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Original Article

Simultaneous determination of 15 flavonoids from different parts of *Scutellaria baicalensis* and its chemometrics analysis

Jie Shen[a,b], Pei Li[a,b], Chun-nian He[a,b,*], Hai-tao Liu[a,b,*], Yan-ze Liu[a,b], Xiao-bo Sun[a,b], Rong Xu[a,b], Pei-gen Xiao[a,b]

[a] *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, China*
[b] *Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Beijing 100193, China*

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ABSTRACT

Objective: The aerial parts of *Scutellaria baicalensis* were used as Huangqin Tea for thousands of years and mainly contain flavonoids which contribute to its bioactivities. However, there is no appropriate quality evaluation method of Huangqin Tea, and three flavanones of isocarthamidin-7-*O*-β-*D*-glucuronide, carthamidin-7-*O*-β-*D*-glucuronide, and isoscutellarein-8-*O*-β-*D*-glucuronide with high contents in the aerial parts have never been defined quantitatively. Here, an HPLC-DAD method for simultaneous determination of 15 flavonoids and systematically compared their contents and distribution in the roots, stems, leaves, and flowers of *S. baicalensis* was established.
Methods: Under the HPLC-DAD chromatographic conditions, 77 batches of samples of *S. baicalensis* were analyzed. Meanwhile, the chromatographic fingerprint of different parts of *S. baicalensis* was established. Subsequently, principal component analysis (PCA), orthogonal projections to latent structures discriminant analysis (OPLS-DA), and clustering heat map were performed based on the contents of 15 flavonoids in different parts of *S. baicalensis*.
Results: The results showed significant differences in the contents and distributions of 15 flavonoids among the different parts of *S. baicalensis*. The chemical composition of stems showed some similarities to leaves, and their contents were all lower than leaves. The contents of isocarthamidin-7-*O*-β–glucuronide [(106.66 66 ± 22.68) mg/g], carthamidin-7-*O*-β-*D*-glucuronide [(19.82 ± 11.17) mg/g], and isoscutellarein-8-*O*-β-*D*-glucuronide [(3.10 ± 1.73) mg/g] were the highest in leaves. The content of apigenin-7-*O*-β-*D*-glucopyranoside and chrysin-7-*O*-β-*D*-glucuronide were the highest in flowers. The contents of baicalin, baicalein, wogonoside, wogonin, alpinetin, and oroxylin A were higher in roots than in other parts.
Conclusion: The method was fully validated and could be effectively used to characterize the contents and distributions of main flavonoids in the different parts of *S. baicalensis*. It may lay a foundation to establish the quality evaluation system for Huangqin Tea.

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1. Introduction

Scutellaria baicalensis Georgi is an indigenous medicinal plant in China. Its dried root (*Scutellariae Radix*) is a typical Chinese materia medica, which was first recorded in *Shennong Bencaojing* (the Classic of Herbal Medicine, written between about 200 and 250 AD) for clearing heat, drying dampness, and detoxifying (Ma, 2013). Many studies have demonstrated that *Scutellariae Radix* possesses a wide range of pharmacological effects, such as antitumor (Cheng et al., 2018; Yu et al., 2018), hepatoprotective (Chen, Liang, Lee, Huang, & Lin, 2014), and antimicrobial effect (Trinh et al., 2018). It is reported that the pharmacodynamics of *Scutellariae Radix* are closely related to the flavones, such as baicalin, wogonoside and their corresponding aglycones baicalein and wogonin (Khan et al., 2017; Wang et al., 2015; Xin, Song, He, & Du, 2013; Yang, Tang, & Liu, 2013).

The important medicinal value of *Scutellariae Radix* creates a great gradually increasing demand and it has been over 5000 tons needed in traditional Chinese medicine market in China (Yan et al., 2017). The biomass of the aerial parts (stem, leaf, and flower) of *S. baicalensis* is more than roots, which are usually discarded during the root harvesting time or only used as Huangqin Tea in limited areas (He, Peng, Xiao, Li, & Xiao, 2011; Yan et al., 2017). It is worth noting that the aerial parts of *S. baicalensis* have been used as Huangqin Tea for thousands of years and mainly contain flavonoids

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* Corresponding authors.
E-mail addresses: cnhe@implad.ac.cn (C.-n. He), htliu0718@126.com (H.-t. Liu).

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which contribute to the capability of preventing sunstroke, drying humidity, and promoting digestion function (He et al., 2011). The modern pharmacological also showed that the total flavonoids of *S. baicalensis* stem-leaf have various potential pharmacological activities, such as anti-oxidant, improving cardiovascular defects, hepatoprotective, neuroprotective, antibacterial, and antitumor activity (Wang, Shen, Xing, Guan, & Xin, 2013). However, there is no quality evaluation method for the Huangqin Tea, which greatly hampered the development of the Huangqin Tea industry and the utilization of the aerial parts of *S. baicalensis* (He, Peng, Xiao, Xu, & Xiao, 2013; Yan et al., 2017).

