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Original Article

Simultaneous characterization and quantification of 17 main compounds in *Rabdosia rubescens* by high performance liquid chromatography

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ARTICLE INFO

Article history:
Received 28 December 2015
Received in revised form
30 April 2016
Accepted 13 May 2016
Available online 26 July 2016

Keywords:
high performance liquid chromatography
oridonin
ponicidin
quantitative analysis
Rabdosia rubescens

ABSTRACT

Rabdosia rubescens is a healthy herbal tea and well-known Chinese medicinal herb. To evaluate the quality of *R. rubescens* from China, a high performance liquid chromatography method with dual-wavelength detection was developed and validated. The method was successfully applied for the simultaneous characterization and quantification of 17 main constituents from four different cultivation regions in China. Under optimal conditions, analysis was performed on a Luna C-18 column and gradient elution with a solvent system of acetonitrile and 0.5% (v/v) acetic acid–water at a flow rate of 1.0 mL/min and wavelength of 220 nm and 280 nm. All standard calibration curves exhibited good linearity ($r^2 > 0.9992$) within the test ranges. The precision was evaluated by intraday and interday tests, which revealed relative standard deviation values within the ranges of 0.57–2.35% and 0.52–3.40%, respectively. The recoveries were in the range of 96.37–101.66%. The relative standard deviation values for stability and repeatability were < 5%. The contents of some compounds were low and varied with different cultivars. The proposed method could serve as a prerequisite for quality control of *R. rubescens* materials and products.

1. Introduction

Rabdosia rubescens (Hemsl.) Hara (family Labiatae), known as Dong-ling-cao in China, is a well-known Chinese medicinal herb [1]. This herb is used to treat stomach ache, pharyngitis, sore throat, and cough. It has also been used as a supplement for the treatment of cancers of the esophagus, gastric cardia, breast, liver, and prostate for the past 30 years [2]. Moreover, *R. rubescens* leaves have long been used in China to make a healthy herbal tea and they are listed in the Chinese Pharmacopoeia [3,4]. The herbal tea is used to clear the throat and

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http://dx.doi.org/10.1016/j.jfda.2016.05.008

the lungs [5]. Phytochemical studies have shown that this plant contains diterpenoids [6], flavonoids [5], phenolic acids, triterpenoids, and volatile oils [7]. The genus *Rabdosia* is rich in diterpenoids, which have been shown to be the main bioactive constituents [8,9]. Clinical studies have indicated that oridonin and ponicidin are major antitumor constituents of *Rabdosia* [10]. Apart from diterpenoids, flavonoids such as cirsiliol and pedalitin are also isolated and identified from *R. rubescens* [10–15]. Phenolic acids such as rosmarinic acid have been shown to mitigate streptozotocin-induced diabetic manifestations by protecting rat tissues against free-radical damage [16]. In recent years, more constituents from *R. rubescens* have been reported to have different bioactivities [17,18].

Quantification of the natural compounds in *R. rubescens* is significant for quality evaluation of the herb. Simple quantitative analysis of one or two active components in an herb does not represent its integral quality. Therefore, simultaneous quantitative analysis of active components is the most direct method for quality control of *R. rubescens*.

Several studies have reported the determination of flavonoids or diterpenoids in *R. rubescens* by high performance liquid chromatography (HPLC)-UV [19–21]. However, most of them are focused on the determination of one or two components from only one or two locally grown cultivars in China. To make full use of the *R. rubescens* resources and further explore the active substances in the herb, we studied the profiles of 17 components in four extracts of *R. rubescens* cultivated from different provinces in China. This will contribute to the comprehensive development and utilization of *R. rubescens* resources.

2. Materials and methods

2.1. Chemicals and reagents

The acetonitrile used was of HPLC-grade (Merck, Darmstadt, Germany). Other reagents, such as acetic acid and water, were of analytical grade (Hengxing Chemical Reagent Co. Ltd., Tianjin, China). Chemical standards of rubescensin K (1, ≥ 95.0% purity), oridonin (2, ≥ 90.0% purity), ponicidin (3, ≥ 98.0% purity), rosthorin (4, ≥ 92.0% purity), enmenol (5, ≥ 98.0% purity), nodifloretin (6, ≥ 98.0% purity), pedalitin (7, ≥ 99.0% purity), penduletin (8, ≥ 98.0% purity), 5, 8, 4′-trihydroxy-6, 7, 3′-trimethoxyflavone (9, ≥ 95.0% purity), 5, 4′-dihydroxy-6, 7, 8, 3′-tetramethoxyflavone (10, ≥ 95.0% purity), cirsiliol (11, ≥ 99.0% purity), quercetin (12, ≥ 95.0% purity), luteolin (13, ≥ 99.0% purity), caffeic acid (14, ≥ 90.0% purity), isoacteoside (15, ≥ 95.0% purity), rosmarinic acid (16, ≥ 95.0% purity), and methyl ursolate (17, ≥ 92.0% purity) were prepared in our laboratory [5,6]. The purity of each compound was determined by HPLC analysis. The chemical structures of these reference compounds are shown in Figure 1.

