Quantitative Analysis of Bioactive Marker Compounds from Cinnamomi Ramulus and Cinnamomi Cortex by HPLC-UV

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Abstract – In this study, quantitative and pattern recognition analysis for the quality evaluation of Cinnamomi Ramulus and Cinnamomi Cortex using HPLC/UV was developed. For quantitative analysis, three major bioactive compounds were determined. The separation conditions employed for HPLC/UV were optimized using an ODS C_{18} column (250 × 4.6 mm, 5 μm) with gradient conditions of acetonitrile and water as the mobile phase, at a flow rate of 1.0 mL/min and a detection wavelength of 265 nm. This method was fully validated with respect to linearity, accuracy, precision, recovery, and robustness. The HPLC/UV method was applied successfully to the quantification of three major compounds in the extract of Cinnamomi Ramulus and Cinnamomi Cortex. The HPLC analytical method for pattern recognition analysis was validated by repeated analysis of thirty eight Cinnamomi Ramulus and thirty five Cinnamomi Cortex samples. The results indicate that the established HPLC/UV method is suitable for quantitative analysis.

Keywords – Cinnamomi Ramulus, Cinnamomi Cortex, *Cinnamomum cassia* Blum, HPLC-UV, Quality control, Monitoring

Introduction

Cinnamomi Ramulus is the dried young branches of *Cinnamomum cassia* Blume (Lauraceae), and other species of the same genus in the Korean Herbal Pharmacopoeia (KHP) and the Chinese Pharmacopoeia (CP). Cinnamomi Ramulus is controlled to contain more than 1.0% of cinnamaldehyde in the CP. Various monoterpenoides, sesquiterpenoids, diterpenoids, sterols, cinnamaldehyde and its analogues, and flavan-3-ols and their oligomers have been isolated from Cinnamomi Ramulus (Fang *et al*., 2007). Many reports have been published highlighting the variety of biological activities of Cinnamomi Ramulus and Cinnamomi Cortex, including anxiolytic-like (Yu *et al*., 2007), anti-atopic dermatitis (Sung *et al*., 1999), anti-

fungal (Giordani *et al*., 2006; Pawar *et al*., 2006), anti-inflammatory (Lee *et al*., 2005), and anti-tumor (Ka *et al*., 2003) activities. Cinnamomi Ramulus and Cinnamomi Cortex are different parts. Cinnamomi Cortex is the bark of *Cinnamomum cassia* Blume (Leuraceae) in the KHP and CP. However, the regulation content has not been set with the KHP and CP. Some HPLC/UV analytical methods have been developed for the analysis of compounds from Cinnamomi Ramulus and its related products. Chen *et al*. (2010) reported on cinnamaldehyde, cinnamic acid, and coumarin as specific markers for the distinction of Cinnamomi Ramulus in Guizhi decoction, but these peaks were not retained well Ding *et al*. (2011) established a HPLC method with complicated gradient condition for the determination of seven compounds in Cinnamomi Ramulus and Cinnamomi Cortex, which was not also effective due to the gradient elution.

The purpose of this study was to establish a stable HPLC method which could quantitatively analyze bioactive major compounds from Cinnamomi Ramulus and Cinnamomi Cortex, and to suggest an analytical

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method resulted from this work as an official analytical method for the KHP. In the present study, a simple, sensitive and precise reverse-phase HPLC/UV method has been developed for the quantitative determination of three marker components, coumarin (**1**), cinnamic acid (**2**), and cinnamaldehyde (**3**) along with pattern-recognition analysis for the quality control of Cinnamomi Ramulus and Cinnamomi Cortex. All of the published HPLC studies have been focused on Cinnamomi Ramulus or Cinnamomi Cortex, respectively. There has been no report to distinguish between Cinnamomi Ramulus and Cinnamomi Cortex. Using this method, the contents of bioactive compounds in thirty eight Cinnamomi Ramulus and thirty five Cinnamomi Cortex samples from China, Vietnam, Indonesia, and Sri Lanka were analyzed and compared.

Experimental

Plant material – Seventy three samples corresponding to thirty eight Cinnamomi Ramulus (R01 - R38; R01 - R10 (China) and R11 - R38 (Vietnam)) and thirty five Cinnamomi Cortex samples (C39 - C73; C39 - C46 (China), C47 - C68 (Vietnam), C69 - C72 (Indonesia) and C73 (Sri Lanka)) cultivated in different regions were provided by the National Center for Standardization of Herbal Medicine.