Though the determination of flavonoids in *Scutellariae Radix* or in the stem-leaf of *S. baicalensis* has been reported by several research groups, no research has been conducted on the contents and distribution of flavonoids as whole tissues and no research has been studied about the quality evaluation method for the Huangqin Tea until now (Chen & Sun, 2017; Seo et al., 2013; Yan et al., 2017). Besides, we have already found that the flavonoid of isoscutellarein-8-*O*-β-*D*-glucuronide and two scarcity flavanones in nature (isocarthamidin-7-*O*-β-*D*-glucuronide and carthamidin-7-*O*-β-*D*-glucuronide) have high contents in the aerial parts in our previous study and there are rare researches till now due to the structural instability of isocarthamidin-7-*O*-β-*D*-glucuronide and carthamidin-7-*O*-β-*D*-glucuronide (He et al., 2011; Li et al., 2017; Seo et al., 2013; Xu, Yang, Zhao, Wang, & Hu, 2012).

In order to lay a foundation to establish the quality evaluation system for the Huangqin Tea and get a deeper understanding about the contents and distribution of flavonoids in different parts (roots, stems, leaves, and flowers) of *S. baicalensis*, this study simultaneously determined 15 major flavonoids in different parts of *S. baicalensis*. Particularly, three flavonoids of isoscutellarein-8-*O*-β-*D*-glucuronide, isocarthamidin-7-*O*-β-*D*-glucuronide, and carthamidin-7-*O*-β-*D*-glucuronide have never been determined simultaneously. In this study, we established the HPLC fingerprint of different parts (roots, stems, leaves, and flowers) of *S. baicalensis* and used the chemometrics analysis (similarity analysis, PCA, OPLS-DA, and clustering heat map) to compare the contents and distributions of 15 flavonoids in different parts of *S. baicalensis*. Moreover, it could effectively promote to understand the flavonoid distribution in different parts of *S. baicalensis*, and provide valuable information for establishing the quality evaluation method for the Huangqin Tea and scientific utilizing the resources of *S. baicalensis*.

2. Materials and methods

2.1. Chemicals

Standards of scutellarin (**3**), apigenin-7-*O*-β-*D*-glucopyranoside (**4**), baicalin (**5**), luteolin (**8**), wogonoside (**9**), alpinetin (**10**), apigenin (**11**), baicalein (**12**), wogonin (**13**), chrysin (**14**), and oroxylin A (**15**) were purchased from the National Institute for Food and Drug Control. Standards of isocarthamidin-7-*O*-β-*D*-glucuronide (**1**), carthamidin-7-*O*-β-*D*-glucuronide (**2**), isoscutellarein-8-*O*-β-*D*-glucuronide (**6**), and chrysin-7-*O*-β-*D*-glucuronide (**7**) were isolated from *S. baicalensis* and identified by UV, IR, NMR spectra, and the purity of products was over 98% determined by HPLC (Compound **1** was a mixture of C (2)-R and -S configuration compounds at the ratio of 1:1. Compound **2** was also a mixture of C (2)-R and -S configuration compounds at the ratio of 1:1, and showed a double peak under the experimental chromatographic conditions).

Methanol and acetonitrile were HPLC analytical grade and purchased from Honeywell Burdick and Jackson (Morristown, NJ, USA). HPLC-grade formic acid was purchased from CNW Technologies GmbH (Dusseldorf, Germany). All aqueous solutions were prepared with ultrapure water produced with a Milli-Q system (18.2 MΩ, Millipore, Bedford, MA, USA).

2.2. Chromatographic condition

A total of 10 μL of each extract was injected into the HPLC system for quantitative analysis. The chromatographic separation of compounds was achieved using a YMC-pack ODS-A analytical column (250 mm × 4.6 mm, 5 μm). Column temperature was maintained at 30 °C. The mobile phase consists of methyl alcohol (A), acetonitrile (B), and 0.1% formic acid (C). Elution was performed at a flow rate of 1.0 mL/min. The program of linearity gradient elution: 0-60 min, A-B-C (15:5:80) to A-B-C (80:10:10) and followed by 15 min column re-equilibration. The detection wavelength was set at 278 nm. Data acquisition and analysis was achieved using Agilent Chemstation software workstation (C.01.06).

2.3. Sample collection and preparation

Seventy-seven batches of samples (34 roots, 15 stems, 15 leaves, and 13 flowers) of different parts of *S. baicalensis* were collected from Shanxi, Hebei, Jilin, Shandong, and Shaanxi Provinces of China. The samples were identified by Peigen Xiao, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College.

All dried samples of *S. baicalensis* were crushed and screened through a 40-mesh sieve. Samples (0.5 g) were macerated with 70% ethanol (50 mL) and placed in an ultrasonic water bath for 30 min. After cooling to room temperature and complementing the reduced volume, and then the extractions solution was filtered through a 0.45 μm membrane filter, and 10 μL extract was injected for each analysis in triplicate.

2.4. Standard solutions

Individual standard stock solution of 15 standards was respectively dissolved in methanol. The mixture stock solution was comprised of 15 standards, of which concentrations were 2.080 mg/mL (**1**), 1.435 mg/mL (**2**), 0.196 mg/mL (**3**), 0.046 mg/mL (**4**), 0.136 mg/mL (**5**), 1.080 (**6**) mg/mL, 0.232 mg/mL (**7**), 0.128 mg/mL (**8**), 0.067 mg/mL (**9**), 0.041 mg/mL (**10**), 0.050 mg/mL (**11**), 0.038 mg/mL (**12**), 0.066 mg/mL (**13**), 0.013 mg/mL (**14**), and 0.016 mg/mL (**15**), respectively. All the stock solutions were stored at 4 °C. The chromatograms of the 15 reference substances were shown in Fig. 1.

