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Simultaneous determination of 13 bioactive compounds in Herba Artemisiae Scopariae (Yin Chen) from different harvest seasons by HPLC–DAD

Xiao-jie Tan[a,b], Qing Li[b], Xiao-hui Chen[b], Zhi-wei Wang[b], Zheng-yuan Shi[b], Kai-shun Bi[a,b,*], Ying Jia[a,**]

[a] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, Liaoning Province, PR China
[b] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, Liaoning Province, PR China

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ABSTRACT

Herba Artemisiae Scopariae is a Chinese herbal medicine widely used for the remedy of liver diseases. A high performance liquid chromatography method coupled with diode array detection was developed to simultaneously determine 13 different bioactive compounds in Herba Artemisiae Scopariae (Yin Chen) including chlorogenic acid (1), 6,7-dihydroxycoumarin (2), caffeic acid (3), 4-hydroxyacetophenone (4), scopoletin (5), rutin (6), hyperoside (7), isoquercitrin (8), scoparone (11), 7-methoxycoumarine (12) and quercetin (13). By using four different wavelengths in the HPLC analysis, the developed method was able to determine the bioactive compounds with excellent resolution, precision and recovery. The method was applied to determine the amounts of the bioactive compounds in nine samples from different cultivated regions and harvest seasons in China, and significant variations were revealed. Chlorogenic acid was the most abundant among the analyzed compounds. The samples harvested in the spring contained higher contents of chlorogenic acid than those collected in other seasons. Other phenolic acids as caffeic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and 4-hydroxyacetophenone accumulated at much higher amounts in about May to July. The samples analyzed contained a much lower level of the amount of other flavonoids and coumarins as rutin, hyperoside, isoquercitrin and scoparone.

1. Introduction

Herba Artemisiae Scopariae (Yin chen), one of the well-known traditional Chinese medicines, is the aerial parts of the *Artemisia capillaris* Thunb. or *Artemisia scoparia* Waldst. et kit. Herba Artemisiae Scopariae has been being widely used for the remedy of liver diseases such as hepatitis, jaundice and fatty liver in traditional oriental medicine. Modern pharmacological studies showed that aqueous extract from *A. capillaris* Thunb. (AEAC) inhibits expression of inflammatory proteins including iNOS, COX-2 and TNF-alpha. Also, nuclear translocation of NF-kappaB and degradation of I-kappaBalpha are blocked by AEAC pretreatment [1]. The aqueous extract of *A. capillaris* Thunb. inhibited the EtOH-, IL-1alpha-, and TNF-alpha-induced cytotoxicity and the EtOH-induced apoptosis of Hep G2 cells [2].

Chemical investigations have shown that phenolic acids, coumarins, flavonoids and 4-hydroxyacetophenone are the major constituents in this herb [3]. Pharmacological studies have revealed that these possess a wide array of pharmacological and biochemical properties, which are believed to be responsible for the antihepatotoxic activity [4,5], neuroprotective and neurotrophic effect, antioxidant activity [6,7], antiviral activity [8], cerebral protection [9,10,12], antiatherogenic [11], the protective effect against hepatic damage [13,14] and cholagogue behavior [15]. The above listed constituents are believed to be the active components in Herba Artemisiae Scopariae, and could be considered as the 'marker compounds' for the chemical evaluation or standardization of Herba Artemisiae Scopariae.

There has been no quantitative analysis for multiple compounds in Herba Artemisiae Scopariae until now. The currently available assay procedure for Herba Artemisiae Scopariae involves HPLC quantitative analyses of 7-methoxycoumarin and capillary electrophoresis with electrochemical detection of 4-hydroxyacetophenone, chlorogenic acid, and caffeic acid [16–22]. These methods can not satisfy the requirements for studies of

* Corresponding author at: School of Pharmacy, Shenyang Pharmaceutical University, PO Box 21, 103 Wenhua Road, Shenyang 110016, PR China. Tel.: +86 24 23928487; fax: +86 24 23896576.
** Corresponding author at: School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, PO Box 21, 103 Wenhua Road, Shenyang 110016, PR China. Tel.: +86 24 23928487; fax: +86 24 23896576.
E-mail addresses: bikaishun@yahoo.com (K.-s. Bi), xiaojiesmail@yahoo.com (Y. Jia).

