Natural Product Sciences
17(4) : 328-336 (2011)

Comparative Analyses of Bioactive Constituents from *Forsythia suspensa* and *Forsythia viridissima* by HPLC-DAD

Tae Hyung Won[1], Lijuan Liao[1], Seung Ho Lee[2], Jong Keun Son[2], and Jongheon Shin[1,*]

[1]*Natural Products Research Institute, College of Pharmacy, Seoul National University, San 56-1, Shillim, Kwanak, Seoul 151-742, Korea*
[2]*College of Pharmacy, Yeungnam University, 214-1Dae-dong Gyeongsan, Gyeongbuk 712-749, Korea*

Abstract – A high-performance liquid chromatography (HPLC) with diode array detector (DAD) method was established for the discrimination of a folk medicine *Forsythia suspensa* and *Forsythia viridissima*. Five and three representative metabolites of the lignan and phenolic glycoside classes were selected for the analysis from *F. suspensa* and *F. viridissima*, respectively. The optimal chromatographic conditions were obtained on an ODS column (5 μm, 4.6 × 250 mm) with the column temperature at 40°C. The mobile phase was composed of methanol and 0.3% acetic acid using an isocratic elution with the flow rate 1 mL/min. Detection wavelength was set at 280 nm. All calibration curves showed good linear regression ($r^2 > 0.996$) within test ranges. Limits of detection (LOD) and limits of quantitation (LOQ) values were lower than 0.096 and 0.291 μg/mL, respectively. The developed method provided satisfactory precision and accuracy with overall intra-day and inter-day variations of 0.07-0.63% and 0.14-0.62%, respectively, and the overall recoveries of 97.79-102.46% for all of the compounds analyzed. In addition, effectiveness of diverse extraction methods was compared to each other for the development of standard analytical method. The verified method was successfully applied to the quantitative determination of representative metabolites in fifty-three commercial *F. suspensa* samples and fifteen commercial *F. viridissima* samples from diverse sources. The overall analytical results showed the unequivocal differences in bioactive constituents between *F. suspensa* and *F. viridissima*.
Keywords – HPLC-DAD, *Forsythia suspensa*, *Forsythia viridissima*, lignan, phenylethanoide glycoside

Introduction

Herbal medicines have been practiced to maintain good health and treat diseases in the Asia communities and recently in worldwide. Increment of worldwide attention and concomitant pharmaceutical research has made it essential to carry out the quality control measurement for the herbal medicines. However, serious hindrance has been attributed to the lack of recognition and regulation of profession, qualified practitioners, quality-controlled herbal medicines, and evidence-based clinical studies (Normile, 2003; Chan, 2005). Thus it is urgently needed to establish a comprehensive qualified evaluation method based on analysis of the whole bioactive compounds in order to accurately reflect the quality of herbal medicines.

The dried fruits of *Forsythia suspensa* and *Forsythia viridissima*, named Forsythia fructus (Oleaceae), are commonly used as herbal medicines. *F. suspensa and F. viridissima* is listed in Korea and Japan Pharmacopoeia but in China Pharmacopoeia only *F. suspensa* is listed. Although there are officially differences, these two species are widely distributed in Korea, China and Japan, East Asia. Traditionally *F. suspensa* has been used as an antipyretic, detoxicant and anti-inflammatory agent. (Piao *et al.*, 2008; Chang *et al.*, 2008). Also *F. suspensa* extract suppresses vomiting, resist hepatic injury, inhibit elastase activity and exhibit diuretic, analgesic, antioxidant, antiendotoxic and antiviral effects (Zhang, 2000; Liu, 2007). Flavonoids, lignans, terpenes, phenylethanoid glycosides, and volatile oils, have been isolated from *F. suspensa* (Li and Feng, 2005).

On the other hand, *F. viridissima* has been used as an anti-inflammatory agent and it has diuretic, antidote, extrusion of pus and antibacterial effects (Lee *et al.*, 2010). Flavonoids, lignans, triterpenoids, and their glycoside derivatives analogous to *F. suspensa* have been isolated from *F. viridissima* (Nishibe *et al.*, 1977; Chiba *et al.*, 1978). However, there are significant differences in the amount and distribution of individual constituent between

*Author for correspondence
Tel: +82-2-880-2484; E-mail: shinj@snu.ac.kr

F. suspensa and *F. viridissima,* such as the lack of as phylligenin, pinoresinol, phyllirin and pinoresinol-β-D-glucoside in *F. viridissima* and arctigenin, matairesinol, arctiin and matairesinoside in *F. suspensa* (Kitagawa *et al.,* 1988; Mariko *et al*., 1979). Despite these differences, both plants have been commonly used as herbal medicines without discrimination. Therefore the quality control for the discrimination between the two species is essential.

In this study, we initially aimed at developing an HPLC-DAD method for the simultaneous identification and quantitation of bioactive constituents in *F. suspensa* and *F. viridissima*. Based upon the prior researches, among the components selected for analysis were five representative compounds from *F. suspensa* and three representative compounds from *F. viridissima* with significant bioactivities and large contents: forsythiaside (**1**) of phenylethanoide glycoside and lariciresinol (**2**), phyllirin (**3**), pinoresinol (**4**), and phylligenin (**5**) of lignan (Fig. 1.) in *F. suspensa* and arctiin (**6**), matairesinol (**7**) and arctigenin (**8**) of lignan in *F. viridissima* (Fig. 2.). The developed methods have been verified for their effectiveness against diverse validation parameters. In addition, the contents of bioactive compounds in fifty-three commercial *F. suspensa* from China and fifteen commercial *F. viridissima* from Korea were analyzed and compared to each other.