2.5. Method validation

The mixture stock solution was diluted to a series of different concentration solutions for constructing the calibration curves. These mixture standard solutions were injected in triplicate, and calibration curves were constructed by plotting the peak area (*Y*-axis) versus the concentration (*X*-axis) of each analyte. The results of all 15 references showed good linearity ($r^2 > 0.9993$). The linear regression equations, correlation coefficients (r^2), and linearity ranges were given in Table 1.

The limit of detection (LOD) and the limit of quantification (LOQ) under the present chromatographic conditions were determined by the serial dilution of a standard solution until the signal-to-noise (S/N) ratio was approximately three or ten, respectively. The values were also given in Table 1.

The precision of the method was determined by the analysis of six consecutive injections using the same sample solution. The relative standard deviation (RSD) was less than 3.5%, indicating the method could be considered reliable.

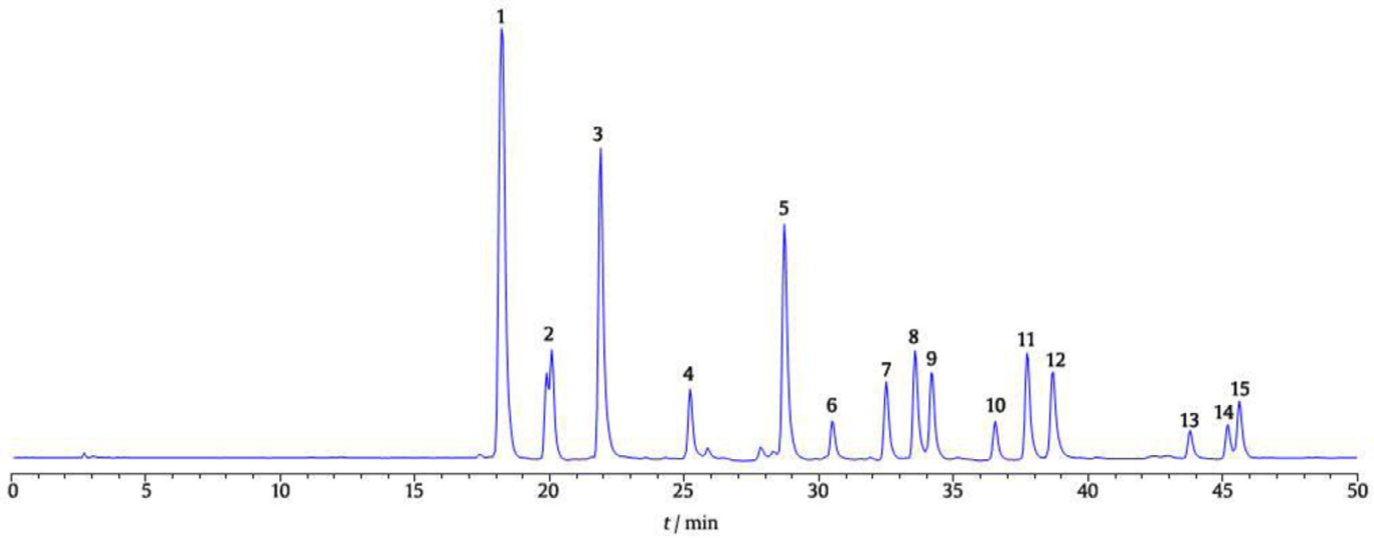

Fig. 1. Chromatograms of 15 reference substances (**1**: isocarthamidin-7-*O*-β-*D*-glucuronide; **2**: carthamidin-7-*O*-β-*D*-glucuronide; **3**: scutellarin; **4**: apigenin-7-*O*-β-*D*-glucopyranside; **5**: baicalin; **6**: isoscutellarein-8-*O*-β-*D*-glucuronide; **7**: chrysin-7-*O*-β-*D*-glucuronide; **8**: luteolin; **9**: wogonoside; **10**: alpinetin; **11**: apigenin;**12**: Baicalein; 1**3**: wogonin; **14**: chrysin; **15**: oroxylin A).

Table 1
Linear relationship with LOD, LOQ, and repeatability of 15 flavonoids.

Compounds	Regressive equation	r^2	Linear range / (μg·mL^{-1})	LOD / (μg·mL^{-1})	LOQ / (μg·mL^{-1})	Repeatability RSD / %
1	$y = 11.92\ x - 73.52$	0.9999	16.25–2080.00	0.48	1.59	0.65
2	$y = 8.90\ x - 4.43$	0.9993	22.42–1435.00	2.80	5.61	2.22
3	$y = 18.45\ x - 9.94$	0.9999	4.90–195.84	0.08	0.20	0.71
4	$y = 15.87\ x + 0.45$	0.9996	1.15–46.00	0.05	0.14	1.58
5	$y = 32.03\ x - 8.49$	0.9998	3.40–136.00	0.05	0.14	1.58
6	$y = 12.89\ x - 108.35$	0.9995	33.75–1080.00	0.30	0.99	2.24
7	$y = 22.52\ x - 3.23$	0.9998	0.58–232.00	0.07	0.22	1.26
8	$y = 9.18\ x - 5.29$	0.9999	3.20–128.00	0.10	0.24	2.56
9	$y = 38.31\ x - 4.42$	0.9996	1.68–67.20	0.03	0.07	2.30
10	$y = 30.97\ x - 1.70$	0.9997	1.02–40.75	0.02	0.07	2.28
11	$y = 22.69\ x - 4.37$	0.9994	1.25–50.00	0.04	0.11	2.61
12	$y = 56.76\ x - 15.96$	0.9998	0.96–38.40	0.03	0.09	1.81
13	$y = 31.23\ x - 1.79$	0.9999	1.65–66.10	0.02	0.07	2.10
14	$y = 60.66\ x - 1.60$	0.9997	0.33–13.06	0.12	0.29	2.46
15	$y = 59.67\ x - 1.37$	0.9999	0.389–15.52	0.13	0.30	1.95

Repeatability of the method was evaluated by analyzing six samples from the same source using the developed method. The repeatability of 15 reference substances of RSD peak area was calculated, which was all less than 2.56%, which showed the excellent repeatability of this method. The values were also given in Table 1.