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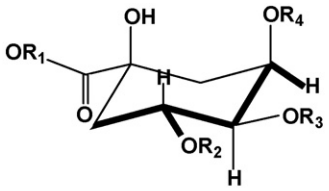
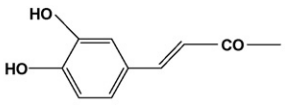


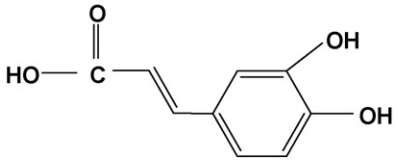


caffeoyl

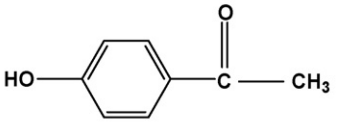
R_1, R_2, R_4= H R_3 = caffeoyl chlorogenic acid (1)

R_1, R_3 = H $R_{2,4}$ = caffeoyl 3, 5-dicaffeoylquinic acid (9)

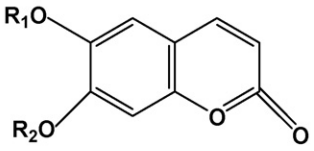
R_1, R_2 = H $R_{3,4}$ = caffeoyl 4, 5-dicaffeoylquinic acid (10)

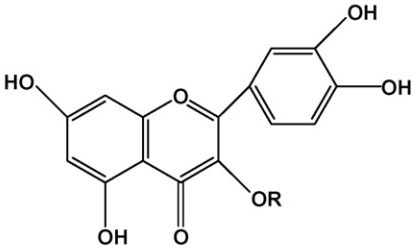


caffeic acid (3)



4-Hydroxyacetophenone (4)





R_1, R_2 = H 6,7-dihydroxycoumarin (2)

R_1 = CH_3 R_2 = H scopoletin (5)

R_1, R_2 = CH_3 scoparone (11)

R_1 = H R_2 = CH_3 7-methoxycoumarine (12)

R=Glc(6-1)Rha Rutin (6)

R=Gal hyperoside (7)

R=Glc isoquercitrin (8)

R=H Quercetin (13)

Fig. 1. Structures of 13 compounds in Herba Artemisiae Scopariae: chlorogenic acid (1), 6,7-dihydroxycoumarin (2), caffeic acid (3), 4-hydroxyacetophenone (4), scopoletin (5), rutin (6), hyperoside (7), isoquercitrin (8), 3,5-dicaffeoylquinic acid(9), 4,5-dicaffeoylquinic acid (10), scoparone (11), 7-methoxycoumarine (12) and quercetin (13).

mechanism of action and quality control of Herba Artemisiae Scopariae. Moreover, due to multiple compounds that might be associated with the therapeutic functions, a single or a few marker compounds could not be responsible for the overall pharmacological activities of the drug. A comprehensive quality evaluation method based on analysis of all bioactive compounds is urgently needed in order to accurately reflect the quality of this herbal drug. In this study, a HPLC method was developed for simultaneous determination of 13 major phenolic acids, flavonoids, and coumarins, namely chlorogenic acid (1), 6,7-dihydroxycoumarin (2), caffeic acid (3), 4-hydroxyacetophenone (4), scopoletin (5), rutin (6), hyperoside (7), isoquercitrin (8), 3,5-dicaffeoylquinic acid (9), 4,5-dicaffeoylquinic acid (10), scoparone (11), 7-methoxycoumarine (12) and quercetin (13). Their structures are shown in Fig. 1.

The harvest season has been shown to affect the therapeutic effect of Herba Artemisiae Scopariae significantly. In the traditional Chinese medicine, it has to be collected around April; the drug collected in May is believed to have no medicinal value at all. However, some modern chemical and pharmacological studies suggested that Herba Artemisiae Scopariae should be collected in August or September, which is the flower bud stage. Our method was successfully applied to nine samples collected from different cultivated regions and harvest seasons.