2.2. Chromatographic conditions and instrumentation

HPLC analysis was performed on an Agilent 1260 LC Series instrument (Santa Clara, CA, USA). A Luna C-18 column (5 μm, 4.6 mm internal diameter × 250; Phenomenex, Torrance, CA, USA) was used in chromatographic analysis. The analytical conditions were as follows: flow rate was 1.0 mL/min; column temperature was maintained at 30°C. The mobile phase was composed of A [0.5% (v/v) acetic acid–water solution] and B (acetonitrile) with a gradient elution: 0 minutes, 100% A; 0–15 minutes, 100–50% A; 15–35 minutes, 50–0% A; 35–40 minutes, 0–100% A. The chromatogram was monitored at a wavelength of 220 nm for Compounds 1–5 and Compounds 15–17, and at 280 nm for Compounds 6–14 during the experiment.

2.3. Plants materials

Four batches of *R. rubescens* were collected from four different provinces in China in October 2014. We collected the whole herb of four samples: Henan (Henan, China; Sample HN), Guangxi (Guangxi, China; Sample GX), Jiangxi (Jiangxi, China; Sample JX), and Sichuan (Sichuan, China; Sample SC). Their botanical origins were identified by the corresponding author (NB), and voucher specimens were deposited in the herbarium of Northwest University, Xi'an, Shaanxi, China.

2.4. Preparation of sample solutions

One hundred grams of each tested sample was ground into fine powder (200 mesh). Dried powder (0.5 g) was extracted under ultrasonication with 25 mL methanol for 50 minutes at 50°C. Subsequently, each extracted solution was filtered, evaporated to dryness, and redissolved in 25 mL methanol. One milliliter was removed and filtered through a 0.45-μm nylon membrane filter (Jiang Tian Unity, Tianjin, China) before injection into the HPLC system for analysis.

2.5. Preparation of standard solutions

The chromatographic purity of the reference Compounds 1–17 was checked at multiple wavelengths by HPLC, UV spectroscopy and other physical properties. Standard stock solution (1.0 mg/mL) was prepared by dissolving the reference compounds in methanol, and the standard stock solution was stored at 4°C in the dark for further analysis. Working standard solutions for calibration curves were prepared by diluting the standard stock solution with methanol at different concentrations, and the concentration ranges for these analytes were as follows: 1, 0.20–100.0 μg/mL; 2, 0.60–200.0 μg/mL; 3, 0.80–200.0 μg/mL; 4, 0.50–150.0 μg/mL; 5, 0.80–200.0 μg/mL; 6, 1.0–200.0 μg/mL; 7, 1.0–200.0 μg/mL; 8, 0.10–150.0 μg/mL; 9, 0.50–200.0 μg/mL; 10, 0.80–200.0 μg/mL; 11, 0.10–150.0 μg/mL; 12, 1.0–200.0 μg/mL; 13, 0.50–150.0 μg/mL; 14, 0.50–150.0 μg/mL; 15, 0.10–150.0 μg/mL; 16, 0.50–200.0 μg/mL; and 17, 0.10–150.0 μg/mL. The standard solutions were filtered through a 0.45-μm nylon membrane filter (Jiang Tian Unity) prior to injection. All solutions were stored in a refrigerator at 4°C before analysis.

2.6. HPLC and extraction method validation

To assess the validity of the method, validation tests were performed. The linearity of standard curves was based on plotting the peak areas versus the corresponding