The stability was evaluated by storing the sample solutions at 25 °C, then analyzed at 0, 6, 12, 18, 24, and 48 h, respectively. RSD of peaks area was less than 2.4%, indicating that the sample solution was stable within the tested time period.

Accuracy was determined by adding the mixed standard solutions on the same concentration levels to the known amount of six sample solutions. Then, the resultant samples were extracted and analyzed with the proposed method, and six copies experiments were performed at each concentration level. The recovery percentages were calculated using the equation: Recovery (%) = [(total detected amount − original amount)/ spiked amount] × 100. Variations are expressed in terms of the relative standard deviation (RSD) of the measurement in all tests. As shown in Table 2, the developed analytical method was reproducible with good accuracy in the range of 96.71%−103.05% (RSD < 2.68%).

2.6. Statistical analysis

ChemPattern Software (Chemmind Technologies Co.V2.0, Beijing, China) was used to establish the HPLC fingerprint. Common pattern and similarity analysis was conducted using a total of 77 batches of 34 roots, 15 stems, 15 leaves, and 13 flowers samples. The total chromatograms of each sample were exported as a .cdf file, then imported into the ChemPattern Software. The “Normalization (observation)” data processing mode was used for common pattern and similarity analysis. Other common pattern parameters were common pattern statistics (mean), common peak screening (all variables), and minimum common peak (0.1%); Spectrogram simulation (Gaussian curve simulation) were as follows.

The principal components analysis (PCA) and orthogonal partial lease squares discriminant analysis (OPLS-DA) were carried out by Simca-P (13.0) Software. In this paper, the 15 target compounds analyzed from the 77 samples composed a data matrix with 77 rows and 15 columns, which was used for PCA and OPLS-DA analysis after normalization.

Clustering heat map of 15 compounds content in the different part of *S. baicalensis* was carried out by Metaboanalyst 3.0.

3. Results

3.1. Establishment of chromatographic fingerprint of S. baicalensis

The reference HPLC-DAD fingerprint of roots, stems, leaves, and flowers of *S. baicalensis* were shown in the Fig. 2A total of 39 common peaks existed in the roots, and according to the retention

Table 2
Accuracy of flavonoids in *S. baicalensis* ($n = 6$)

Analyte[a]	Original/ mg	Spiked/ mg	Detected/ mg	Recovery/ %	Average recovery/ %	RSD/%
1	1.68	1.38	3.09	102.32	102.32	2.67
	1.03	1.38	2.37	97.21		
	1.33	1.38	2.72	100.38		
	1.39	1.38	2.72	96.27		
	1.06	1.38	2.41	97.75		
	1.40	1.38	2.70	93.92		
2	0.11	0.12	0.23	103.05	103.05	0.78
	0.11	0.12	0.23	102.04		
	0.14	0.12	0.26	100.99		
	0.08	0.12	0.20	102.31		
	0.10	0.12	0.23	102.96		
	0.08	0.12	0.20	101.11		
3	7.62	6.37	13.93	99.11	98.94	0.93
	7.45	6.37	13.84	100.13		
	7.21	6.37	13.54	99.25		
	7.28	6.37	13.61	99.33		
	7.41	6.37	13.68	98.42		
	7.24	6.37	13.45	97.44		
4	0.00	0.03	0.03	100.01	101.28	1.58
	0.00	0.03	0.03	101.53		
	0.00	0.03	0.03	102.45		
	0.00	0.03	0.03	98.77		
	0.00	0.03	0.03	103.07		
	0.00	0.03	0.03	101.84		
5	0.71	0.54	1.24	98.14	97.50	1.52
	0.70	0.54	1.22	96.29		
	0.68	0.54	1.21	99.26		
	0.68	0.54	1.21	98.14		
	0.69	0.54	1.21	95.18		
	0.68	0.54	1.21	97.96		
6	0.04	0.05	0.09	96.81	96.81	2.11
	0.04	0.05	0.09	101.99		
	0.03	0.05	0.08	102.09		
	0.04	0.05	0.09	100.43		
	0.04	0.05	0.09	97.42		
	0.04	0.05	0.08	99.85		
7	5.64	5.08	10.80	101.57	99.35	2.68
	5.52	5.08	10.46	97.36		
	5.34	5.08	10.36	98.78		
	5.39	5.08	10.27	96.01		
	5.48	5.08	10.52	99.17		
	5.36	5.08	10.60	103.21		
8	0.00	0.04	0.05	104.35	100.88	2.56
	0.00	0.04	0.04	101.37		
	0.00	0.04	0.04	99.99		
	0.00	0.04	0.04	98.40		
	0.00	0.04	0.05	103.20		
	0.00	0.04	0.04	97.94		
9	0.02	0.04	0.06	95.96	97.38	2.15
	0.02	0.04	0.06	99.10		
	0.02	0.04	0.06	100.45		
	0.02	0.04	0.06	95.06		
	0.02	0.04	0.06	97.75		
	0.02	0.04	0.06	95.96		
10	0.01	0.05	0.06	93.68	96.78	2.39
	0.01	0.05	0.06	99.23		
	0.01	0.05	0.06	95.02		
	0.01	0.05	0.06	98.28		
	0.01	0.05	0.06	95.59		
	0.01	0.05	0.06	98.85		
11	0.06	0.06	0.12	100.90	98.03	2.61
	0.06	0.06	0.11	97.67		
	0.06	0.06	0.11	93.91		
	0.06	0.06	0.11	96.59		
	0.06	0.06	0.11	100.18		
	0.06	0.06	0.11	98.92		
12	0.04	0.04	0.07	97.62	97.84	1.13
	0.04	0.04	0.07	98.94		
	0.03	0.04	0.07	98.15		
	0.03	0.04	0.07	96.03		
	0.03	0.04	0.07	97.35		
	0.03	0.04	0.07	98.94		
13	0.01	0.03	0.03	94.86	96.71	2.58
	0.01	0.03	0.03	93.68		
	0.01	0.03	0.04	98.81		
	0.01	0.03	0.03	94.86		
	0.01	0.03	0.04	98.81		
	0.01	0.03	0.04	99.21		
14	0.07	0.10	0.17	100.68	99.89	2.04
	0.07	0.10	0.17	99.22		
	0.07	0.10	0.17	96.59		
	0.07	0.10	0.17	100.88		
	0.07	0.10	0.17	102.63		
	0.07	0.10	0.17	99.32		
15	0.00	0.03	0.04	103.15	100.91	1.69
	0.00	0.03	0.03	99.71		
	0.00	0.03	0.03	98.57		
	0.00	0.03	0.04	102.29		
	0.00	0.03	0.04	100.28		
	0.00	0.03	0.04	101.43		