2. Experimental

2.1. Chemicals, reagents and materials

Acetonitrile was of HPLC grade from Yu-wang Chemical Factory (Shandong, China). Phosphoric acid was of HPLC grade from Beijing Reagent Company (Beijing, PR China). For samples from different growing months, two batches of samples of 2-year-old plants were collected. Batch A samples were collected on the same day of every month from April to August in 2007 from the same field of Shenyang, Liaoning province (higher latitude). Batch B samples were collected on the same day of every month from March to June in 2007 from the same field of Xi'an, Shaanxi province (lower latitude). The samples were authenticated by Qi-shi Sun, Professor of Pharmacognosy, Shenyang Pharmaceutical University, according to the morphological characteristics. Voucher specimens (No. Yinchen-X-0319-Yinchen-S-0819) were deposited at Herbarium of Shenyang Pharmaceutical University. Standard compounds **4**–**7**, **11**–**13** were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Standard compounds **1**–**3**, **8**–**10** were isolated from the aerial parts of the *A. scoparia* Waldst. et kit. *A. scoparia* Waldst. et kit. (5 kg) by extracting with 70% EtOH at room temperature. It was concentrated in vacuo and then extracted with petroleum (60–90 °C fraction),

Table 1
Detection wavelength (λ), linear regression data, LOD, precision and repeatability of 13 compounds in Herba Artemisiae Scopariae

Analyte[a]	λ (nm)	Regression equation[b]	r^2	Linear range (μg ml^{-1})	LOD (μg ml^{-1})[c]	Precision		Repeatability R.S.D. (%)
						Intra-day R.S.D. (%)[d]	Inter-day R.S.D. (%)	
1	325	$y = 14.51x + 131.2$	1.0000	77.82–3112	0.082	0.4	0.6	2.9
2	345	$y = 22.04x + 1.349$	0.9999	0.73–29.09	0.065	0.8	2	tr
3	325	$y = 27.65x - 8.961$	1.0000	1.47–58.76	0.048	0.6	1.2	3.6
4	280	$y = 38.52x - 0.8900$	1.0000	2.00–80.00	0.048	0.4	0.8	2.8
5	345	$y = 23.58x - 2.894$	0.9999	0.73–29.09	0.065	0.7	0.4	tr
6	355	$y = 9.683x - 2.796$	0.9999	1.83–73.09	0.095	0.9	0.8	2.6
7	355	$y = 13.38x - 8.610$	1.0000	5.54–221.4	0.074	0.4	1.4	2.9
8	355	$y = 13.21x - 3.125$	0.9999	1.98–79.27	0.079	0.4	1.4	4
9	325	$y = 4.520x - 29.14$	0.9999	46.40–1856	0.619	0.4	1.5	2.8
10	325	$y = 6.441x - 31.53$	0.9999	88.09–3524	0.211	0.4	1.1	3.2
11	345	$y = 30.70x - 26.34$	0.9999	0.73–290.9	0.097	0.4	0.5	2.5
12	325	$y = 31.84x - 11.56$	0.9999	0.73–29.09	0.048	0.3	1.2	2.3
13	355	$y = 29.25x - 0.4250$	0.9999	0.73–29.09	0.048	0.7	1.8	tr

"tr": below the linear range of calibration.
[a] The notation for analyte refers to Fig. 1.
[b] The regression equations were constructed by plotting the peak areas *versus* the concentration (μg ml^{-1}) of each analyte. Each regression equation included six data points.
[c] LOD refers to the limits of detection.
[d] R.S.D. (%) = (S.D./mean) × 100.

$CHCl_3$, EtOAc and n-BuOH. The $CHCl_3$ (130 g) and EtOAc fraction (70 g) were subjected to repeated chromatography on silica gel column and eluted with cyclohexane–EtOAc and $CHCl_3$–MeOH gradient solvent system. Further purification was performed by using preparative HPLC with MeOH–H_2O solvent system and Sephadex LH20 column to yield compounds 1 (15 mg), 2 (5 mg), 3 (10 mg), 8 (12 mg), 9 (20 mg) and 10 (30 mg), among which compounds 2, 9 and 10 were isolated from *A. capillaris* Thunb. for the first time. The structures were confirmed by their UV, MS, ^{1}H NMR and ^{13}C NMR data compared with the data from literature [23–27]. The purities of these isolated chemicals were determined to be more than 95% by normalization of peak areas detected by HPLC–DAD.

2.2. Sample preparation

The samples were powdered to a homogeneous size in a mill, passed through a 40-mesh sieve. The accurately weighed powder (0.5 g) was extracted by refluxing with 100 ml methanol–water (60:40, v/v) for 1 h. The samples were evaporated to dryness on a rotary evaporator, and then the extract was transferred into a 10-ml volumetric flask which was made up to volume with extraction solvent and was filtered through a 0.45-μm membrane prior to injection into the HPLC system.