Reagents – All of the standard compounds were provided by the National Center for Standardization of Herbal Medicine. Structures of coumarin (**1**), cinnamic acid (**2**), and cinnamaldehyde (**3**) were unambiguously identified by comparisons of NMR and MS data, with the published data, such as. The standard compound structures are shown in Fig. 1. The purity of standard compounds was estimated to be higher than 95% based on HPLC and LC-MS/MS analysis. Methanol and acetonitrile of HPLC grade were purchased from Merck K GaA (Darmstadt, Germany). All other chemicals used were of analytical grade unless otherwise noted. Distilled water was prepared using a Milli-Q purification system (Millipore, Bed-ford, MA, USA).

Sample preparation – To determine the contents of three marker compounds and for pattern recognition analysis of Cinnamomi Ramulus and Cinnamomi Cortex, the dried branches or cortex powder were used for each extraction. Powder samples of Cinnamomi Ramulus or Cinnamomi Cortex (0.5 g) were mixed with 50 mL of 50% methanol containing 1,000 ppm of I.S. in a vial. The mixture was then sonicated for 30 min. The solution was weighed again, and the loss in weight was made up with 50% methanol. The solution was filtered through a 0.21-μm membrane filter (Whatman) and the filtrate was used as the test solution. 10 μL of the test solution was then injected into the HPLC system.

HPLC/UV conditions – The HPLC equipment was a Gilson HPLC system (Gilson, USA) with Gilson 321 pumps, a Gilson 151 UV detector and a Gilson 231 XL autosampler. Phenomenex Gemini (250 × 4.6 mm, 5 μm), YMC ODS-H80 (250 × 4.6 mm, 5 μm) and Shodex ODS pak (250 × 4.6 mm, 5 μm) columns were tested with the guard column filled with the same stationary phase. A (100% acetonitrile) and B (water) were used as the mobile phase using a gradient condition (0 min, 10% A; 5 min, 20% A; 35 min, 50% A; 50 min, 100% A) to analyze samples. The mobile phase was filtered under vacuum through a 0.45-μm membrane filter and degassed prior to use. The analysis was carried out at a flow rate of 1.0 mL/min with the detection wavelength set to 265 nm, and the total run time was 50 min. All compounds could be resolved with baseline separation at 265 nm with the maximum absorption. Hence, characteristic chromatographic patterns were obtained at 265 nm. The chromatograms were processed using software Empower Pro software, build 1154 (Waters, Milford, MA).

Analytical method validation – The standards (4 mg) of coumarin (**1**), cinnamic acid (**2**), and cinnamaldehyde (**3**) were each accurately weighed and then dissolved with 10 mL of 100% methanol to produce stock standard solutions of 400 ppm, respectively. The internal standard (4-hydroxycinnamic acid) of 5 mg was accurately weighed and then dissolved with 10 mL of 100% methanol to produce stock solution of 500 ppm. The calibration curves were made by diluting the stock solutions with 100% methanol. The reference solution of the three standard compounds at concentrations of 0.1 – 400 μg/mL was analyzed by HPLC/UV. The regression equations were
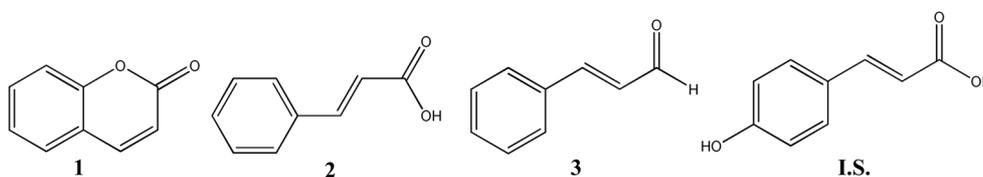

Fig. 1. Chemical structures of standards.
1 coumarin, **2** cinnamic acid, **3** cinnamaldehyde, **I.S.** 4-hydroxycinnamic acid

calculated in the form of $y = ax + b$, where y and x correspond to peak area ratio for an internal standard and compound concentration, respectively. The recovery tests were executed by mixing a powdered sample (0.5 g) with three control levels of the reference compounds (near the LOQ, medium and higher concentrations from the calibration). The mixture was then extracted by sonication with 50 mL of 50% methanol for 30 minutes. The extract solution was filtered through a 0.45 μm membrane. The HPLC/UV analysis experiments were performed in triplicate for each control level. The data was compared with those from the standard solution and extracted sample. Precision and accuracy were determined by multiple analysis (n = 5) of quality control samples prepared at lower, medium and higher concentrations spanning the calibration range.