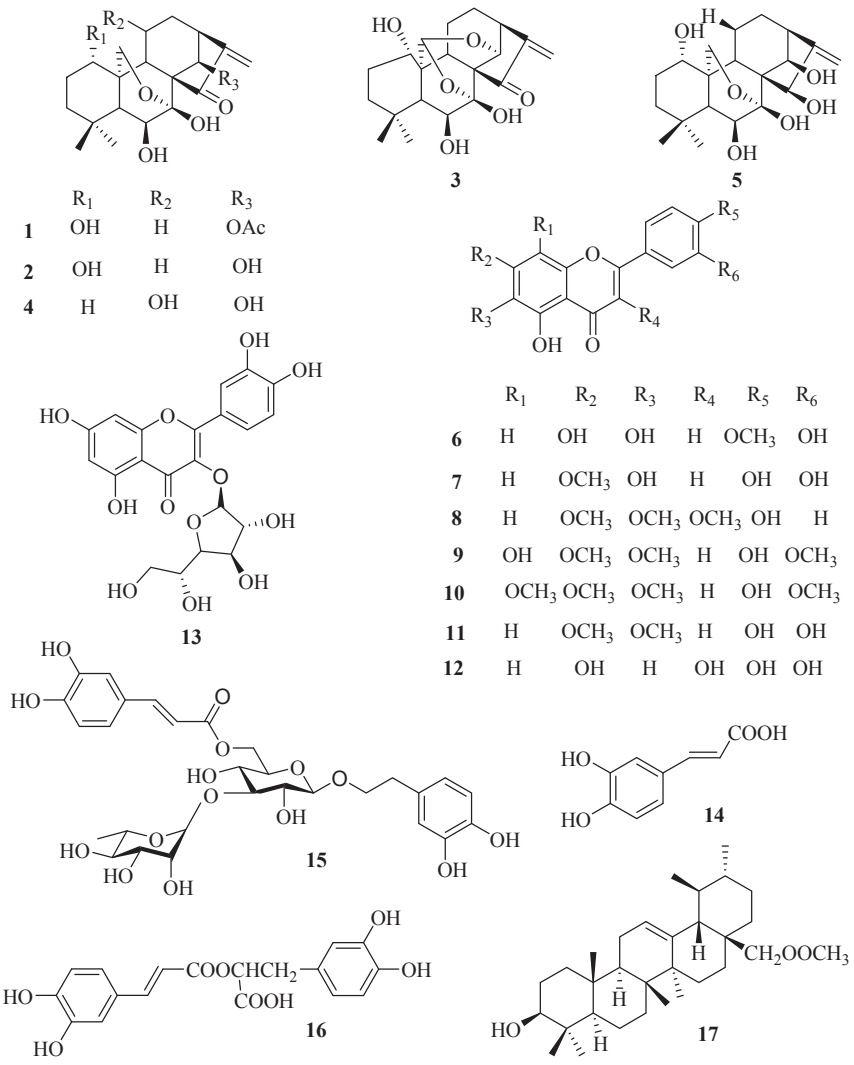

Figure 1 – Chemical structure of the 17 identified components of *Rabdosia rubescens*.

concentrations of each analyte. Linearity test solutions for the assay method prepared from stock solution (1 mg/mL). The lowest concentration of working solution for calibration use was diluted with methanol to a series of appropriate concentrations. They were then analyzed until the signal-to-noise ratio for each compound was about 3 for the limit of detection (LOD) and 10 for the limit of quantification (LOQ). Data on precision expressed as intra- and interday relative standard deviation (RSD) were determined from three independently prepared sample solutions of one extract from the same source. The results were calculated by average of the RSD ($n = 3$, each) obtained on three different days and RSD of the three average values ($n = 3$, each) obtained on three different days, respectively. To evaluate the stability of the solution, one of the aforementioned sample solutions was tested at 0 hours, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 24 hours and 48 hours after preparation. To confirm the repeatability, three samples from the same source were extracted, and each of the three extracts was analyzed and variations expressed by the RSD. A recovery test was performed to evaluate the accuracy of this method. A known amount of the mixed standard solution was added to 0.5 g of a sample quantified previously, then analyzed using the described method. Three replicates were performed for the test.

To obtain the optimal extraction efficiency, factors including the particle size of crude drug, solvent volume, extraction time and temperature of the extraction performance were evaluated. It was found that 200 mesh powders, 25 mL methanol, and ultrasonication for 50 minutes at 50°C yielded the optimum extraction efficiency.

2.7. *Identification and quantification*

Identification of the 17 components was carried out by comparing the HPLC retention time and UV spectra of target peaks with those of reference compounds. Quantification was performed based on linear calibration plots of the peak areas versus the concentration.

3. Results and discussion

3.1. Optimization of the extraction method

As a simple and efficient extraction method, ultrasonic extraction was used in this experiment. To determine the optimizing extraction conditions, different solvents such as ethyl acetate, ethanol and methanol were investigated, and results indicated that methanol was an ideal solvent, which showed the highest extraction rate. Various factors including the particle size of crude drug, extraction volume, extraction time and extraction temperature were also investigated. A series of experiments was performed to obtain the optimum results as follows: particle size of sample powders (60 mesh, 100 mesh, 150 mesh, 200 mesh, and 300 mesh) was 200 mesh, extraction volume (10 mL, 20 mL, 25 mL, 30 mL and 40 mL) was 25 mL, extraction time (30 minutes, 40 minutes, 50 minutes, 60 minutes, and 90 minutes) was 50 minutes, and extraction temperature (28°C, 30°C, 40°C, 50°C, and 60°C) was 50°C (Figure S1).

3.2. Optimization of HPLC conditions

In order to obtain the most useful chemical information and the best separation of *R. rubescens*, the mobile phase compositions (methanol–water and acetonitrile–water), different concentrations of acid (phosphoric acid and acetic acid), gradient elution procedure and detection wavelength were optimized. The results showed that 0.5% (v/v) acetic acid–-water solution narrowed peak shape and improved tailing. The chromatographic separation for the analytes with acetonitrile was superior to that with methanol. According to the result of HPLC-UV analysis of 17 target compounds, the maximum absorption wavelength of diterpenoid compounds was 220 nm, and of flavonoids and depsides was 280 nm. Hence, we choose 220 nm and 280 nm as the detection wavelengths.