Experimental

Plant materials – Fifty-three samples of *Forsythia suspensa* and fifteen samples *Forsythia viridissima* grown in different regions were provided by the National Center for Standardization of Herbal Medicine, such as C-1 (Pan'an, China), C-2~C-5 (Hanam, China), C-6~C-19 (Shanxi, China), C-20~C-53 (unidentified, China), K-1~K-2 (Buyeo, Korea), K-3 (Jeonbuk, Korea), K-4 (Jeonju, Korea), K-5~K-12 (Kyungbuk, Korea), K-13~K-15 (unidentified, Korea).

Reagents, chemicals and samples – forsythiaside (**1**), lariciresinol (**2**), phyllirin (**3**), pinoresinol (**4**), phillygenin (**5**), arctiin (**6**), matairesinol (**7**), and arctigenin (**8**), isolated and purified from *Forsythia suspensa* and *Forsythia viridissima* by a series of chromatographic procedures were provided from National Center for Standardization of Herbal Medicine, Korea and the structures were elucidated by comparison of spectral data (UV, IR, MS, 1H-NMR, 13C-NMR) with the literature

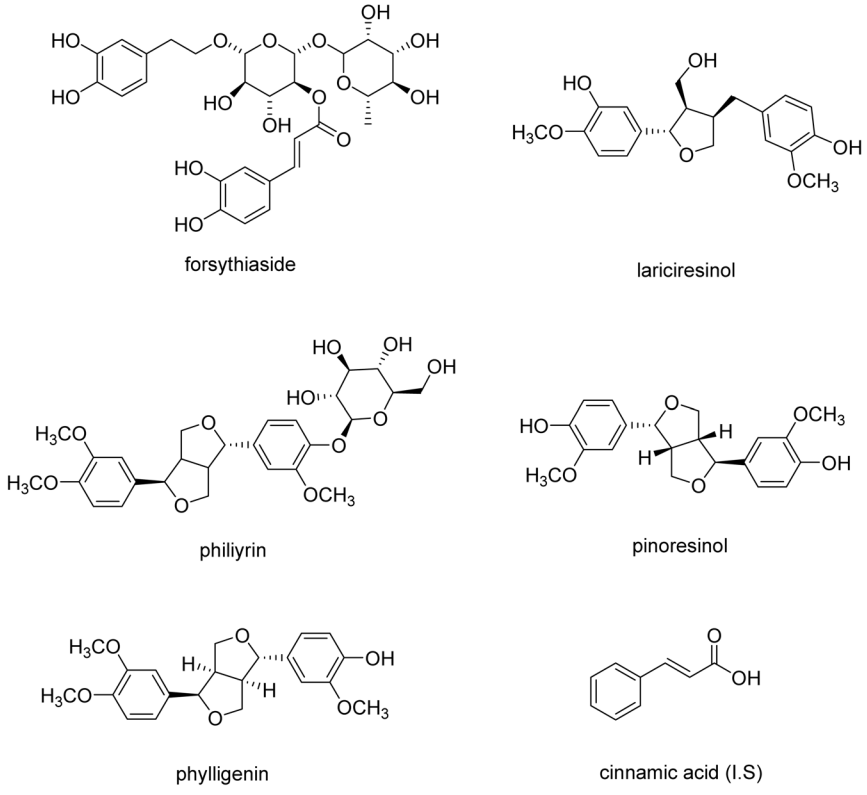

Fig. 1. Chemical structures of the standard compounds from *Forsythia suspensa.*

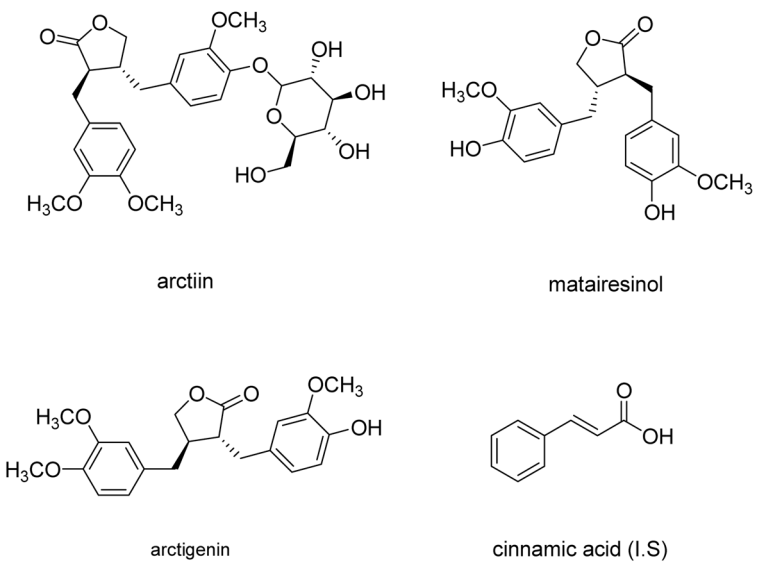

Fig. 2. Chemical structures of the standard compounds from *Forsythia viridissima*.

data. The purities of these compounds were determined to be higher than 96% by normalization of the peak area detection by HPLC analysis. Cinnamic acid as an internal standard compound was obtained from Sigma-Aldrich (USA).

Acetonitrile, methanol, and the de-ionized water (J. T. Baker, USA) used in this work were of HPLC grade and ethanol (Duksan, Korea) was analytical grade.