Pattern recognition analysis – To evaluate the phytochemical equivalency among the thirty eight Cinnamomi Ramulus and thirty five Cinnamomi Cortex samples, pattern recognition analysis was conducted. We used three marker compound peaks (coumarin (**1**), cinnamic acid (**2**) and cinnamaldehyde (**3**)) for pattern recognition analysis. Pattern recognition analysis was conducted using software package R-2.11.0.

Results and Discussion

Optimization of chromatographic conditions – The HPLC conditions were selected by the requirement for obtaining the chromatograms with a better resolution of the adjacent peaks within a short retention time. For optimization of the chromatographic conditions, the effect of the composition of mobile phase on the separation was examined. Mobile phase of water-methanol did not result in satisfactory separation of structurally similar compounds. Acetonitrile as an organic modifier demonstrated a significant improvement in separation. We tested the addition of 0.1%, 1% and 10% acids (acetic acid, formic acid and phosphoric acid) to the mobile phase. The addition of 0.1% phosphoric acid to the mobile phase resulted in good resolution of all the compounds, as well as satisfactory peak symmetry and shape. The typical chromatograms of the samples and standard mixture are shown in Fig. 2, from which one can observe that all target compounds and the internal standard are completely separated in 50 minutes. 4-Hydroxycinnamic acid (**I.S.**) was selected as an internal standard. The chromatographic peaks of the analytes in sample solution were identified by comparing their retention times with
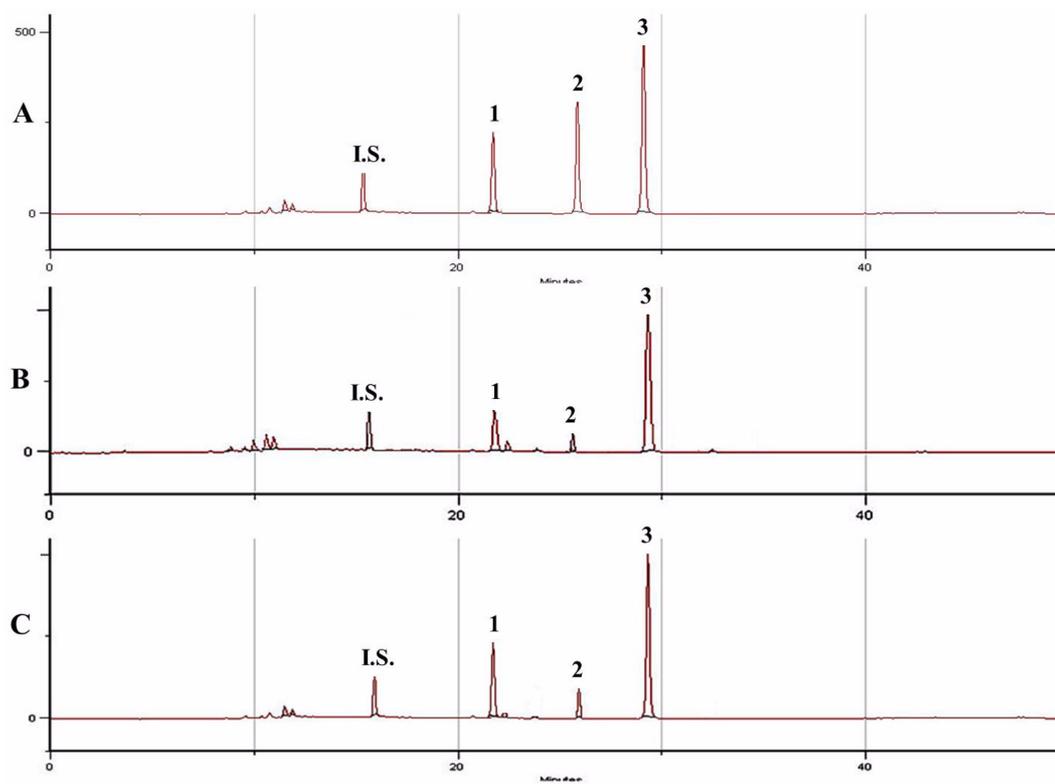
Fig. 2. HPLC chromatograms of standard mixture (**A**), Cinnamomi Ramulus (**B**), and Cinnamomi Cortex (**C**).
1 coumarin, **2** cinnamic acid, **3** cinnamaldehyde, **I.S.** 4-hydroxycinnamic acid.

those of the reference standards and further confirmed by spiking samples with the reference compounds (Fig. 2). All compounds could be resolved with baseline separation at 265 nm with the maximum absorption shown for three constituents. Hence, characteristic chromatographic patterns were obtained at 265 nm.