3.3. Validation results

The quantitative analysis method was validated in terms of the linearity, LOD, LOQ, precision, repeatability, stability, and accuracy. The results demonstrated that all calibration curves exhibited excellent linear regression with the determination coefficients (r^2) ranging from 0.9992 to 0.9999, and the calibration ranges adequately covered variations in the amounts of the compounds investigated in the samples. The overall LODs and LOQs of the 17 compounds were < 0.19 μg/mL and < 0.65 μg/mL, respectively (Table 1). Intraday and interday precision, repeatability and stability were evaluated and expressed by the RSD values. The results demonstrated that the values of precision were all < 3.40% (Table 2), and repeatability and stability were < 5% (Table 3). The overall recoveries lay between 96.37% and 101.66% for the 17 reference compounds, with RSD values < 3.07% (Table 4). The contents of the analyzed compounds are summarized in Table 5. The results indicated that the established method was accurate and reliable enough for the determination of the 17 compounds in *R. rubescens*.

3.4. Identification of 17 compounds in R. rubescens

According to the HPLC fingerprint, 17 constituents (1–17) were selected for the quantitative analysis (Figure 1). Identification of these components was based on comparing their HPLC retention times and UV spectra with those of reference compounds. Seventeen constituents in *R. rubescens* were unequivocally identified as rubescensin K, oridin, ponicidin, rosthorin, enmenol, nodifloretin, pedalitin, penduletin, 5, 8, 4′-trihydroxy-6, 7, 3′-trimethoxyflavone, 5, 4′-dihydroxy-6, 7, 8, 3′-tetramethoxyflavone, cirsiliol, quercetin, luteolin, caffeic acid, isoacteoside, rosmarinic acid, and methyl ursolate (Figure 2). Compounds 2 and 5 were critical pairs in chromatography, and we differentiated them by UV spectra. The

Table 1 – Calibration curves and LOD and LOQ data of 17 compounds investigated by high performance liquid chromatography ($n = 3$).

Compound	Calibration curves[a]	R^2	Linear range (μg/mL)	LOD[b] (μg/mL)	LOQ[b] (μg/mL)
Rubescensin K	$y = 3.94x - 1.54$	0.9996	0.2–100	0.02	0.09
Oridonin	$y = 14.14x + 8.47$	0.9992	0.6–200	0.08	0.35
Ponicidin	$y = 20.01x + 2.09$	0.9997	0.8–200	0.16	0.48
Rosthorin	$y = 19.50x + 5.02$	0.9993	0.5–150	0.05	0.21
Enmenol	$y = 46.09x + 0.90$	0.9997	0.8–200	0.14	0.55
Nodifloretin	$y = 43.59x - 421.70$	0.9993	1.0–200	0.09	0.34
Pedalitin	$y = 60.95x - 20.50$	0.9999	1.0–200	0.18	0.65
Penduletin	$y = 48.66x + 48.41$	0.9998	0.1–150	0.007	0.02
5,8,4′-Trihydroxy-6,7,3′-trimethoxyflavone	$y = 28.50x - 71.10$	0.9996	0.5–200	0.04	0.15
5,4′-Dihydroxy-6,7,8,3′-tetramethoxyflavone	$y = 21.01x - 30.41$	0.9995	0.8–200	0.12	0.40
Cirsiliol	$y = 22.33x - 18.87$	0.9999	0.1–150	0.01	0.05
Quercetin	$y = 61.57x - 104.89$	0.9995	1.0–200	0.19	0.59
Luteolin	$y = 181.82x - 31.79$	0.9999	0.5–150	0.05	0.14
Caffeic acid	$y = 70.84x - 79.69$	0.9995	0.5–150	0.05	0.22
Isoacteoside	$y = 10.24x - 2.63$	0.9996	0.1–150	0.003	0.01
Rosmarinic acid	$y = 50.70x - 2.10$	0.9995	0.5–200	0.06	0.29
Methylursolate	$y = 24.28x + 0.65$	0.9993	0.1–150	0.01	0.07

LOD = limit of detection; LOQ = limit of quantification.
[a] y is the value of peak area, and x is the value of the reference compound concentration (μg/mL).
[b] LOD and LOQ were determined at signal/noise ratio of about 3 and 10, respectively.

Table 2 – Intraday and interday precision of the developed method ($n = 3$).