times of reference substance and UV spectrum, nine compounds can be identified, the peak number of 8, 11, 20, 26, 30, 33, 35, 37, and 38 were identified as compounds **1**, **3**, **5**, **9**, **10**, **12**, **13**, **14**, and **15**, respectively (Fig. 2A). The chemical composition of the stems was similar to the leaves, while peaks of compound **7** were absent in the stems. There existed 28 common peaks in the stems, mainly centering on the retention time at 14–35 min, and six peaks of 9, 12, 14, 16, 20, and 22 can be identified as compounds **1**, **2**, **3**, **4**, **5**, and **6**, respectively (Fig. 2B). As for the leaves, seven peaks of 11, 13, 14, 18, 20, 22, and 25 can be identified as compounds **1**, **2**, **3**, **4**, **5**, **6**, and **7**, respectively (Fig. 2C). Lastly, a total of 36 common peaks in the flowers and thirteen peaks of 9, 12, 14, 17, 20, 22, 27, 28, 29, 31, 32, 33, and 36 can be identified as compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, and **14**, respectively (Fig. 2D).

3.2. Similarity analysis

The similarities of different parts of *S. baicalensis* were evaluated by comparing each common pattern fingerprint. The similarity values of roots, stems, leaves, and flowers were more than 0.92, 0.98, 0.98, and 0.91, respectively. This result indicated that the different parts of *S. baicalensis* were consistent, stable, and can meet the fingerprinting technical requirements.

3.3. Multivariate statistical analysis

The PCA and OPLS-DA were performed based on the contents of 15 target compounds from HPLC-DAD profiles. In the PCA score map (Fig. 3A), the roots and the aerial parts were completely separated. In the aerial part, the part of flowers is completely separated with stems and leaves, but the stem and leaf compositions were slightly overlap. It impacted that the chemical composition in stems and leaves may be similar. Based on the S-plot of OPLS-DA (Fig. 3B), it can be seen that the compounds, isocarthamidin-7-*O*-β-*D*-glucuronide (**1**) and baicalin (**5**) may be the main differentially expressed compounds between the above and below ground parts of *S. baicalensis*. Also, the flavonoids 2, 3, 4, 9, and 12 may also contribute to some effect on the difference among the different parts of this plant.