Instrumentation and chromatographic conditions – The HPLC system was consisted of Agilent 1200 series equipped with an autosampler, a column oven, a binary pump, a diode array detector (Agilent Technologies, Waldbronn, Germany) and a degasser (Agilent Technologies, Tokyo, Japan). The Chemstation software (Agilent Technologies, Avondale, CA, USA) was used to operate this HPLC-DAD system. Separation was performed on a Waters Sunfire^{TM} C^{18} (5 μm, 4.6 mm × 250 mm) analytical column. An isocratic method was used mobile phase A (0.3% acetic acid): B (methanol) in the ratio of 55 : 45 (*v/v*). Standard or sample solutions of 10 μL were directly injected to the HPLC system and the mobile phase flow rate was 1 mL/min and the column temperature was set at 40°C.

Preparation of test sample – In order to achieve quantitative extraction method, variables involved in the procedure such as extraction method, solvent, and extraction time were optimized. Ultrasonication, vortex and reflux methods were compared to each other for the selection of the optimal extraction method (Table 1) at 50% aqueous methanol solvent for 60 min. Aqueous methanol or ethanol solutions were tried as the extraction solvent (Table 2) using ultrasonication method at 60 min. Yields were also compared for the extraction times of 10, 20, 30, 60, and 90 min to determine the optimal extraction time (Table 3) using ultrasonication method at 50% aqueous methanol. As a result, 50% aqueous methanol for 60 min using ultrasonication was chosen for the best extraction condition for *F. suspensa* and *F. viridissima.*

Table 1. Comparison of effectiveness of extraction methods against standard metabolites in *F. suspensa* and *F. viridissima* (w/w, %, n = 3)

Compounds	Sonication	Vortex	Reflux
1	3.65 ± 0.04	3.34 ± 0.05	3.58 ± 0.01
2	0.08 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
3	0.32 ± 0.01	0.55 ± 0.01	0.76 ± 0.01
4	0.28 ± 0.02	0.28 ± 0.01	0.25 ± 0.01
5	0.32 ± 0.01	0.29 ± 0.01	0.23 ± 0.01
6	0.76 ± 0.01	2.27 ± 0.02	3.88 ± 0.02
7	0.66 ± 0.01	1.77 ± 0.01	3.03 ± 0.01
8	0.56 ± 0.01	2.01 ± 0.01	2.89 ± 0.02

Accurately weighed plant powder of 100 mg were extracted with 50 mL of 50% aqueous methanol adding 50 ppm internal standard 300 μL, by means of sonication at room temperature for 60 min. After the filtration through 0.2 μm membrane filter, to an aliquot of 1 mL of the filtrate was evaporated under vacuum, and then dissolved in 55% aqueous methanol prior to analysis.

Calibration - Stock solution (1 mg/mL) of the forsythia-

Table 2. Comparison of effectiveness of extraction solvent against standard metabolites in *F. suspensa* and *F. viridissima* (w/w, %, n = 3)

Compounds	70% MeOH	50% MeOH	70% EtOH	50% EtOH
1	2.90 ± 0.03	3.65 ± 0.04	3.60 ± 0.01	3.13 ± 0.02
2	0.08 ± 0.01	0.08 ± 0.00	0.07 ± 0.01	0.08 ± 0.01
3	0.27 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.28 ± 0.01
4	0.27 ± 0.02	0.28 ± 0.02	0.28 ± 0.01	0.28 ± 0.01
5	0.24 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.28 ± 0.01
6	0.57 ± 0.01	0.76 ± 0.01	0.71 ± 0.01	0.58 ± 0.01
7	1.89 ± 0.01	2.27 ± 0.02	1.95 ± 0.03	1.93 ± 0.02
8	3.29 ± 0.04	3.88 ± 0.02	3.28 ± 0.06	3.15 ± 0.04

Table 3. Comparison of effectiveness of extraction time against standard metabolites in *F. suspensa* and *F. viridissima* (w/w, %, n = 3)

Compounds	10 min	20 min	30 min	60 min	90 min
1	2.38 ± 0.01	2.89 ± 0.02	3.33 ± 0.01	3.65 ± 0.04	3.48 ± 0.01
2	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.00	0.13 ± 0.01
3	0.20 ± 0.01	0.23 ± 0.00	0.24 ± 0.01	0.32 ± 0.00	0.24 ± 0.01
4	0.19 ± 0.00	0.23 ± 0.01	0.24 ± 0.01	0.28 ± 0.01	0.26 ± 0.01
5	0.19 ± 0.00	0.25 ± 0.01	0.26 ± 0.01	0.32 ± 0.01	0.29 ± 0.01
6	0.53 ± 0.01	0.55 ± 0.01	0.59 ± 0.01	0.76 ± 0.01	0.65 ± 0.01
7	1.68 ± 0.01	1.83 ± 0.01	2.02 ± 0.01	2.27 ± 0.01	1.97 ± 0.02
8	2.94 ± 0.01	3.08 ± 0.01	3.32 ± 0.01	3.38 ± 0.01	3.25 ± 0.03

side (**1**), lariciresinol (**2**), phyllirin (**3**), pinoresinol (**4**) and phylligenin (**5**) isolated from *F. suspensa* and arctiin (**6**), matairesinol (**7**), arctigenin (**8**) isolated from *F. viridissima* were prepared individually in methanol, and different concentration (4, 8, 15, 20, 25, and 30 μg/mL) of these were loaded onto an HPLC for the preparation of the calibration function. The calibration function of individual compound was calculated with peak area (y), concentration (x, μg/mL), and mean values (n = 3) ± standard deviation.