2.3. HPLC–DAD analysis

An Agilent 1100 liquid chromatograph system (Agilent Technologies, Palo Alto, CA, USA) consisting of a quaternary pump, an autosampler and a photodiode array detector coupled with Agilent Chemstation was used. Separations were carried out with a Gemini C_{18} reversed-phase column (250 mm × 4.6 mm, 5 μm) (Phenomenex Sciences Instrument Co., Ltd., USA). The mobile phase consisted of 0.04% phosphoric acid aqueous (A) and acetonitrile (B). The gradient program was as follows: 0–12 min, linear gradient 10–12% B; 12–17 min linear gradient 12–16% B; 17–40 min linear gradient 16–25% B; 40–50 min linear gradient 25–38% B. Chromatography was performed at 35 °C. The flow rate was 1.0 ml/min and aliquots of 5 μl were injected. The UV detection wavelength was set at 280, 325, 345 and 355 nm and absorption spectra of compounds were recorded between 200 and 400 nm. The compounds were identified by comparing their retention times and UV spectra with those of the markers.

3. Results and discussion

3.1. Extraction procedure

Various extraction methods, solvents and times were evaluated to obtain the best extraction efficiency. The results revealed that refluxing was better than ultrasonic bath extraction, so the further experiments were carried out with refluxing. Various solvents including water, methanol–water (20:80, v/v; 40:60, v/v; 60:40, v/v; 80:20, v/v) and methanol were screened. Methanol–water (60:40, v/v) exhibited complete extraction of all the major constituents. No second extraction step was found necessary.

3.2. Optimization of chromatographic conditions

Various compositions of mobile phase were tried to obtain chromatograms with good resolution of adjacent peaks. Acetonitrile and 0.04% phosphoric acid in the gradient mode were chosen to give the desired separation and acceptable tailing factor within the running time of 50 min. As the three types of compounds show different UV absorption properties, different detection wavelengths were simultaneously set to monitor these compounds in a single run, viz. 280 nm for 4, 325 nm for 1, 3, 9, 10 and 12, 345 nm for 2, 5 and 11, 355 nm for 6, 7, 8 and 13 to provide sufficient sensitivity for each analytes.

3.3. Calibration curves, limits of detection and quantification

Standard stock solutions containing 13 analytes were prepared and diluted to appropriate concentrations for plotting the calibration curves. At least six concentrations of the 13 analyte solutions were analyzed in triplicate, and then the calibration curves were constructed by plotting the peak areas *versus* the concentration of each analyte. The calculated results are given in Table 1. All the analytes showed good linearity ($r^2 > 0.999$) in a relatively wide concentration range.

The working solutions of the analytes were further diluted with methanol to yield a series of appropriate concentrations. The limits of detection (LOD) for each investigated compounds (S/N < 3) can be seen in Table 1.

Table 2
Recoveries of 13 compounds in Herba Artemisiae Scopariae

Analyte[a,b]	Initial amount (mg)	Added amount (mg)	Detected amount (mg)	Recovery (%)[c]	R.S.D. (%)[d]
1	2.012	1.070	3.133	104.7	1.8
		2.140	4.213	102.9	0.8
		3.210	5.288	102.1	1.1
2	tr	0.020	0.021	102.8	1.7
		0.040	0.041	101.6	1.0
		0.060	0.058	95.9	0.7
3	0.033	0.020	0.053	99.2	4.1
		0.040	0.074	102.3	2.4
		0.061	0.090	94.8	0.7
4	0.042	0.023	0.065	99.7	2.6
		0.045	0.086	97.0	1.5
		0.068	0.108	97.3	0.5
5	tr	0.015	0.015	103.4	3.5
		0.029	0.031	106.9	1.7
		0.044	0.045	103.4	3.3
6	0.021	0.027	0.049	105.2	3.4
		0.054	0.077	104.9	0.8
		0.080	0.104	103.1	3.5
7	0.082	0.041	0.124	102.6	1.8
		0.081	0.162	98.5	0.6
		0.122	0.201	97.4	0.1
8	0.052	0.036	0.089	102.6	9.1
		0.073	0.127	103.6	1.3
		0.109	0.165	103.9	3.3
9	0.324	0.278	0.602	100.5	4.5
		0.555	0.887	101.6	4.7
		0.833	1.127	96.4	3.3
10	3.604	2.153	5.839	103.8	2.5
		4.307	8.067	103.6	1.1
		6.460	10.277	103.3	0.7
11	0.037	0.020	0.058	105.2	1.2
		0.040	0.076	98.6	2.0
		0.060	0.096	98.4	4.9
12	0.087	0.045	0.133	102.9	3.6
		0.090	0.181	104.0	4.0
		0.135	0.225	102.1	1.3
13	tr	0.013	0.014	105.0	2.1
		0.027	0.028	103.6	0.8
		0.040	0.041	102.8	1.9