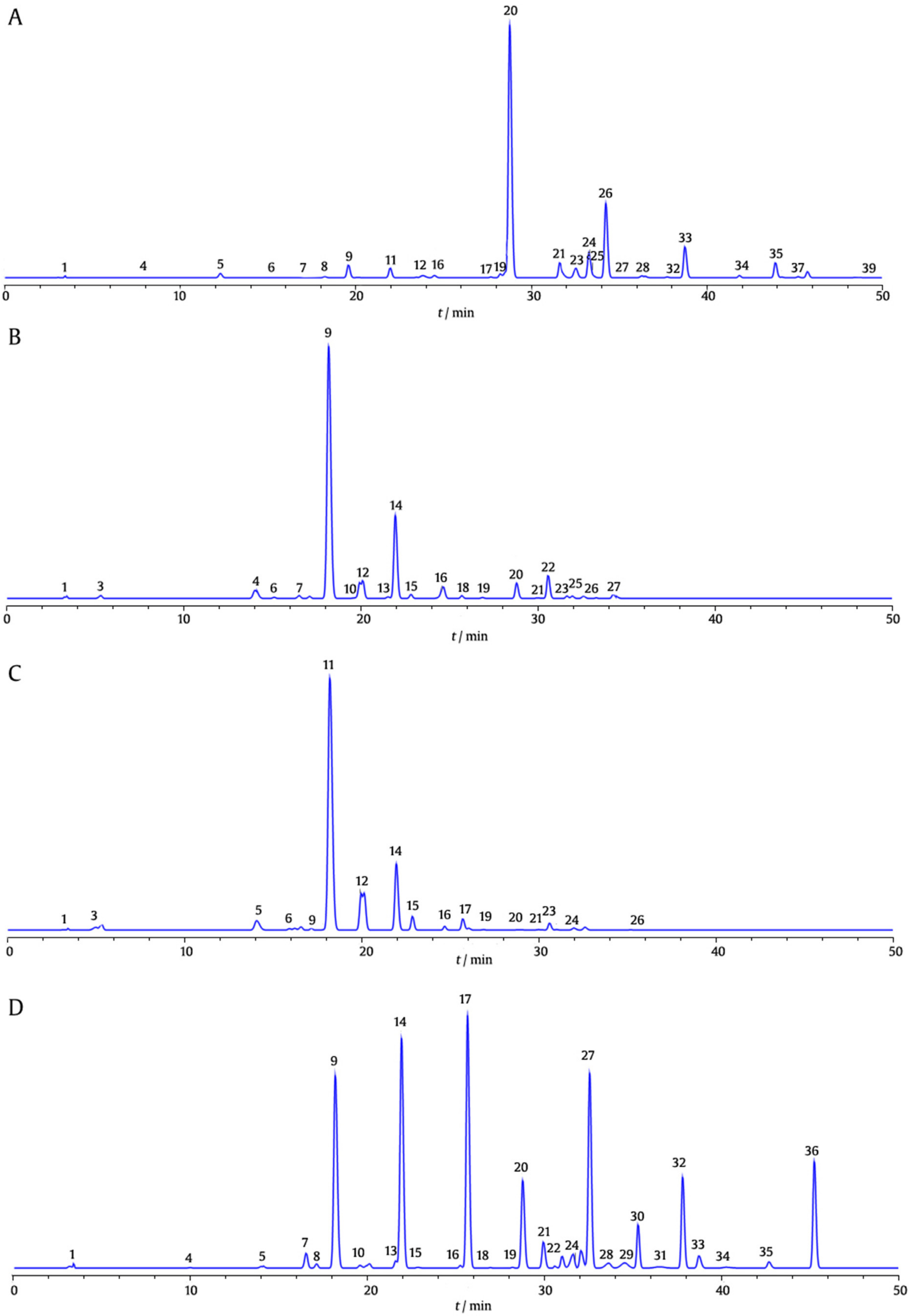

Fig. 2. Reference HPLC-DAD fingerprint chromatograms of different parts of *S. baicalensis* (A: roots; B: stems; C: leaves; D: flowers).

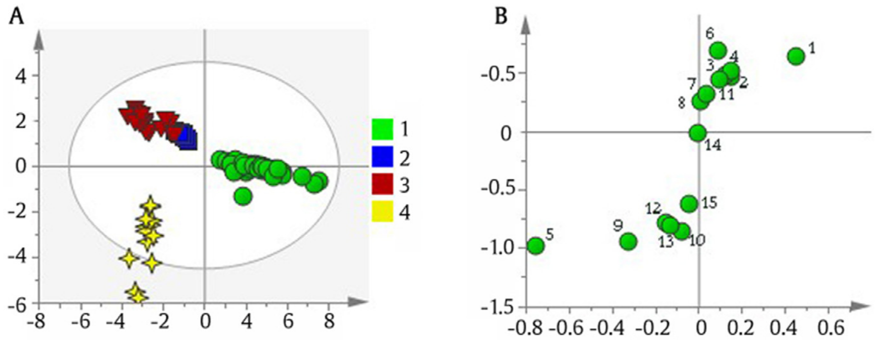

Fig. 3. A: PCA score map of different part of *S. baicalensis* (1: roots; 2: stems; 3: leaves; 4: flowers); B: S-Plot score map of roots and aerial parts of *S. baicalensis* (1: isocarthamidin-7-*O*-β-*D*-glucuronide; 2: carthamidin-7-*O*-β-*D*-glucuronide; 3: scutellarin; 4: apigenin-7-*O*-β-*D* glucopyranoside; 5: baicalin; 9: wogonoside; 12: baicalein; 13: wogonin).

3.4. Contents of 15 flavonoids in different parts of S. baicalensis

The clustering heat map of content of 15 flavonoids in different parts of *S. baicalensis* (Fig. 4) showed that the chemical composition and content have significant differences between the root and the aerial parts of *S. baicalensis*. In addition, the chemical composition of stems and leaves showed some comparability, while their contents and species showed remarkable differences to flowers.