Optimization of sample preparation conditions – Eight extracting solvents, 100% ethanol, 75% ethanol, 50% ethanol, 25% ethanol, 100% methanol, 75% methanol, 50% methanol and 25% methanol were compared with regard to the contents of compounds using sonication for 30 minutes. When a sample (R01) was extracted with 50% methanol, the contents of compounds were higher than those of other extracting solvents. Therefore, we employed 50% methanol as an extracting solvent throughout this work. Two extraction methods, ultra-sonication and reflux using 50% methanol as an extraction solvent, were compared with regard to the contents of compounds. When sonication extraction method was used, the contents of compounds were higher than those of reflux. To determine the time needed to obtain complete extractions, extractions of a sample were performed for four different lengths of time (10, 20, 30, 40, and 60 minutes). 50% methanol solvent and sonication extraction method were also employed. When the extraction time was 30 min, the contents of compounds were nearly the same as those for 40 min. Therefore,

when extraction time was 30 min, all of the compounds were sufficiently extracted (all extraction data not shown).

Validation – 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 (Table 1). The limits of detection (LOD) and limits of quantitation (LOQ) were evaluated based on the lowest detectable peak in the chromatogram having a signal-to-noise (S/N) ratios of 3 and 10, respectively. Under our experimental conditions, we listed LOD and LOQ in Table 1. The obtained values for both LOD and LOQ for these three standards were shown to be low enough to detect traces of these compounds in either crude extract or its preparation.

The extraction recovery test was performed by extracting a known amount of the three compounds from the Cinnamomi Ramulus and Cinnamomi Cortex powder samples. A known amount of each standard compound at three levels was mixed with the sample powder and extracted with 50% methanol, as described in the experimental section. The recovery of each standard ranged from 99.89 to 100.75%, and the RSD was less than 0.68%. The average recovery was represented by the formula: R (%) = [(amount from the sample spiked standard – amount from the sample)/amount from the spiked standard] × 100.

Intra-assay precision and accuracy were determined

Table 1. Calibration graphs, linear ranges, LOD and LOQ

Analytes	Liner range (μg/mL)	Slope (a)	Intercept (b)	Correlation coefficient (r^2)	LOD (ng/mL)	LOQ (ng/mL)
Coumarin (**1**)	0.1~40	0.0137	0.0027	0.9999	12	36
Cinnamic acid (**2**)	0.1~40	0.1128	0.0548	0.9999	13	39
Cinnamaldehyde (**3**)	0.1~400	0.0346	0.0029	0.9994	15	26

Table 2. Precision and accuracy of analytical results

Analytes	Fortified conc. (μg/mL)	Sample conc. (μg/mL)	Intraday (n = 5)				Sample conc. (μg/mL)	Inter-day (n = 3)			
			Observed (μg/mL)	S.D.	Accuracy (%)	Precision (%)		Observed (μg/mL)	S.D.	Accuracy (%)	Precision (%)
Coumarin (**1**)	0.4	41.45	42.68	0.01	101.98	0.29	41.39	42.60	0.01	101.95	1.04
	20.0	42.66	62.12	0.17	99.14	0.86	42.11	62.17	0.17	100.09	0.83
	40.0	43.33	82.33	0.11	98.80	0.27	43.75	82.70	0.23	98.75	0.56
Cinnamic acid (**2**)	0.2	1.72	1.88	0.01	98.09	0.24	1.66	1.87	0.01	100.39	1.06
	1.0	1.74	2.69	0.01	98.35	0.97	1.70	2.68	0.02	99.11	1.50
	2.0	1.72	3.70	0.01	99.38	0.08	1.75	3.70	0.01	98.66	0.72
Cinnamaldehyde (**3**)	0.4	226.87	227.29	0.01	100.01	0.65	186.43	186.72	0.02	99.94	0.41
	80.0	192.72	267.21	0.22	97.98	0.27	188.10	266.73	1.08	99.49	1.34
	160.0	182.04	344.98	0.03	100.86	0.02	182.20	345.01	0.55	100.82	0.34

Table 3. Robustness of column, temperature and flow rate on theoretical plate (N), capacity factor (k'), separation factor (α) and resolution (Rs)