"tr": below the linear range of calibration.
[a] The notation for analyte refers to Fig. 1.
[b] Triplicate assay at each concentration level.
[c] Recovery R.S.D.(%) = 100 × (amount found-original amount)/amount spiked; the data presented as average of three determinations.
[d] R.S.D. (%) = (S.D./mean) × 100.

3.4. Precision, accuracy and stability

The intra- and inter-day precisions were determined by analyzing known concentrations of the 13 analytes in 5 replicates during a single day and by duplicating the experiments on 3 successive days. In order to confirm the repeatability, 5 different working solutions prepared from the same sample obtained from Liaoning Province were analyzed. The relative standard deviation (R.S.D.) was taken as a measure of precision and repeatability. The results are shown in Table 1, indicating that the intra-, inter-day and repeatability R.S.D. values of the 13 compounds were all less than 4.0%, which showed good reproducibility of the developed method.

Recovery tests were carried out to further investigate the accuracy of the method by adding three concentration levels of the mixed standard solutions to known amounts of Herba Artemisiae Scopariae samples. The resultant samples were then extracted and analyzed with the described method. The average percentage recoveries were evaluated by calculating the ratio of detected amount *versus* added amount. The recovery of the method was in the range of 98.8–104.4%, with R.S.D. less than 3.8% as shown in Table 2. Considering the results, the method was deemed to be accurate.

Stability of sample solution was tested at room temperature. The sample solution was analyzed in triplicate every 8 h within 48 h. The analytes were found to be very stable in methanol solution (R.S.D. < 3.5%) over the tested period.

3.5. Sample analysis

The method was applied to simultaneously determine 13 constituents in 9 collected samples of Herba Artemisiae Scopariae collected at various harvesting times and from various cultivation regions in China. Representative chromatograms are shown in

Table 3
Contents in mg/g of 13 compounds in Herba Artemisiae Scopariae

No.	Source	Harvesting month	1[a,b]	2	3	4	5	6	7	8	9	10	11	12	13
1	Shenyang, Liaoning	April	27.972 ± 0.711	–	0.167 ± 0.005	0.467 ± 0.012	0.035 ± 0.001	0.454 ± 0.010	0.876 ± 0.016	0.231 ± 0.009	0.262 ± 0.007	6.319 ± 0.190	0.089 ± 0.002	0.020 ± 0.000	tr
2	Shenyang, Liaoning	May	7.218 ± 0.188	–	0.101 ± 0.003	0.716 ± 0.012	0.034 ± 0.001	0.517 ± 0.012	0.368 ± 0.010	0.066 ± 0.002	0.405 ± 0.009	6.097 ± 0.170	0.051 ± 0.001	0.012 ± 0.000	tr
3	Shenyang, Liaoning	June	5.855 ± 0.133	tr	0.168 ± 0.005	0.544 ± 0.008	0.031 ± 0.001	0.437 ± 0.009	0.121 ± 0.003	0.052 ± 0.002	0.732 ± 0.016	7.427 ± 0.187	0.059 ± 0.001	0.020 ± 0.000	tr
4	Shenyang, Liaoning	July	4.511 ± 0.111	0.021 ± 0.000	0.158 ± 0.005	0.788 ± 0.011	0.037 ± 0.001	0.299 ± 0.006	0.111 ± 0.003	0.069 ± 0.002	0.531 ± 0.013	10.522 ± 0.288	0.104 ± 0.002	0.081 ± 0.002	tr
5	Shenyang, Liaoning	August	4.553 ± 0.127	–	0.126 ± 0.004	0.511 ± 0.008	0.054 ± 0.002	1.396 ± 0.035	1.755 ± 0.049	0.435 ± 0.017	0.208 ± 0.006	5.612 ± 0.174	3.050 ± 0.073	0.023 ± 0.001	0.515 ± 0.010
6	Xi'an, Shaanxi	March	22.804 ± 0.381	–	0.181 ± 0.005	1.026 ± 0.015	tr	0.524 ± 0.010	0.466 ± 0.010	0.291 ± 0.009	0.146 ± 0.003	4.874 ± 0.116	0.122 ± 0.002	0.002 ± 0.000	tr
7	Xi'an, Shaanxi	April	18.130 ± 0.228	–	0.261 ± 0.006	0.994 ± 0.017	0.031 ± 0.001	0.358 ± 0.006	0.444 ± 0.008	0.326 ± 0.009	0.123 ± 0.002	4.079 ± 0.075	0.179 ± 0.003	0.002 ± 0.000	tr
8	Xi'an, Shaanxi	May	4.701 ± 0.115	–	0.111 ± 0.003	2.608 ± 0.062	tr	0.673 ± 0.015	0.368 ± 0.009	0.195 ± 0.007	0.285 ± 0.007	8.744 ± 0.240	0.064 ± 0.001	0.035 ± 0.001	0.070 ± 0.000
9	Xi'an, Shaanxi	June	9.668 ± 0.261	0.013 ± 0.000	0.157 ± 0.005	1.290 ± 0.034	0.040 ± 0.001	1.005 ± 0.024	2.082 ± 0.056	0.444 ± 0.017	0.397 ± 0.010	5.901 ± 0.177	2.618 ± 0.060	0.008 ± 0.000	0.152 ± 0.000