The determination results (Table 3) showed that the flavonoids of isocarthamidin-7-*O*-β-*D*-glucuronide [**1**, (106.66 ± 22.68) mg/g], carthamidin-7-*O*-β-*D*-glucuronide [**2**, (19.82 ± 11.17) mg/g] and isoscutellarein-8-*O*-β-*D*-glucuronide [**6**, 3.10 ± 1.73) mg/g] had the highest contents in leaves. The contents of apigenin7-*O*-β-*D*-glucopyranoside [**4**, (18.1 ± 4.85) mg/g] and chrysin-7-*O*-β-*D*-glucuronide [**7**, (9.82 ± 5.51) mg/g] in flowers were the highest. The flavonoids of baicalin [**5**, (90.8 ± 23.66) mg/g], wogonoside [**9**, (17.39 ± 6.64) mg/g], alpinetin [**10**, (1.15 ± 0.61) mg/g], baicalein [**12**, (4.39 ± 2.98) mg/g], wogonin [**13**, (3.48 ± 1.98) mg/g] and oroxylin A [**15**, (0.53 ± 0.52) mg/g] have higher content in the roots. Scutellarin (**3**) has higher content in flowers [(14.92 ± 3.62) mg/g] and leaves [(14.78 ± 3.68) mg/g]. Three flavonoids, luteolin (**8**), apigenin (**11**), and chrysin (**14**) showed lower content in *S. baicalensis*. The flavone of luteolin (**8**) was only detected in the flowers with low content of (0.37 ± 0.39) mg/g. Though the flavone of apigenin (**11**) was detected both in the leaves [(0.02 ± 0.08) mg/g] and flowers [(1.99 ± 1.52) mg/g], the content in the leaves was too low and only detected in the Jilin Province leaf sample. Chrysin (**14**) showed low content in the roots [(0.19 ± 0.23) mg/g], leaves [(0.01 ± 0.01) mg/g], and flowers [(0.51 ± 0.47) mg/g].

4. Discussion

Combined with the results of the multivariate statistical analysis and clustering heat map analysis, we can speculate that

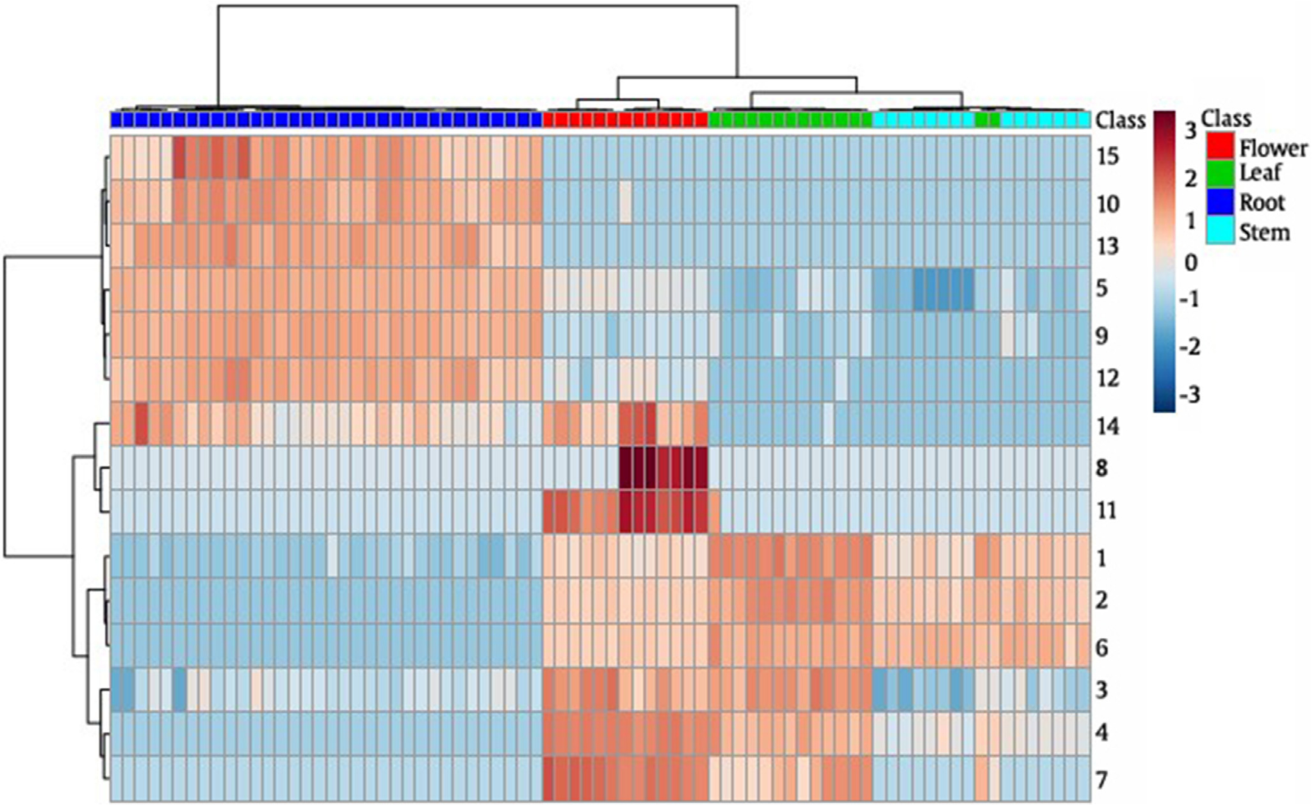

Fig. 4. Clustering heat map of 15 compounds in different part of *S. baical*ensis.

Table 3
Contents of 15 compounds in different parts of *S. baicalensis* (mean ± SD, $mg.g^{-1}$).