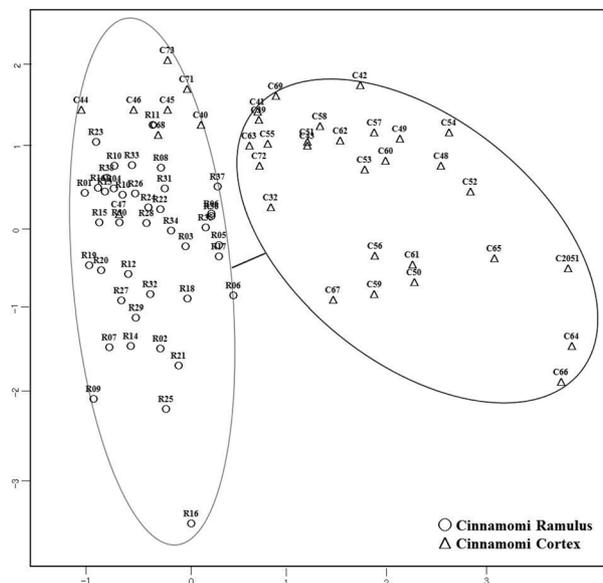| Cinnamomi Ramulus		Analytes		
		Coumarin (**1**)	Cinnamic cid (**2**)	Cinnamaldehyde (**3**)
		mean ± S.D.	mean ± S.D.	mean ± S.D.
Column	*Theoretical plate (N)*			
	YMC	69722 ± 95	101596 ± 120	108239 ± 107
	Phenomenex	66951 ± 153	107603 ± 131	95949 ± 125
	Shodex	81705 ± 149	126742 ± 99	115111 ± 122
	Capacity factor (k')			
	YMC	5.60 ± 0.01	6.55 ± 0.01	8.04 ± 0.01
	Phenomenex	4.46 ± 0.01	5.22 ± 0.01	6.16 ± 0.01
	Shodex	5.05 ± 0.02	6.22 ± 0.02	7.10 ± 0.01
	Separation factor (α)			
	YMC	1.81 ± 0.02	1.17 ± 0.00	1.23 ± 0.00
	Phenomenex	1.65 ± 0.01	1.17 ± 0.00	1.18 ± 0.00
	Shodex	1.58 ± 0.02	1.23 ± 0.00	1.14 ± 0.00
	Resolution (Rs)			
	YMC	25.61 ± 0.18	9.14 ± 0.03	13.35 ± 0.04
	Phenomenex	18.70 ± 0.04	7.76 ± 0.05	8.95 ± 0.02
	Shodex	21.15 ± 0.03	12.73 ± 0.03	8.89 ± 0.03
Temperature	*Theoretical plate (N)*			
	25	69722 ± 95	101996 ± 120	108239 ± 107
	30	69889 ± 103	101310 ± 141	108949 ± 115
	35	68971 ± 122	100948 ± 129	100467 ± 94
	40	70112 ± 142	101914 ± 193	111574 ± 104
	Capacity factor (k')			
	25	5.60 ± 0.01	6.55 ± 0.01	8.04 ± 0.01
	30	5.66 ± 0.04	6.71 ± 0.02	8.10 ± 0.01
	35	5.37 ± 0.01	6.50 ± 0.02	8.11 ± 0.02
	40	5.48 ± 0.02	6.58 ± 0.01	8.16 ± 0.01
	Separation factor (α)			
	25	1.81 ± 0.02	1.17 ± 0.00	1.23 ± 0.00
	30	1.86 ± 0.00	1.21 ± 0.01	1.26 ± 0.01
	35	1.82 ± 0.02	1.15 ± 0.00	1.14 ± 0.00
	40	1.91 ± 0.01	1.09 ± 0.02	1.16 ± 0.00
	Resolution (Rs)			
	25	25.61 ± 0.18	9.14 ± 0.03	13.35 ± 0.04
	30	25.96 ± 0.10	9.20 ± 0.01	13.41 ± 0.03
	35	25.88 ± 0.19	9.21 ± 0.01	13.32 ± 0.01
	40	25.92 ± 0.16	9.18 ± 0.02	13.24 ± 0.04
Flow rate	*Theoretical plate (N)*			
	0.9	69710 ± 92	101989 ± 112	108226 ± 100
	1.0	69891 ± 104	101321 ± 120	108267 ± 112
	1.1	68969 ± 119	100953 ± 125	108444 ± 92
	Capacity factor (k')			
	0.9	5.61 ± 0.01	6.52 ± 0.01	8.02 ± 0.01
	1.0	5.65 ± 0.02	6.72 ± 0.02	8.11 ± 0.01
	1.1	5.57 ± 0.02	6.53 ± 0.01	8.10 ± 0.02
	Separation factor (α)			
	0.9	1.80 ± 0.01	1.18 ± 0.01	1.25 ± 0.01
	1.0	1.82 ± 0.01	1.18 ± 0.01	1.22 ± 0.01
	1.1	1.81 ± 0.02	1.20 ± 0.01	1.21 ± 0.02
	Resolution (Rs)			
	0.9	25.63 ± 0.15	9.13 ± 0.02	13.34 ± 0.02
	1.0	25.85 ± 0.12	9.22 ± 0.01	13.37 ± 0.02
	1.1	25.84 ± 0.16	9.19 ± 0.01	13.33 ± 0.01

