.063 %;

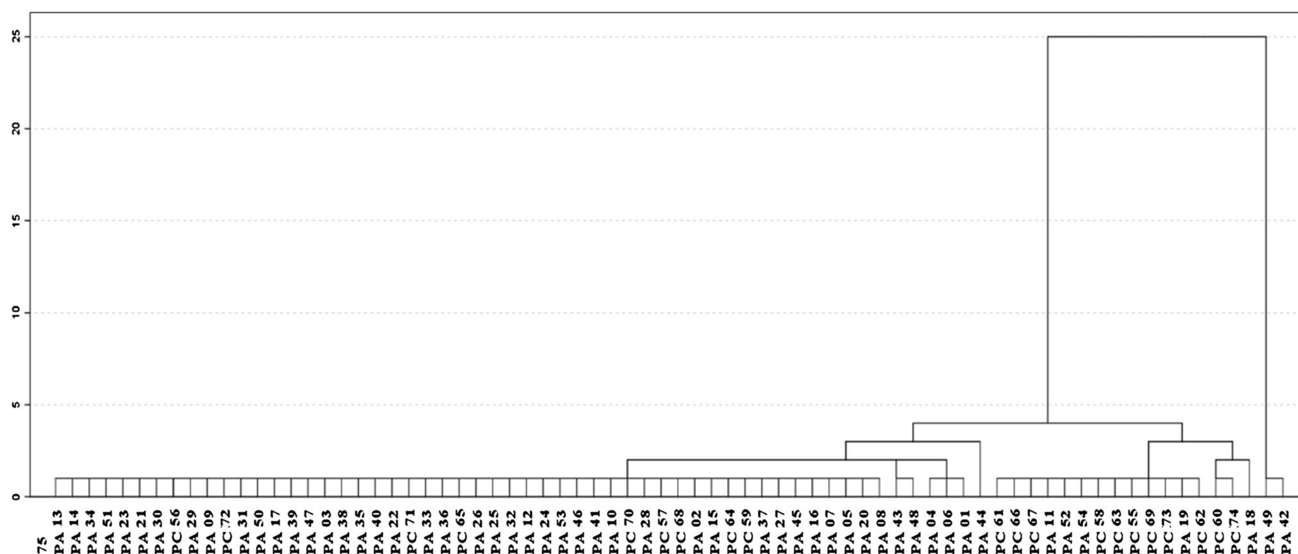

Fig. 4 Hierarchical clustering analysis of for Perillae Folium. *PA P. frutescens* Britton var. *acuta. PC P. frutescens* Britton var. *crispa* Decaisne

range of 0–0.643 %) in PA samples. Additionally, the total average contents of elemicin, perillaldehyde, and dillapiole were very similar with each other (0.059, 0.050 and 0.056 %, respectively). Nevertheless, using elemicin, perillaldehyde, and dillapiole as marker compounds for quality regulation was inappropriate, because these three compounds were not detected in fifty-three, three and twenty-nine samples of 74 Perillae Folium samples, respectively. In addition, the standard deviations from the total sample means for elemicin, perillaldehyde, and dillapiole were so large (about two times or much larger than each total mean), but the standard deviation of rosmarinic acid showed a similar value as its total mean. The content of each marker compound in the samples is summarized in Table 4. Lee and Yang reported that growth behavior and perillaldehyde concentration were influenced by planting/growing seasons, and primary leaves at upper positions have a higher concentration of perillaldehyde than those in lower positions (Lee and Yang 2006). Due to this content variation of perillaldehyde, it was considered inadequate to use perillaldehyde as a marker compound for Perillae Folium in J.P. In contrast, rosmarinic acid was found in all the samples. According to the quantitative analysis results, we concluded that rosmarinic acid was reasonable as a marker compound for quality regulation of Perillae Folium.

Pattern recognition analysis

A hierarchical clustering analysis of 74 samples was performed using IBM SPSS Statistics 19.0 software (Fig. 4). Pattern recognition analysis was conducted using a combination of four marker compounds (rosmarinic acid, elemicin, perillaldehyde, and dillapiole). However, PA and PC could not be identified by the pattern-recognition analysis. Additionally, the Korean and Chinese products did not appear to be significantly different in pattern-recognition analysis. We attempted a lot of different calculation methods in pattern-recognition analysis such as prediction analysis of microarrays and principal component analysis, etc., but the results were unable to discriminate between the genes or origins of Perillae Folium. This result suggested that PA and PC samples could be used for the circulation of traditional medicine, because those two genes are not significantly different. To distinguish between PA and PC samples, other components or methods such as gene analysis should be used.

Conclusion

In this study, a fully validated HPLC method was developed for quality control of Perillae Folium. Based on validation results, this HPLC method was demonstrated to be a simple, accurate and reasonable analytical method for simultaneous quantification. The developed method was successfully applied to quantify four bioactive marker compounds in 74 batches of Perillae Folium collected from different locations in Korea and China. Our results suggest that rosmarinic acid can be used as a marker compound for quality evaluation of Perillae Folium.

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Conflict of interest The authors have no conflicts of interest that are directly relevant to the content of this study.

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