"–": below the LOD.
"tr": below the linear range of calibration.
[a] The notation for analyte refers to Fig. 1.
[b] Content = mean ± S.D., $n = 3$. (R.S.D. < 5.0%).

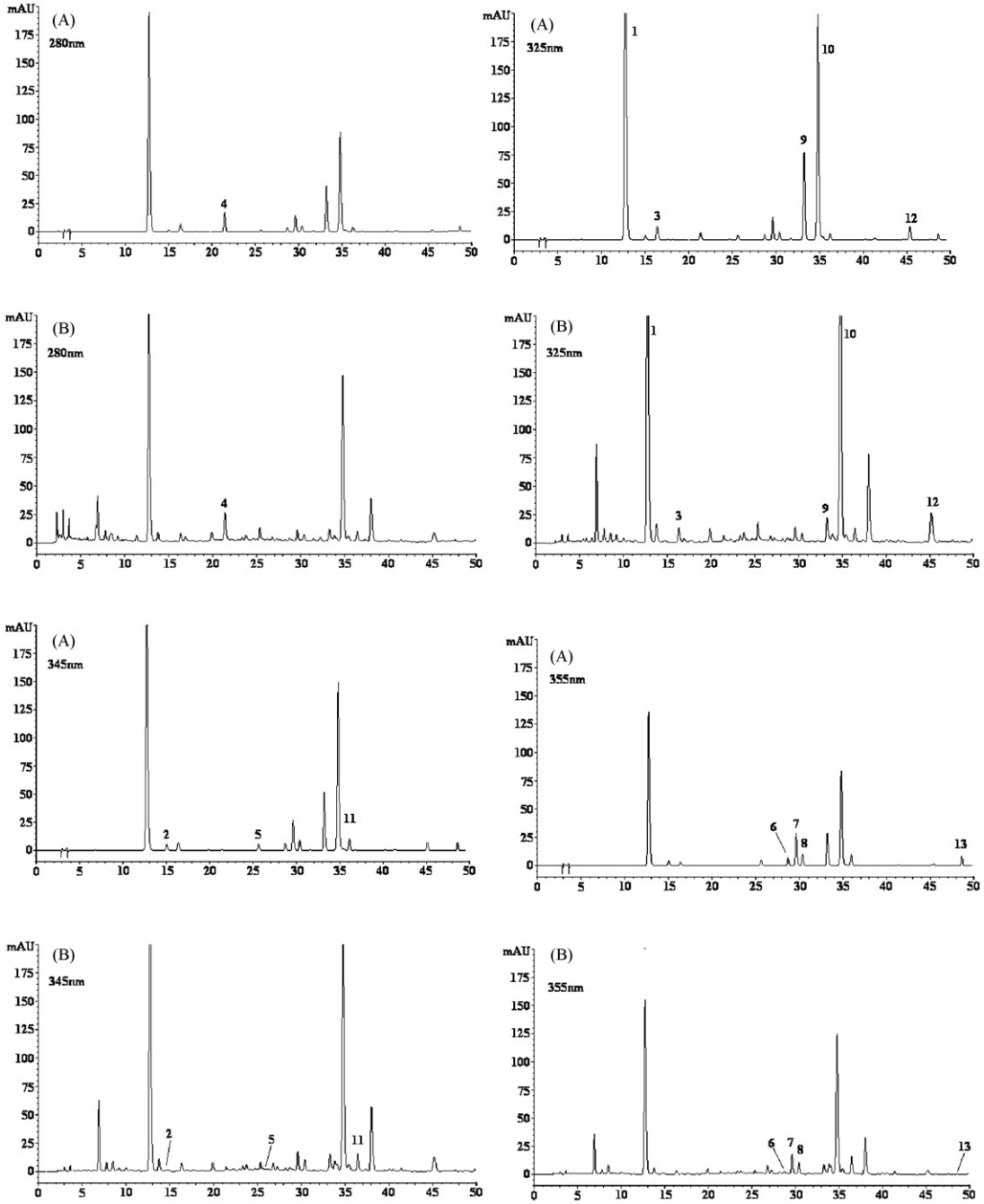


Fig. 2. HPLC chromatograms of (A) standard mixture: chlorogenic acid (1) (389.09 μg ml^{-1}), 6,7-dihydroxycoumarin (2) (3.64 μg ml^{-1}), caffeic acid (3) (7.34 μg ml^{-1}), 4-hydroxyacetophenone (4) (10.00 μg ml^{-1}), scopoletin (5) (3.64 μg ml^{-1}), rutin (6) (9.14 μg ml^{-1}), hyperoside (7) (27.68 μg ml^{-1}), isoquercitrin (8) (9.91 μg ml^{-1}), 3,5-dicaffeoylquinic acid (9) (232.00 μg ml^{-1}), 4,5-dicaffeoylquinic acid (10) (440.45 μg ml^{-1}), scoparone (11) (3.64 μg ml^{-1}), 7-methoxycoumarine (12) (3.64 μg ml^{-1}) and quercetin (13) (3.64 μg ml^{-1}); (B) Herba Artemisiae Scopariae (Shenyang, Liaoning, China): chlorogenic acid (1) (2.012 mg/g), 6,7-dihydroxycoumarin (2) (tr), caffeic acid (3) (0.033 mg/g), 4-hydroxyacetophenone (4) (0.042 mg/g), scopoletin (5) (tr), rutin (6) (0.021 mg/g), hyperoside (7) (0.082 mg/g), isoquercitrin (8) (0.052 mg/g), 3,5-dicaffeoylquinic acid (9) (0.324 mg/g), 4,5-dicaffeoylquinic acid (10) (3.604 mg/g), scoparone (11) (0.037 mg/g), 7-methoxycoumarine (12) (0.087 mg/g) and quercetin (13) (tr).

Fig. 2. The contents of the 13 compounds in the samples were quantified and the results are shown in Table 3 with the mean values of three replicate injections. Variations of the 13 compounds content in Herba Artemisiae Scopariae of different harvesting months are shown in Fig. 3.

The role of different harvesting times was determined. Among the analyzed compounds, the content of chlorogenic acid and 4,5-dicaffeoylquinic acid was the highest at a concentration of about 4.70–27.97 and 0.40–10.52 mg/g, respectively. Table 3 and Fig. 3 show that the amount of chlorogenic acid was decreasing with time remarkably. Other phenolic acids as caffeic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and 4-hydroxyacetophenone together accumulated at much higher amounts between May and July. The samples analyzed contained much lower level of the amount of other flavonoids and coumarins as rutin, hyperoside, isoquercitrin, scoparone, which increased with time. The scoparone, especially, increased significantly in autumn. The content of 6,7-dihydroxycoumarin, scopoletin, 7-