from the variability of multiple analyses (n = 5) of quality control samples analyzed within the same analytical run. The remaining quality control samples had intra-assay precision below 0.97% and accuracy between 97.98% and 101.19%. Inter-assay precision and accuracy were evaluated from the variability of multiple analyses (n = 5) of quality control samples analyzed on a single analytical run and extended for five consecutive days. The remaining quality control samples had inter-assay precision lower than 1.34% and accuracy between 98.66% and 101.95%. This above data reflect that the developed method is highly reproducible. Precision and accuracy data are presented in Table 2.

The robustness was determined in order to evaluate the reliability of the established HPLC method. All of the parameters were maintained so there would not be any interference with the other peaks for the Cinnamomi Ramulus. The experimental conditions, such as the column temperature, column species and flow rates, were intentionally altered. The theoretical plate (N), capacity factor (k'), separation factor (α) and resolution (Rs) were evaluated. To evaluate the suitability, three different columns, YMC ODS-H80, Phenomenex Gemini and Shodex ODS pak, were compared with regard to four analytical factors (N, k', α and Rs) at a column temperature of 25 °C. The result showed that the four analytical factors did not differ greatly, depending on the column species. Four different column temperatures, 25, 30, 35 and 40 °C, were compared with regard to four analytical factors using YMC ODS-H80 column. The result showed that four analytical factors did not differ greatly, depending on the column. Three different flow rates, 0.9, 1.0, and 1.1 mL/min, were compared with regard to four analytical factors using a YMC ODS-H80 column at 25 °C. The results showed that the four analytical factors did not differ greatly, depending on the flow rates. We performed optimization by changing the chromatographic parameters, but the four analytical factors did not differ greatly, indicating that these experiment conditions were sufficiently robust (Table 3).

The sample stability test was determined with a standard mixture solution at 0, 0.5, 1, 2, 5, 10, 15, and 30 days. During this period, the solutions were stored at room temperature and 4 °C in dark conditions. The resulting data indicated that all marker analytes remained stable more than 98.56% during the experimental period (data not shown).

Sample analysis – The developed HPLC/UV method was then applied to the simultaneous determination of the three compounds, coumarin (**1**), cinnamic acid (**2**), and

Fig. 3. Fanny of Cinnamomi Ramulus (R01 - R38) & Cinnamomi Cortex (C39 - C73).

cinnamaldehyde (**3**) in the Cinnamomi Ramulus and the Cinnamomi Cortex. The quantity of each compound present in samples was determined and the results are summarized in Table 4. Each sample was analyzed in triplicate to ensure the reproducibility of the quantitative result. The results indicated that coumarin (0.02~0.23%), cinnamic acid (0.03~0.19%) and cinnamaldehyde (0.91 ~3.15) were found in Cinnamomi Ramulus, and coumarin (0.02~0.98%), cinnamic acid (0.01~0.10%) and cinnamaldehyde (0.77~5.49) were found in Cinnamomi Cortex. The most abundant component was cinnamaldehyde. The content of cinnamaldehyde in Cinnamomi Cortex (3.20%; average value of C39 - C73) was higher than that in Cinnamomi Ramulus (1.75%; average value of R01 - R38) (Table 5). These quantitative analysis results for Cinnamomi Ramulus and Cinnamomi Cortex samples will be reflected in the content regulation of marker compounds for Cinnamomi Ramulus and Cinnamomi Cortex in the next KHP revision.