Compound	Concn (μg/mL)	RT[a] (min)	Intraday			RT[a] (min)	Interday		
			Detected [a] (μg/mL)	Accuracy (%)	RSD (%)		Detected [a] (μg/mL)	Accuracy (%)	RSD (%)
Rubescensin K	2.61	22.09 ± 0.007	2.64 ± 0.03	101.15 ± 1.15	1.14	22.12 ± 0.004	2.67 ± 0.02	102.30 ± 0.77	0.64
Oridonin	117.45	18.06 ± 0.010	115.91 ± 2.72	98.69 ± 2.32	2.35	18.12 ± 0.011	115.96 ± 2.41	100.04 ± 2.05	2.08
Ponicidin	32.10	20.09 ± 0.003	31.04 ± 0.28	96.70 ± 0.87	0.91	20.02 ± 0.013	31.04 ± 0.19	96.70 ± 0.59	0.60
Rosthorin	7.41	17.22 ± 0.011	7.39 ± 0.07	99.73 ± 0.94	0.95	17.27 ± 0.015	7.38 ± 0.06	99.86 ± 0.81	0.83
Enmenol	43.77	18.19 ± 0.012	44.22 ± 1.04	101.03 ± 2.38	2.35	18.26 ± 0.017	44.10 ± 0.60	100.75 ± 1.37	1.37
Nodifloretin	33.62	17.58 ± 0.014	33.55 ± 0.19	99.80 ± 0.57	0.57	17.65 ± 0.010	33.55 ± 0.18	99.80 ± 0.54	0.52
Pedalitin	10.94	19.64 ± 0.012	10.44 ± 0.13	95.42 ± 1.19	1.25	19.69 ± 0.020	10.43 ± 0.15	99.90 ± 1.37	1.44
Penduletin	2.09	27.30 ± 0.008	2.08 ± 0.03	99.52 ± 1.44	1.44	27.39 ± 0.012	2.06 ± 0.05	98.56 ± 2.39	2.22
5,8,4-Trihydroxy-6,7,3′-trimethoxyflavone	12.02	26.10 ± 0.011	12.06 ± 0.13	100.33 ± 1.08	1.08	26.14 ± 0.06	12.07 ± 0.20	100.42 ± 1.66	1.66
5,4-Dihydroxy-6,7,8,3′-tetramethoxyflavone	35.34	31.57 ± 0.017	35.17 ± 0.35	99.52 ± 0.99	1.01	31.51 ± 0.015	35.14 ± 0.12	99.43 ± 0.34	3.40
Cirsiliol	24.81	25.21 ± 0.015	24.34 ± 0.16	98.11 ± 0.64	0.66	25.28 ± 0.020	24.28 ± 0.25	97.86 ± 1.01	1.03
Quercetin	nd	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin	7.41	22.87 ± 0.012	7.44 ± 0.07	100.40 ± 0.94	0.94	22.76 ± 0.004	7.43 ± 0.05	100.27 ± 0.67	0.63
Caffeic acid	20.17	13.14 ± 0.014	19.65 ± 0.13	97.42 ± 0.64	0.66	13.07 ± 0.016	19.65 ± 0.05	97.42 ± 0.25	1.67
Isoacteoside	31.14	14.72 ± 0.010	30.96 ± 0.41	99.42 ± 1.32	1.32	14.63 ± 0.011	30.36 ± 0.50	97.50 ± 1.61	1.66
Rosmarinic acid	41.85	16.12 ± 0.012	42.16 ± 0.25	100.74 ± 0.60	0.59	16.14 ± 0.021	42.19 ± 0.27	100.81 ± 0.65	0.65
Methylursolate	3.52	6.53 ± 0.061	3.50 ± 0.05	99.43 ± 1.42	1.43	6,58 ± 0.015	3.50 ± 0.10	99.43 ± 2.84	2.70

Concn = concentration; nd = not determined; RSD = relative standard deviation; RT = retention time.
[a] Data are presented as mean ± standard deviation.

maximum UV absorption wavelength of Compound 2 was at 240 nm. The maximum UV absorption wavelength of Compound 5 was at 220 nm (Figure S2).

3.5. *Quantification of the 17 compounds in* R. rubescens

The established HPLC method was subsequently applied to a simultaneous determination of the 17 constituents in four methanol extracts of the herb from different major *R. rubescens* producing regions in China. The contents of the analyzed compounds are summarized in Table 5. The content of each compound varied significantly among the different regions, which ranged from 0.096 mg/g to 5.872 mg/g. The genus *Rabdosia* is rich in diterpenoids [7,8], and the content of analyzed diterpenoids (Analytes 1–5) was in the range of 0.202–5.872 mg/g. Among them, oridonin and ponicidin (Analytes 2 and 3) are generally considered the highly active ingredients, especially for anti-cancer activity [22–26]. Oridonin was the richest among five diterpenoids and its average

Table 3 – Analysis of the stability and repeatability of the developed method.