Result and discussion

For the simultaneously determination of five bioactive compounds in *Forsythia suspensa* and three bioactive compounds in *Forsythia viridissima*, we firstly investigated the optimal chromatographic conditions. Various mixtures of water, methanol and acetonitrile were tested as a mobile phase. The optimal wavelength for detection was tested at 210, 230, 250, 280, and 300 nm, respectively. Considering the significantly distinct UV maxima between the two types of analytes, optimum detection wavelength was decided at 280 nm where all of the eight representative compounds showed relatively good absorptions. The temperature for detection was tested at 25, 30, 35, 40, and 45°C. Optimum detection temperature was decided at 40°C where the baseline was stable and all of the compounds had good theoretical plate, capacity factor, separation factor, and resolution (Table 4 and Table 5). The presences of forsythiaside (**1**), lariciresinol (**2**), phyllirin (**3**), pinoresinol (**4**) and phillygenin (**5**) in *F. suspensa* and arctiin (**6**), matairesinol (**7**), and arctigenin (**8**) in *F. viridissima* were verified by comparing each retention time and UV spectrum with those of each standard compound and spiking with authentic standards. As a result, an isocratic methanol - 0.3% acetic acid solvent system in the ratio 55 : 45 (*v/v*) at 280 nm and 40 °C gave the desired separation within the running time of 35 min (Fig. 3 and Fig. 4).

A methanol stock solution, containing eight standard compounds, was prepared and diluted to a series of appropriate concentrations for the construction of calibration curves. The curves showed good linearity and the correlation coefficients were found to be in the range of 0.996 - 0.999 for all the compounds, over the concentration ranges 4 - 30 μg/L. Limits of detection and limits of quantitation were determined by means of serial dilution based on a signal-to-noise (S/N) ratio of 3 : 1 and 10 : 1, respectively. LOD and LOQ were less than 0.096 and 0.291 mg/L, respectively (Table 6).

Table 4. Comparison of robustness of *F. suspensa* metabolites with temperature of column oven.

Temperature (°C)	Analytes				
	1	**2**	**3**	**4**	**5**
Theoretical plate (N)					
25°C	4818.00 ± 248.59	10306.67 ± 20.40	9093.67 ± 29.67	12299.33 ± 235.03	14630.67 ± 215.96
30°C	4864.00 ± 221.72	10841.00 ± 72.27	9779.33 ± 66.79	12853.67 ± 143.91	14982.00 ± 147.03
35°C	4196.00 ± 238.03	11423.67 ±196.04	10359.00 ± 65.51	13495.67 ± 68.50	15748.67 ± 43.32
40°C	3554.33 ± 660.49	11841.00 ± 54.74	10792.00 ± 131.24	14010.00 ± 189.95	16130.67 ± 2.08
45°C	3314.33 ± 247.46	12216.67 ± 241.54	11115.00 ± 169.43	14174.00 ± 203.60	16345.00 ± 142.24
Capacity factor (k')					
25°C	12.40 ± 0.07	24.92 ± 0.05	33.45 ± 0.06	41.72 ± 0.03	116.09 ± 0.08
30°C	11.25 ± 0.10	22.66 ± 0.08	30.46 ± 0.09	37.51 ± 0.09	101.91 ± 0.15
35°C	10.28 ± 0.10	20.70 ± 0.08	27.80 ± 0.09	33.85 ± 0.09	89.69 ± 0.12
40°C	9.53 ± 0.06	19.03 ± 0.04	25.52 ± 0.05	30.74 ± 0.05	79.52 ± 0.01
45°C	8.83 ± 0.08	17.58 ± 0.08	23.51 ± 0.09	28.04 ± 0.10	70.76 ± 0.20
Separation factor (α)					
25°C	–	2.01 ± 0.01	1.34 ± 0.00	1.25 ± 0.00	2.78 ± 0.00
30°C	–	2.01 ± 0.01	1.34 ± 0.00	1.23 ± 0.00	2.72 ± 0.01
35°C	–	2.01 ± 0.01	1.34 ± 0.00	1.22 ± 0.00	2.65 ± 0.00
40°C	–	2.00 ± 0.01	1.34 ± 0.00	1.20 ± 0.00	2.59 ± 0.01
45°C	–	1.99 ± 0.01	1.34 ± 0.00	1.19 ± 0.00	2.52 ± 0.00
Resolution (Rs)					
25°C	–	13.93 ± 0.12	6.91 ± 0.02	5.52 ± 0.02	27.44 ± 0.21
30°C	–	14.13 ± 0.08	7.14 ± 0.02	5.36 ± 0.03	27.23 ± 0.05
35°C	–	13.79 ± 0.12	7.29 ± 0.03	5.18 ± 0.00	27.26 ± 0.02
40°C	–	13.11 ± 0.51	7.37 ± 0.02	4.98 ± 0.03	27.00 ± 0.07
45°C	–	12.88 ± 0.22	7.39 ± 0.05	4.74 ± 0.03	26.49 ± 0.06

Table 5. Comparison of robustness of *F. viridissima* metabolites with temperatures of column oven