Pattern recognition analysis – To evaluate the phytochemical equivalency between the seventy three samples corresponding to thirty eight Cinnamomi Ramulus and thirty five Cinnamomi Cortex samples, pattern recognition analysis was conducted. We used three marker compound peaks (coumarin (**1**), cinnamic acid (**2**), and cinnamaldehyde (**3**)) for pattern recognition analysis. From the pattern analysis of Fanny (Fig, 3), we indicated that all of the samples were clustered largely into two groups, A (Cinnamomi Ramulus) and B (Cinnamomi

Table 4. Contents (wt %, n = 3) of three components in Cinnamomi Ramulus (R01 - R38) and Cinnamomi Cortex (C39 - C73) samples

Sample No	mean ± S.D. 1	2	3	Sample No	mean ± S.D. 1	2	3	Sample No	mean ± S.D. 1	2	3	Sample No	mean ± S.D. 1	2	3	Sample No	mean ± S.D. 1	2	3
R01	0.03 ± 0.00	0.06 ± 0.00	0.91 ± 0.03	R16	0.22 ± 0.03	0.19 ± 0.00	2.16 ± 0.08	R31	0.02 ± 0.00	0.06 ± 0.00	2.29 ± 0.08	C46	0.03 ± 0.00	0.03 ± 0.00	1.54 ± 0.05	C61	0.36 ± 0.01	0.08 ± 0.00	4.50 ± 0.08
R02	0.08 ± 0.00	0.12 ± 0.01	2.24 ± 0.08	R17	0.18 ± 0.02	0.08 ± 0.00	2.36 ± 0.07	R32	0.06 ± 0.00	0.10 ± 0.01	2.08 ± 0.07	C47	0.04 ± 0.01	0.07 ± 0.00	1.28 ± 0.03	C62	0.31 ± 0.01	0.03 ± 0.00	3.32 ± 0.05
R03	0.09 ± 0.00	0.08 ± 0.00	2.38 ± 0.05	R18	0.10 ± 0.00	0.10 ± 0.00	2.42 ± 0.05	R33	0.04 ± 0.01	0.05 ± 0.00	1.50 ± 0.05	C48	0.36 ± 0.5	0.04 ± 0.00	4.67 ± 0.13	C63	0.66 ± 0.00	0.07 ± 0.00	5.49 ± 0.03
R04	0.03 ± 0.00	0.05 ± 0.00	1.04 ± 0.03	R19	0.07 ± 0.00	0.07 ± 0.00	0.94 ± 0.03	R34	0.17 ± 0.02	0.07 ± 0.01	1.56 ± 0.04	C49	0.33 ± 0.05	0.03 ± 0.00	4.16 ± 0.07	C64	0.64 ± 0.01	0.10 ± 0.00	5.45 ± 0.01
R05	0.10 ± 0.00	0.08 ± 0.01	2.86 ± 0.06	R20	0.05 ± 0.00	0.09 ± 0.00	1.28 ± 0.05	R35	0.16 ± 0.01	0.07 ± 0.00	2.16 ± 0.06	C50	0.48 ± 0.06	0.08 ± 0.01	3.76 ± 0.10	C65	0.55 ± 0.00	0.07 ± 0.00	4.62 ± 0.08
R06	0.11 ± 0.01	0.10 ± 0.01	3.15 ± 0.02	R21	0.22 ± 0.01	0.12 ± 0.01	1.54 ± 0.06	R36	0.18 ± 0.02	0.06 ± 0.00	2.09 ± 0.06	C51	0.48 ± 0.04	0.02 ± 0.00	1.60 ± 0.05	C66	0.98 ± 0.01	0.10 ± 0.00	3.40 ± 0.02
R07	0.06 ± 0.00	0.12 ± 0.02	1.55 ± 0.05	R22	0.04 ± 0.00	0.05 ± 0.00	1.34 ± 0.04	R37	0.19 ± 0.01	0.06 ± 0.00	2.02 ± 0.02	C52	0.39 ± 0.03	0.05 ± 0.00	5.10 ± 0.07	C67	0.60 ± 0.02	0.08 ± 0.00	1.20 ± 0.04
R08	0.07 ± 0.00	0.05 ± 0.00	1.90 ± 0.07	R23	0.04 ± 0.00	0.05 ± 0.00	0.92 ± 0.02	R38	0.04 ± 0.00	0.05 ± 0.00	1.37 ± 0.03	C53	0.36 ± 0.02	0.04 ± 0.00	3.45 ± 0.02	C68	0.02 ± 0.01	0.04 ± 0.00	2.04 ± 0.04
R09	0.07 ± 0.00	0.14 ± 0.02	1.28 ± 0.03	R24	0.05 ± 0.00	0.07 ± 0.00	1.87 ± 0.06	C39	0.09 ± 0.01	0.03 ± 0.00	3.20 ± 0.09	C54	0.29 ± 0.02	0.03 ± 0.00	5.20 ± 0.09	C69	0.36 ± 0.00	0.01 ± 0.00	1.75 ± 0.04
R10	0.04 ± 0.00	0.06 ± 0.00	1.34 ± 0.02	R25	0.18 ± 0.01	0.14 ± 0.00	1.81 ± 0.03	C40	0.18 ± 0.02	0.03 ± 0.00	1.88 ± 0.03	C55	0.11 ± 0.01	0.04 ± 0.00	3.28 ± 0.05	C70	0.12 ± 0.01	0.04 ± 0.00	3.03 ± 0.05
R11	0.14 ± 0.01	0.03 ± 0.00	1.13 ± 0.03	R26	0.06 ± 0.00	0.06 ± 0.00	1.61 ± 0.03	C41	0.03 ± 0.00	0.03 ± 0.00	3.48 ± 0.09	C56	0.46 ± 0.02	0.07 ± 0.01	3.17 ± 0.03	C71	0.11 ± 0.00	0.02 ± 0.00	2.02 ± 0.02
R12	0.09 ± 0.01	0.09 ± 0.00	1.43 ± 0.04	R27	0.07 ± 0.00	0.10 ± 0.00	1.44 ± 0.02	C42	0.24 ± 0.02	0.01 ± 0.00	3.91 ± 0.08	C57	0.28 ± 0.01	0.03 ± 0.00	4.08 ± 0.09	C72	0.27 ± 0.00	0.04 ± 0.00	2.09 ± 0.04
R13	0.05 ± 0.00	0.06 ± 0.00	1.21 ± 0.03	R28	0.07 ± 0.00	0.07 ± 0.01	1.71 ± 0.03	C43	0.36 ± 0.03	0.09 ± 0.00	3.91 ± 0.07	C58	0.21 ± 0.01	0.03 ± 0.00	3.49 ± 0.05	C73	0.06 ± 0.00	0.01 ± 0.00	1.81 ± 0.04
R14	0.06 ± 0.01	0.12 ± 0.01	1.82 ± 0.02	R29	0.02 ± 0.00	0.11 ± 0.01	2.15 ± 0.05	C44	0.02 ± 0.00	0.03 ± 0.00	0.77 ± 0.02	C59	0.27 ± 0.01	0.03 ± 0.00	2.99 ± 0.09				
R15	0.23 ± 0.01	0.06 ± 0.00	2.42 ± 0.09	R30	0.07 ± 0.00	0.07 ± 0.01	1.17 ± 0.01	C45	0.03 ± 0.00	0.03 ± 0.00	2.26 ± 0.05	C60	0.30 ± 0.02	0.04 ± 0.00	4.11 ± 0.08				