Compound	Stability ($n = 8$)			Repeatability ($n = 3$)		
	RT (min)[a]	Content (mg/g)[a]	RSD (%)	RT (min)[a]	Content (mg/g)[a]	RSD (%)
Rubescensin K	22.05 ± 0.016	0.134 ± 0.003	2.20	19.98 ± 0.021	2.146 ± 0.005	2.50
Oridonin	18.08 ± 0.006	5.873 ± 0.091	1.55	18.08 ± 0.009	5.932 ± 0.121	1.95
Ponicidin	20.14 ± 0.008	1.569 ± 0.026	1.67	19.99 ± 0.012	1.589 ± 0.027	1.44
Rosthorin	17.21 ± 0.009	0.370 ± 0.015	4.04	17.19 ± 0.011	0.393 ± 0.018	4.54
Enmenol	18.23 ± 0.014	2.216 ± 0.043	1.95	18.18 ± 0.019	2.258 ± 0.038	1.75
Nodifloretin	17.63 ± 0.017	1.698 ± 0.022	1.27	17.58 ± 0.018	1.734 ± 0.036	2.57
Pedalitin	19.65 ± 0.018	0.539 ± 0.006	1.14	19.63 ± 0.021	0.566 ± 0.006	1.74
Penduletin	27.35 ± 0.017	0.103 ± 0.004	3.52	27.33 ± 0.015	0.111 ± 0.004	3.92
5,8,4-Trihydroxy-6,7,3′-trimethoxyflavone	26.05 ± 0.015	0.598 ± 0.010	1.61	26.02 ± 0.019	0.602 ± 0.016	1.91
5,4′-Dihydroxy-6,7,8,3′-tetramethoxyflavone	31.52 ± 0.018	1.760 ± 0.019	1.08	31.49 ± 0.020	1.760 ± 0.019	3.30
Cirsiliol	25.24 ± 0.019	1.277 ± 0.016	1.27	25.20 ± 0.023	1.313 ± 0.027	2.27
Quercetin	nd	nd	nd	nd	nd	nd
Luteolin	22.84 ± 0.015	0.384 ± 0.008	2.19	22.77 ± 0.011	0.391 ± 0.005	1.99
Caffeic acid	13.18 ± 0.015	1.021 ± 0.008	0.82	12.96 ± 0.013	1.044 ± 0.011	0.91
Isoacteoside	14.75 ± 0.013	1.536 ± 0.023	1.52	14.54 ± 0.023	1.555 ± 0.029	1.92
Rosmarinic acid	16.17 ± 0.016	2.149 ± 0.020	0.92	16.08 ± 0.022	2.159 ± 0.033	1.32
Methylursolate	6.51 ± 0.067	0.174 ± 0.002	0.91	6.48 ± 0.069	0.184 ± 0.008	3.31

nd = not determined; RSD = relative standard deviation; RT = retention time.
[a] Data are presented as the mean ± standard deviation.

Table 4 – Recovery data of the developed method ($n = 3$).

Compound	Concentration of analyte			Recovery (%)	RSD (%)
	Original [a] (μg/mL)	Spiked [a] (μg/mL)	Found [a] (μg/mL)		
Rubescensin K	1.07 ± 0.04	2.20	3.29 ± 0.05	100.61 ± 1.46	1.44
Oridonin	46.78 ± 1.05	40.30	88.52 ± 1.50	101.66 ± 1.73	1.69
Ponicidin	9.43 ± 0.43	12.10	21.87 ± 0.13	101.56 ± 0.61	0.60
Rosthorin	3.20 ± 0.03	3.50	6.46 ± 0.11	96.37 ± 1.61	1.67
Enmenol	13.13 ± 0.50	12.50	25.34 ± 0.22	98.86 ± 0.88	0.88
Nodifloretin	10.26 ± 0.03	12.10	22.06 ± 0.26	98.64 ± 1.15	1.17
Pedalitin	5.12 ± 0.01	10.30	15.25 ± 0.10	98.88 ± 0.63	0.63
Penduletin	1.07 ± 0.05	1.60	2.59 ± 0.02	97.00 ± 0.75	0.77
5,8,4′-Trihydroxy-6,7,3′-trimethoxyflavone	5.93 ± 0.03	10.20	16.16 ± 0.18	100.21 ± 1.12	1.11
5,4′-Dihydroxy-6,7,8,3′-tetramethoxyflavone	10.67 ± 0.05	12.30	22.96 ± 0.17	99.94 ± 0.73	0.73
Cirsiliol	12.61 ± 0.62	10.10	22.60 ± 0.22	99.53 ± 0.96	0.96
Quercetin	nd	nd	nd	nd	nd
Luteolin	4.31 ± 0.02	3.20	7.37 ± 0.23	98.14 ± 3.02	3.07
Caffeic acid	9.98 ± 0.06	10.10	19.75 ± 0.30	98.37 ± 1.49	1.51
Isoacteoside	15.72 ± 0.12	12.60	28.15 ± 0.25	99.41 ± 0.90	0.90
rosmarinic acid	12.54 ± 0.34	12.80	25.30 ± 0.18	99.86 ± 0.72	0.73
methylursolate	2.09 ± 0.02	3.10	5.09 ± 0.03	98.06 ± 0.59	0.60

nd = not determined; RSD = relative standard deviation.
[a] Data are presented as the mean ± standard deviation.