Temperature (°C)	Analytes		
	6	**7**	**8**
Theoretical plate (N)			
25°C	9688.33 ± 130.86	12868.33 ± 119.64	14528.67 ± 169.01
30°C	10301.67 ± 98.44	13465.33 ± 141.51	15087.33 ± 201.39
35°C	10894.33 ± 20.31	14315.33 ± 176.14	15570.67 ± 118.73
40°C	11412.00 ± 6.24	14624.67 ± 188.60	16076.00 ± 150.57
45°C	12034.00 ± 26.23	15163.67 ± 31.18	16330.00 ± 181.25
Capacity factor (k')			
25°C	28.08 ± 0.02	48.62 ± 0.03	82.54 ± 0.05
30°C	25.60 ± 0.01	43.03 ± 0.01	72.96 ± 0.02
35°C	23.43 ± 0.00	38.29 ± 0.01	64.78 ± 0.04
40°C	21.57 ± 0.01	34.31 ± 0.02	57.92 ± 0.04
45°C	19.94 ± 0.02	30.90 ± 0.03	51.99 ± 0.05
Separation factor (α)			
25°C	–	1.78 ± 0.00	1.14 ± 0.00
30°C	–	1.68 ± 0.00	1.13 ± 0.00
35°C	–	1.63 ± 0.00	1.12 ± 0.00
40°C	–	1.59 ± 0.00	1.11 ± 0.00
45°C	–	1.55 ± 0.00	1.10 ± 0.00
Resolution (Rs)			
25°C	–	14.00 ± 0.01	4.02 ± 0.02
30°C	–	13.57 ± 0.04	3.78 ± 0.02
35°C	–	13.19 ± 0.05	3.55 ± 0.01
40°C	–	12.63 ± 0.04	3.29 ± 0.02
45°C	–	12.16 ± 0.01	3.08 ± 0.02

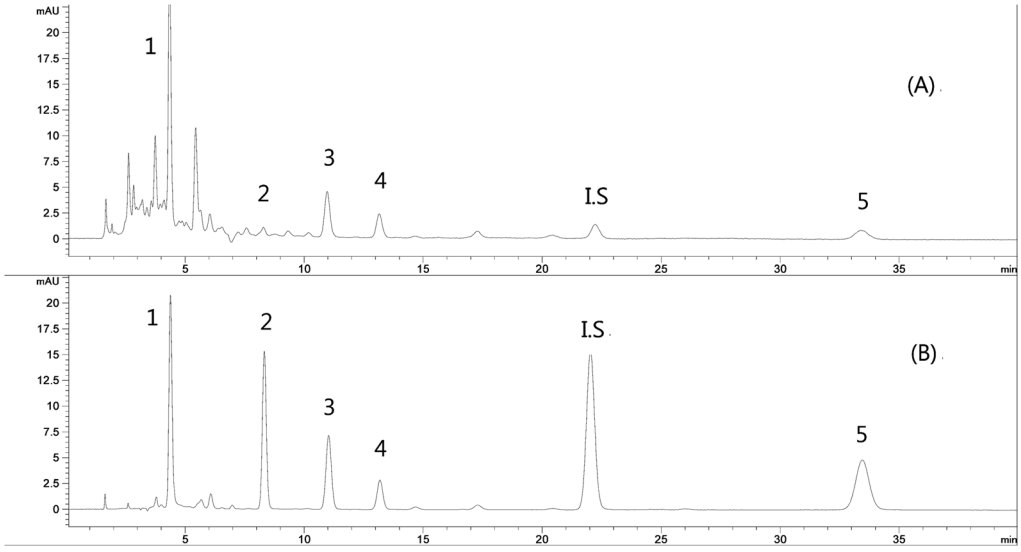

Fig. 3. HPLC chromatograms of the standard extract of *Forsythia suspensa* (A) and standard mixture (B).

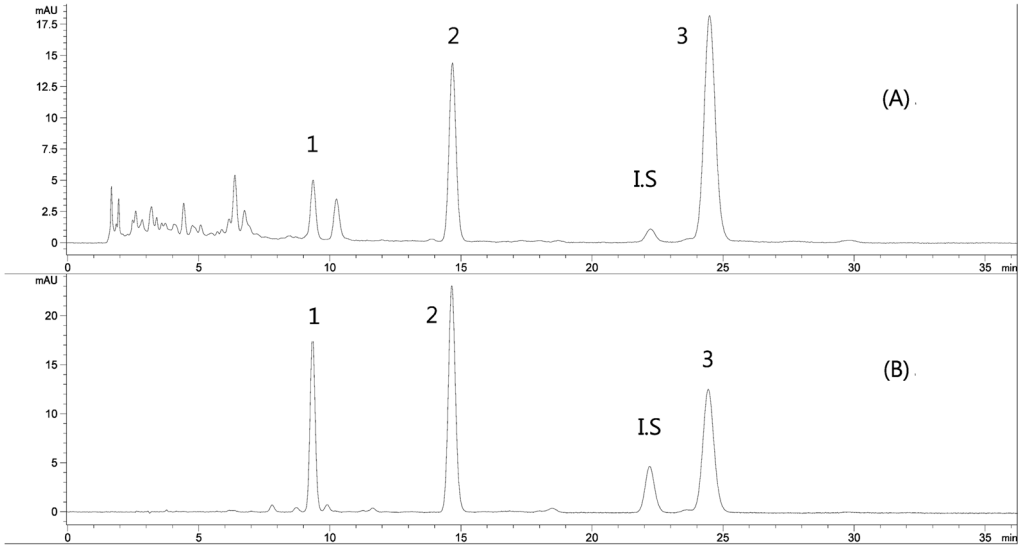

Fig. 4. HPLC chromatograms of the standard extract of *Forsythia viridissima* (A) and standard mixture (B).