1 coumarin, **2** cinnamic acid, **3** cinnamaldehyde

Table 5. Average contents (wt %, n = 3) of three components in Cinnamomi Ramulus and Cinnamomi Cortex samples

	Coumarin (**1**)	Cinnamic acid (**2**)	Cimmanaldehyde (**3**)
	mean ± S.D.	mean ± S.D.	mean ± S.D.
Cinnamomi Ramulus (n = 38)[a]	0.09 ± 0.06	0.08 ± 0.03	1.75 ± 0.54
Cinnamomi Cortex (n = 35)[b]	0.30 ± 0.22	0.04 ± 0.03	3.21 ± 1.28

[a] R01 – R38; [b] C39 – C73

Cortex). In Fanny analysis, the thirty eight Cinnamomi Ramulus samples clustered into one group. However, the Cinnamomi Cortex samples did not cluster into a complete group. Some samples (C40, C44 - C47, C68, C71, and C73) of Cinnamomi Cortex were comprised in the group of Cinnamomi Ramulus.

In conclusion, a rapid and optimized chromatographic method with UV detection was designed for the quality control of Cinnamomi Ramulus and Cinnamomi Cortex, which are well-known Korean traditional medicines. Validation data indicate that the developed analytical method is suitable for measuring the concentrations of three compounds for application to the pattern recognition analysis of Cinnamomi Ramulus and Cinnamomi Cortex. The contents of the most abundant component, cinnamaldehyde, were 0.91~3.15% in Cinnamomi Ramulus and 0.77~5.49% in Cinnamomi Cortex.

The developed HPLC/UV method for quantitative analysis of major bioactive compounds, along with a pattern-recognition method, can provide promising prospect for comprehensive quality control of Cinnamomi Ramulus and Cinnamomi Cortex. Our results confirm that coumarin, cinnamic acid and cinnamaldehyde can serve as family marker compounds to distinguish between Cinnamomi Ramulus and Cinnamomi Cortex. In the pattern recognition analysis we indicated that all of the samples were largely clustered into two groups A (Cinnamomi Ramulus) and B (Cinnamomi Ramulus).

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