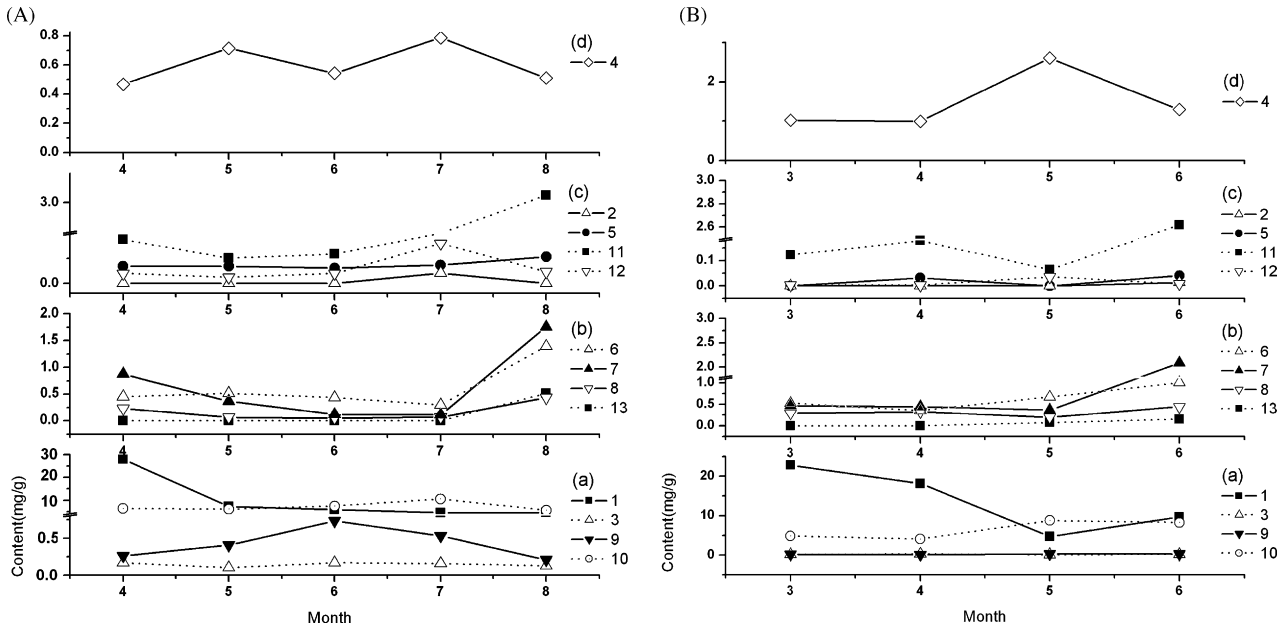


Fig. 3. Variations of the 13 compounds content in Herba Artemisiae Scopariae in different harvesting months. (A) Samples collected on the 19th of every month from April to August in 2007 from the same field of Shenyang, Liaoning. (B) Samples collected on the 19th of every month from March to June in 2007 from the same field of Xi'an, Shaanxi. (a) Phenolic acids: chlorogenic acid (1), 6,7-dihydroxycoumarin (2), caffeic acid (3), 3,5-dicaffeoylquinic acid (9) and 4,5-dicaffeoylquinic acid (10). (b) Flavonoids: rutin (6), hyperoside (7), isoquercitrin (8) and quercetin (13). (c) Coumarins: 6,7-dihydroxycoumarin (2), scopoletin (5), scoparone (11) and 7-methoxycoumarine (12). (d) 4-Hydroxyacetophenone (4).

methoxycoumarine and quercetin was the lowest and close to trace amount. 13 compounds in the samples from different cultivation regions showed a similar trend.

4. Discussion and conclusion

Based on the results that capillarisin and 6,7-dimethylescuretin reached maximum concentrations in the leaf at the point when the flower bud formation in the capitulum. Ikenaga et al. suggests that the most appropriate time to harvest *A. capillaris* for use as a crude drug is between the flower bud stage and early flower stage, from late August to early September [28]. This suggestion is contradictory with the principle of using Herba Artemisiae Scopariae in China over thousands of years as described in the ancient ballad that "March herb Yinchen and April useless straw, May firewood."

One essential difference of traditional Chinese herbal medicines from synthetic drugs is that their therapeutic effects are due to the joint contribution of multi-components, not only a few ones. These multiple constituents in the herb are may work 'synergistically' and could hardly be separated into active parts. Considering the clinical practice of a long time the choleretic constituents in Herba Artemisiae Scopariae would be difficult to identify. Our method and results are hoped to contribute to clarifying the mystery of the mechanism of action of Herba Artemisiae Scopariae and increase its application.

In this study, an HPLC–DAD method for the qualification and quantification of phenolic acids, coumarins and flavonoids and 4-hydroxyacetophenone in Herba Artemisiae Scopariae has been developed and successfully applied to nine samples collected at various harvesting times and from various cultivation regions in China. This method is validated for good accuracy, repeatability and precision, and can be used to evaluate the quality of the drug. The results will be helpful to further discussion of the Herba Artemisiae Scopariae harvest seasons. Moreover, based on this multi-components assay method, further studies of phytochemistry, pharmacology, pharmacodynamics and statistics are expected to follow.

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