Table 6. Calibration curves, LOD and LOQ of the standard compounds in *F. suspensa* and *F. viridissima*

Compounds	Range (μg/mL)	regression equation[a)]	r^2	LOD[b)] (μg/mL)	LOQ[c)] (μg/mL)
1	4 ~ 30	$y = 0.24177x - 0.07272$	0.996	0.067	0.205
2	4 ~ 30	$y = 0.36543x - 0.19401$	0.999	0.054	0.163
3	4 ~ 30	$y = 0.23330x - 0.11506$	0.999	0.094	0.285
4	4 ~ 30	$y = 0.22127x - 0.02661$	0.999	0.096	0.291
5	4 ~ 30	$y = 0.25599x - 0.10564$	0.998	0.082	0.247
6	4 ~ 30	$y = 0.19132x - 0.14105$	0.999	0.064	0.194
7	4 ~ 30	$y = 0.05259x - 0.21936$	0.999	0.066	0.201
8	4 ~ 30	$y = 0.04505x - 0.21331$	0.999	0.087	0.265

a) y: peak area, x: concentration of the analyte (μg/mL).
b) LOD, limit of detection. c) LOQ, limit of quantification.

Table 7. Precision and accuracy of the standard compounds in *F. suspensa* and *F. viridissima*

Compounds	Spiked amount (μg)	Intra-day (n = 5)		Inter-day (n = 5)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1	4	101.14	0.25	101.29	0.19
	8	100.64	0.26	101.66	0.26
	12	99.82	0.25	99.95	0.46
2	2	99.29	0.30	99.04	0.29
	4	100.58	0.48	101.42	0.23
	8	99.97	0.24	100.37	0.37
3	2	99.91	0.47	101.38	0.46
	4	98.35	0.48	100.48	0.32
	8	98.24	0.10	98.82	0.25
4	2	98.43	0.51	99.01	0.62
	4	99.82	0.49	100.01	0.39
	8	101.06	0.11	101.60	0.30
5	4	100.95	0.43	99.90	0.29
	8	100.45	0.03	101.20	0.35
	12	99.24	0.18	99.48	0.32
6	4	99.04	0.43	98.52	0.22
	8	101.43	0.28	101.30	0.17
	12	98.47	0.34	99.24	0.22
7	2	99.20	0.63	98.73	0.31
	4	101.05	0.07	101.89	0.58
	8	99.75	0.23	100.20	0.23
8	4	101.18	0.51	99.79	0.30
	8	101.24	0.14	102.46	0.14
	12	97.79	0.21	98.40	0.27

The accuracy and precision tests were carried out by measurement of the intra-day and inter-day variability and recovery of these constituents. The measurement of intra-day and inter-day variability was utilized to determine the precision of this method. The intra-day variation was determined by analyzing in the triplicate same mixed standard methanol solution for three times within 1 day while for the inter-day variability test, the solution was examined in triplicate for consecutive 3 days. The average percentage recoveries were evaluated by calculating the ratio of detected amount versus the added amount and the mean recovery of each compound was 97.79 - 102.46%. The relative standard deviation (RSD) was taken as a tool of precision. The RSD of intra-day and inter-day variability was less than 0.63% (Table 7).

The developed method was applied for the measurement of concentration of standard components in commercial *F. suspensa* and *F. viridissima*. Fifty-three specimens of *F. suspensa* from various areas of China and fifteen specimens *F. viridissima* from various areas of Korea were analyzed. The test samples were prepared as described for the development of analytical method and injected in triplicate. The results are summarized in Table 8 and Table 9. It was found that contents of standard compounds in various regional samples vary significantly by sources and *F. suspensa* standard compounds were almost not detected in *F. viridissima* and vice versa. Among the five components in *F. suspensa*, forsythiaside had remarkably higher concentrations than others (forsythiaside, 0.15~11.31%; lariciresinol, 0.03~0.21%; phyllirin, 0.02~0.75%; pinoresinol, 0.01~0.23%; phillygenin, 0.04~0.23%). Among the components in *F. viridissima*, arctigenin had higher concentrations than all the other compounds but unremarkably. (arctiin, 0.40~1.60%; matairesinol, 0.63~1.94%; arctigenin, 0.63~4.26%). These results clearly demonstrated the significant differences in bioactive components between *F. suspensa* and *F. viridissima*, each representing Forsythia fructus in different geographical regions. Overall, based on eight standard compounds in *F. suspensa* and *F. viridissima*,

Table 8. Content of the standard compounds in samples of commercial *F. suspensa* from diverse sources (w/w, %)