content in the samples from four regions was 5.657 mg/g. The highest content of oridonin was in Sample JX (5.872 mg/g), whereas the lowest content was in Sample GX (5.439 mg/g). Ponicidin varied significantly among the samples from different regions. Its content in Sample HN was the highest (2.637 mg/g), followed by Samples GX, SC, and JX with contents of 2.030 mg/g, 1.861 mg/g, and 1.605 mg/g, respectively. In our previous study, rubescensin K (Analyte 1) was identified as a new natural product [6]. It was found in all four samples and its average content was 2.306 mg/g and was higher than ponicidin.

Apart from diterpenoids, the differences of the flavonoids (Analytes 6–13) in various regions may also be significant. The results showed that the content of the total flavonoids in four samples ranged from 0.096 mg/g to 4.698 mg/g. In all samples, nodifloretin and 5, 4′-dihydroxy-6, 7, 8, 3′-tetramethoxyflavone (Analytes 6 and 10) were prevalent components, with mean contents of 2.113 mg/g and 2.414 mg/g, respectively. The results from four samples also revealed that the contents of the seven flavonoids, especially cirsiliol (Analyte 11), varied considerably. The content of cirsiliol reached as high as 4.698 mg/g in Sample HN cultivated in Henan province. It was only 1.240 mg/g in Sample JX cultivated in Jiangxi. Quercetin (Analyte 12) was only be detected in Sample HN with a mean content of 3.501 mg/g. Our present study showed that there was a significant regional variability across China in the

Table 5 – Contents of Compounds 1–17 in *Rabdosia rubescens* collected from four different provinces in China.

Compound	Content of compounds (mg/g, $n = 3$) [a]			
	Sample GX	Sample JX	Sample SC	Sample HN
Rubescensin K	2.124 ± 0.05	2.611 ± 0.04	2.340 ± 0.05	2.149 ± 0.05
Oridonin	5.439 ± 0.121	5.872 ± 0.087	5.818 ± 0.083	5.499 ± 0.109
Ponicidin	2.030 ± 0.003	1.605 ± 0.017	1.861 ± 0.008	2.637 ± 0.024
Rosthorin	0.202 ± 0.004	0.372 ± 0.002	0.520 ± 0.002	0.558 ± 0.009
Enmenol	1.787 ± 0.029	2.188 ± 0.027	1.987 ± 0.032	1.029 ± 0.020
Nodifloretin	2.140 ± 0.001	1.681 ± 0.009	2.313 ± 0.003	2.321 ± 0.008
Pedalitin	0.308 ± 0.001	0.547 ± 0.004	0.622 ± 0.001	0.356 ± 0.003
Penduletin	0.096 ± 0.003	0.104 ± 0.003	0.112 ± 0.003	0.109 ± 0.005
5,8,4-Trihydroxy-6,7,3′-trimethoxyflavone	0.451 ± 0.003	0.601 ± 0.005	0.487 ± 0.003	0.228 ± 0.001
5,4′-Dihydroxy-6,7,8,3′-tetramethoxyflavone	2.393 ± 0.003	1.767 ± 0.018	1.919 ± 0.009	3.578 ± 0.011
Cirsiliol	2.476 ± 0.005	1.240 ± 0.011	1.332 ± 0.004	4.698 ± 0.010
Quercetin	nd	nd	nd	3.501 ± 0.005
Luteolin	0.521 ± 0.001	0.370 ± 0.003	0.482 ± 0.001	0.294 ± 0.001
Caffeic acid	0.652 ± 0.002	1.009 ± 0.006	0.698 ± 0.002	0.339 ± 0.003
Isoacteoside	1.261 ± 0.006	1.557 ± 0.016	1.665 ± 0.011	0.183 ± 0.004
Rosmarinic acid	1.421 ± 0.001	2.093 ± 0.007	2.451 ± 0.002	0.210 ± 0.004
Methylursolate	0.147 ± 0.004	0.176 ± 0.003	0.163 ± 0.001	0.173 ± 0.002

nd = not determined.
[a] Data are presented as the mean ± standard deviation.