Sample	**1**	**2**	**3**	**4**	**5**
c-1	4.26 ± 0.28	0.06 ± 0.00	0.24 ± 0.02	0.06 ± 0.01	0.07 ± 0.00
c-2	0.18 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.01	0.04 ± 0.00
c-3	0.78 ± 0.01	0.06 ± 0.00	0.12 ± 0.00	0.10 ± 0.01	0.07 ± 0.00
c-4	0.15 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.06 ± 0.00
c-5	0.96 ± 0.03	0.05 ± 0.00	0.20 ± 0.00	0.17 ± 0.00	0.16 ± 0.00
c-6	5.28 ± 0.05	0.05 ± 0.00	0.31 ± 0.00	0.08 ± 0.01	0.08 ± 0.00
c-7	9.13 ± 0.16	0.03 ± 0.00	0.69 ± 0.01	0.01 ± 0.01	0.05 ± 0.00
c-8	0.23 ± 0.01	0.03 ± 0.00	0.06 ± 0.00	0.04 ± 0.01	0.09 ± 0.04
c-9	5.52 ± 0.52	0.05 ± 0.01	0.38 ± 0.04	0.07 ± 0.00	0.07 ± 0.00
c-10	0.34 ± 0.01	0.03 ± 0.00	0.09 ± 0.01	0.05 ± 0.00	0.05 ± 0.00
c-11	1.96 ± 0.07	0.05 ± 0.00	0.20 ± 0.01	0.18 ± 0.00	0.17 ± 0.00
c-12	0.20 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.05 ± 0.00
c-13	4.07 ± 0.06	0.05 ± 0.00	0.19 ± 0.00	0.11 ± 0.00	0.08 ± 0.00
c-14	4.69 ± 0.05	0.05 ± 0.00	0.40 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
c-15	0.23 ± 0.01	0.03 ± 0.00	0.08 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
c-16	6.42 ± 0.04	0.05 ± 0.00	0.36 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
c-17	8.93 ± 0.26	0.10 ± 0.00	0.48 ± 0.01	0.12 ± 0.00	0.12 ± 0.00
c-18	0.90 ± 0.02	0.05 ± 0.00	0.09 ± 0.00	0.13 ± 0.00	0.15 ± 0.00
c-19	0.53 ± 0.02	0.03 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
c-20	1.98 ± 0.03	0.16 ± 0.00	0.17 ± 0.01	0.21 ± 0.00	0.23 ± 0.00
c-21	2.24 ± 0.03	0.18 ± 0.00	0.21 ± 0.00	0.15 ± 0.00	0.12 ± 0.00
c-22	0.24 ± 0.01	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.05 ± 0.00
c-23	0.25 ± 0.05	0.05 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.05 ± 0.01
c-24	1.52 ± 0.02	0.19 ± 0.00	0.29 ± 0.00	0.21 ± 0.00	0.19 ± 0.00
c-25	0.36 ± 0.01	0.08 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
c-26	6.19 ± 0.16	0.21 ± 0.01	0.33 ± 0.01	0.09 ± 0.00	0.07 ± 0.00
c-27	1.32 ± 0.01	0.11 ± 0.00	0.11 ± 0.00	0.09 ± 0.00	0.08 ± 0.00
c-28	2.21 ± 0.05	0.17 ± 0.01	0.20 ± 0.01	0.21 ± 0.00	0.14 ± 0.00
c-29	1.72 ± 0.09	0.17 ± 0.01	0.19 ± 0.01	0.18 ± 0.00	0.11 ± 0.00
c-30	0.27 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.08 ± 0.00
c-31	3.47 ± 0.14	0.16 ± 0.00	0.27 ± 0.00	0.17 ± 0.00	0.14 ± 0.00
c-32	3.82 ± 0.50	0.17 ± 0.02	0.28 ± 0.02	0.10 ± 0.01	0.10 ± 0.00
c-33	0.22 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.06 ± 0.00
c-34	0.29 ± 0.01	0.04 ± 0.00	0.10 ± 0.00	0.07 ± 0.00	0.12 ± 0.00
c-35	3.78 ± 0.26	0.05 ± 0.01	0.22 ± 0.02	0.08 ± 0.00	0.08 ± 0.00
c-36	0.46 ± 0.04	0.03 ± 0.00	0.08 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
c-37	2.68 ± 0.05	0.04 ± 0.00	0.23 ± 0.00	0.13 ± 0.00	0.13 ± 0.00
c-38	11.31 ± 0.49	0.08 ± 0.03	0.75 ± 0.03	0.01 ± 0.00	0.05 ± 0.00
c-39	2.66 ± 0.02	0.05 ± 0.01	0.26 ± 0.00	0.09 ± 0.00	0.07 ± 0.00
c-40	1.10 ± 0.05	0.04 ± 0.00	0.18 ± 0.00	0.08 ± 0.02	0.11 ± 0.00
c-41	0.58 ± 0.01	0.05 ± 0.00	0.09 ± 0.00	0.08 ± 0.01	0.07 ± 0.00
c-42	0.36 ± 0.01	0.00 ± 0.00	0.06 ± 0.00	0.01 ± 0.01	0.04 ± 0.00
c-43	0.48 ± 0.04	0.00 ± 0.00	0.08 ± 0.00	0.06 ± 0.02	0.07 ± 0.00
c-44	1.49 ± 0.45	0.04 ± 0.00	0.14 ± 0.01	0.07 ± 0.00	0.06 ± 0.00
c-45	0.39 ± 0.01	0.00 ± 0.00	0.12 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
c-46	2.24 ± 0.02	0.05 ± 0.00	0.20 ± 0.01	0.23 ± 0.00	0.19 ± 0.00
c-47	5.54 ± 0.17	0.05 ± 0.00	0.35 ± 0.01	0.07 ± 0.00	0.08 ± 0.00
c-48	3.73 ± 0.01	0.05 ± 0.00	0.27 ± 0.00	0.09 ± 0.00	0.08 ± 0.00
c-49	3.79 ± 0.06	0.05 ± 0.00	0.23 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
c-50	4.09 ± 0.06	0.05 ± 0.00	0.39 ± 0.01	0.09 ± 0.00	0.09 ± 0.00
c-51	5.86 ± 0.11	0.06 ± 0.00	0.41 ± 0.01	0.09 ± 0.00	0.08 ± 0.00
c-52	0.40 ± 0.02	0.00 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
c-53	4.39 ± 0.29	0.06 ± 0.01	0.34 ± 0.02	0.09 ± 0.00	0.09 ± 0.00

Table 9. Content of the standard compounds in samples of commercial *Forsythia viridissima* from diverse sources (w/w, %)