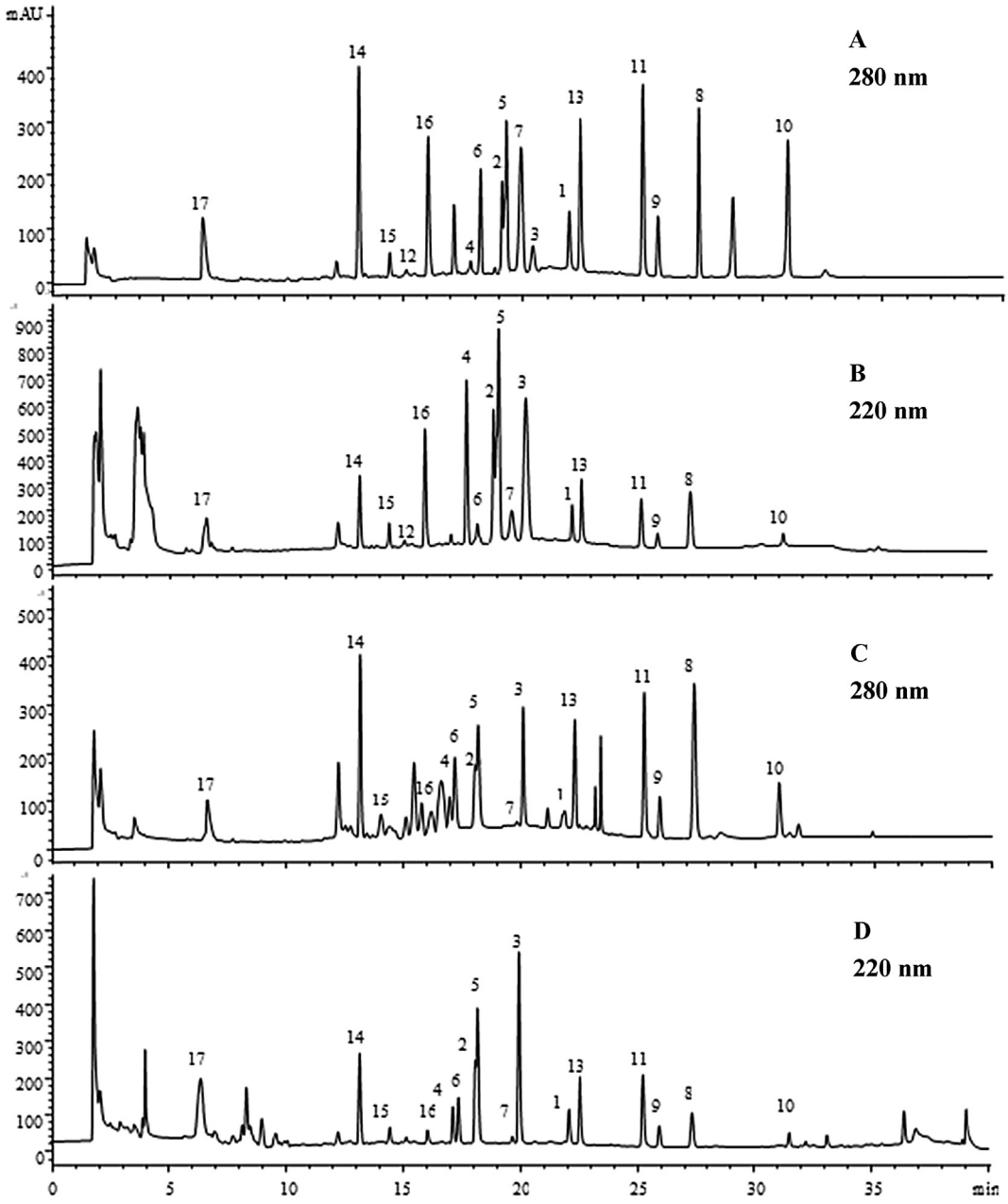

Figure 2 – High performance liquid chromatography of solution of standards (A) 280 nm and (B) 220 nm, and samples (C) at 280 nm and (D) 220 nm. Peaks: 1, rubescensin K; 2, oridonin; 3, ponicidin; 4, rosthorin; 5, enmenol; 6, nodifloretin; 7, pedalitin; 8, penduletin; 9, 5,8,4′-trihydroxy-6,7,3′-trimethoxyflavone; 10, 5,4′-dihydroxy-6,7,8,3′-tetramethoxyflavone; 11, cirsiliol; 12, quercetin; 13, luteolin; 14, caffeic acid; 15, isoacteoside; 16, rosmarinic acid; 17, methyl ursolate.

flavonoid composition of *R. rubescens*. For the depsides analyzed, it was clearly seen that the content of rosmarinic acid (Analyte 16) in Sample GX was obviously lower than that in other three samples.

In summary, our results showed that there was a regional variability across China for the composition of *R. rubescens*. The variation may be due to their genetic origin, growing environment and storage conditions. A simple, accurate and reliable method coupled with dual-wavelength detection could be used for developing the HPLC fingerprint of *R. rubescens* and simultaneous determination of 17 compounds. The method could serve as a prerequisite for quality control of *R. rubescens* products and the assessment of different samples.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2016.05.008.

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