Sample	**6**	**7**	**8**
k-1	0.46 ± 0.04	1.75 ± 0.11	4.26 ± 0.26
k-2	0.87 ± 0.01	0.99 ± 0.01	1.00 ± 0.03
k-3	0.69 ± 0.03	1.81 ± 0.07	3.83 ± 0.23
k-4	0.87 ± 0.04	1.58 ± 0.01	3.13 ± 0.03
k-5	1.60 ± 0.05	0.93 ± 0.03	2.49 ± 0.07
k-6	0.45 ± 0.00	1.26 ± 0.01	2.11 ± 0.02
k-7	0.61 ± 0.00	1.10 ± 0.01	1.75 ± 0.01
k-8	0.97 ± 0.01	1.48 ± 0.00	2.04 ± 0.03
k-9	1.54 ± 0.01	1.09 ± 0.01	1.31 ± 0.01
k-10	1.15 ± 0.03	1.37 ± 0.03	1.38 ± 0.03
k-11	0.41 ± 0.01	1.95 ± 0.01	3.99 ± 0.02
k-12	0.91 ± 0.00	0.83 ± 0.00	0.88 ± 0.01
k-13	1.23 ± 0.01	1.33 ± 0.00	1.41 ± 0.00
k-14	1.61 ± 0.02	0.67 ± 0.01	0.63 ± 0.01
k-15	0.63 ± 0.01	0.49 ± 0.00	0.67 ± 0.01

HPLC-DAD methods we developed can confirm two species easily and swiftly. Therefore it will be effectively used to screen and discriminate a great number of sample.

Conclusion

Discrimination of two herbal medicines, *Forsythia suspensa* and *Forsythia viridissima*, was achieved by the development of simple and versatile analytical methods using HPLC-DAD. These were applied for the simultaneous identification and quantification of forsythiaside (**1**), lariciresinol (**2**), phyllirin (**3**), pinoresinol (**4**) and phylligenin (**5**) in *F. suspensa* and arctiin (**6**), matairesinol (**7**) and arctigenin (**8**) in *F. viridissima*, respectively. Various validation parameters such as specificity, linearity, detection limit, quantitation limit, accuracy and precision were successfully obtained and individually validated. In addition, effectiveness of diverse extraction methods was compared to each other. This method was successfully applied for the measurement of concentrations of standard components from diverse sources between *F. suspensa* and *F. viridissima*, thus demonstrating its potential for the quality control of Forsythia fructus, a widely used but mixed traditional medicine.

Acknowledgements

This research was supported by a grant (09112 KFDA815) from the National Center for Standardization of Herbal Medicine funded by the Korea Food Drug Administration, Republic of Korea (2009).

References

Chan, K., Chinese medicinal materials and their interface with Western medical concepts. *J. Ethnopharmacol.* **96**, 1-18 (2005).

Chang, M.J., Huang, T.M., Min, B.S., Kim, J.C., Woo, M.H., Choi, J.S., Lee, H.K., and Bae, K., Lignans from the fruits of *Forsythia suspensa* (Thunb) Vahl protect high-density lipoprotein during oxidative stress. *Biosci. Biotechnol Biochem*. **72**, 2750-2755 (2008).

Chiba, M., Hisada, S., and Nishibe, S., Studies on the Chinese crude drug "Forsythia Fructus" (III). On the constituents of fruits of *Forsythia viridissima* and *F. suspensa*. *Shoyakugaku Zashi* **32**, 194-197 (1978).

Kitagawa, S., Hisada, S., and Nishibe, S., Phenolic compounds from *Forsythia* leaves. *Phytochem*. **23**, 16-35 (1984).

Lee, E.B. and Keum, H.J., Pharmacological studies on Forsythiae Fructus. *Kor. J. Pharmacogn*. **19**, 262-269 (1988).

Lee, J.Y., Moon, H., and Kim, C.J., Effects of hydroxyl pentacyclic triterpene acids from *Forsythia viridissima* on asthmatic responses to ovalbumin challenge in conscious guinea pigs. *Biol. Pharm. Bull.* **33**, 230-237 (2010).

Li, Q. and Feng, W.S., Recent development of chemical components of *Forsythia suspensa*. *J. Henan Univ. Chin. Med.* **20**, 78-80 (2005).

Liu, M., Recent research on the pharmacological effects of *Forsythia suspensa*. *Mod. Med. Health.* **23**, 2438-2439 (2007).

Mariko, C., Tsukamoto, H., Hisada, S., and Nishibe, S., Studies on the Chinese crude drug "Forsythia fructus". IV. On the constituents of fruits of *Forsythia koreana* and Forsythia fructus from Korea on the market. *Shoyakugaku Zashi.* **33**, 150-154 (1979).

Nishibe, S., Chiba, M., and Hisada, S., Studies on the Chinese crude drug "Forsythia Fructus" (I). On the constituents Forsythia Fructus on the market. *Yakugaku Zashi* **97**, 1134-1137 (1977).

Normile, M., The new face of traditional Chinese medicine. *Science* **299**, 188-190 (2003).

Piao, X.L., Jang, M.H., Cui, J., and Piao, X.S., Lignans from the fruits of *Forsythia suspensa Bioorg. Med. Chem Lett*. **18**, 1980-1984 (2008).

Zhang, H.Y., Research on the chemical constituents and pharmacological activity of *Forsythia suspensa*. *J. Chin. Med. Mater.* **23**, 657-660 (2000).

Received August 26, 2011
Revised October 20, 2011
Accepted October 25, 2011