Analyte^{a}	Roots ($n = 34$)	Stems ($n = 15$)	Leaves ($n = 15$)	Flowers ($n = 13$)
1	2.01 ± 0.85^{c}	22.88 ± 7.82^{b}	106.66 ± 22.68^{a}	20.81 ± 6.87^{b}
2	0.00^{c}	2.69 ± 1.30^{b}	19.82 ± 11.17^{a}	1.91 ± 0.4^{b}
3	5.37 ± 1.19^{b}	4.14 ± 1.32^{b}	14.78 ± 3.68^{a}	14.92 ± 3.62^{a}
4	–	0.20 ± 0.12^{c}	3.64 ± 3.16^{b}	18.1 ± 4.85^{a}
5	90.8 ± 23.66^{a}	0.31 ± 0.47^{b}	0.51 ± 0.52^{b}	3.66 ± 1.63^{b}
6	0.00^{d}	2.15 ± 0.66^{b}	3.10 ± 1.73^{a}	0.86 ± 0.13^{c}
7	–	–	1.45 ± 1.57^{b}	9.82 ± 5.51^{a}
8	0.00^{b}	–	–	0.37 ± 0.39^{a}
9	17.39 ± 6.64^{a}	0.06 ± 0.06^{b}	0.06 ± 0.1^{b}	0.08 ± 0.03^{b}
10	1.15 ± 0.61^{a}	–	–	0.01 ± 0.03^{b}
11	–	–	0.02 ± 0.08^{b}	1.99 ± 1.52^{a}
12	4.39 ± 2.98^{a}	–	0.01 ± 0.02^{b}	0.16 ± 0.14^{b}
13	3.48 ± 1.98^{a}	–	–	–
14	0.19 ± 0.23^{a}	–	0.01 ± 0.01^{b}	0.51 ± 0.47^{a}
15	0.53 ± 0.52^{a}	0.00^{b}	0.00^{b}	0.00^{b}

–: means undetected compound;
Number $^{a}/^{b}/^{c}/^{d}$, means within same row with different letter mark among content levels groups were significantly different ($P < 0.05$).

isocarthamidin-7-*O*-β-*D*-glucuronide (**1**), carthamidin-7-*O*-β-*D*-glucuronide (**2**), scutellarin (**3**), apigenin-7-*O*-β-*D*-glucopyranoside (**4**), baicalin (**5**), wogonoside (**9**), and baicalein (**12**) were the main differentially expressed flavonoids between the below and above ground parts of *S. baicalensis*. Flavonoids of **1** – **4** were higher expressed in the aerial part and all of them had 4′-OH group on their B-rings, and flavonoids of **1** and **2** were two flavanone derivatives. It is worth noting that flavonoids of **5**, **9**, **11**, and **12** were higher expressed in the roots and all of them have no 4′-OH group on their B-rings (Fig. 5). The different synthesized pathway of flavonoids may contribute to this phenomenon. The previous studies have shown that flavones are synthesized usually by the flavonoid pathway, which is a part of phenylpropanoid metabolism, recently a specialized pathway for 4′-deoxyflavone biosynthesis occurred in the roots of *S. baicalensis*, which result in the specific 4′-deoxyflavones higher expression levels in roots than in aerial organs (Yang et al., 2016; Zhao et al., 2016; Zhao, Chen, & Martin, 2016).

Based on the literatures, the pharmacodynamics of the roots are closely related to high levels of baicalin, baicalein, wogonoside, and wogonin. (Khan et al., 2017; Wang et al., 2015; Xin et al., 2013; Yang et al., 2013). The pharmacodynamics of the aerial parts may also relate to high levels of flavones. In this study, it found that six flavone glycosides isocarthamidin-7-*O*-β-*D*-glucuronide (**1**), carthamidin-7-*O*-β-*D*-glucuronide (**2**), scutellarin (**3**), apigenin-7-*O*-β-*D*-glucopyranoside (**4**), isoscutellarein-8-*O*-β-*D*-glucuronide (**6**), chrysin-7-*O*-β-*D*-glucuronide (**7**) and two flavone aglycones luteolin (**8**), apigenin (**11**) showed highest contents in the aerial parts. Furthermore, the stems, leaves, and flowers share common compounds of **1** – **4** and **6**. The modern pharmacology research shows the anti-oxidant abilities as follow: baicalein > apigenin > scutellarin > chrysin > baicalin > apigenin-7-*O*-β-*D*-glucoside > chrysin-7-*O*-β-*D*-glucuronide > isocarthamidin-7-*O*-β-*D*-glucuronide (Li et al., 2017). Especially, scutellarin showed other bioactivities, such as neuroprotective, anti-inflammatory and against LPS-induced cognitive deficits (Baluchnejadmojarad, Zeinali, & Roghani, 2017), and isocarthamidin-7-*O*-β-*D*-glucuronide have the ability of anti-tumor (Yang et al., 2003). These flavonoids which have high content and well bioactivities in the aerial parts of *S. baicalensis* may be the quality evaluation index of Huangqin Tea.

Furthermore, this study also found that the contents of scutellarin (breviscapine, a cerebrovascular and cardiovascular drug) in the stem, leaf, and flower of *S. baicalensis* were (4.14 ± 1.32), (14.78 ± 3.68), and (14.92 ± 3.62) mg/g, respectively, with the total content similar to *Erigeron breviscapus* (18.1 mg/g) (Su, Lv, & Zhang, 2016). The aerial parts of *S. baicalensis* could be a good choice when extracting large amounts of scutellarin.

The biomass of the aerial parts of *S. baicalensis* is huge, and it has been used as Huangqin Tea in the folk to prevent sunstroke,

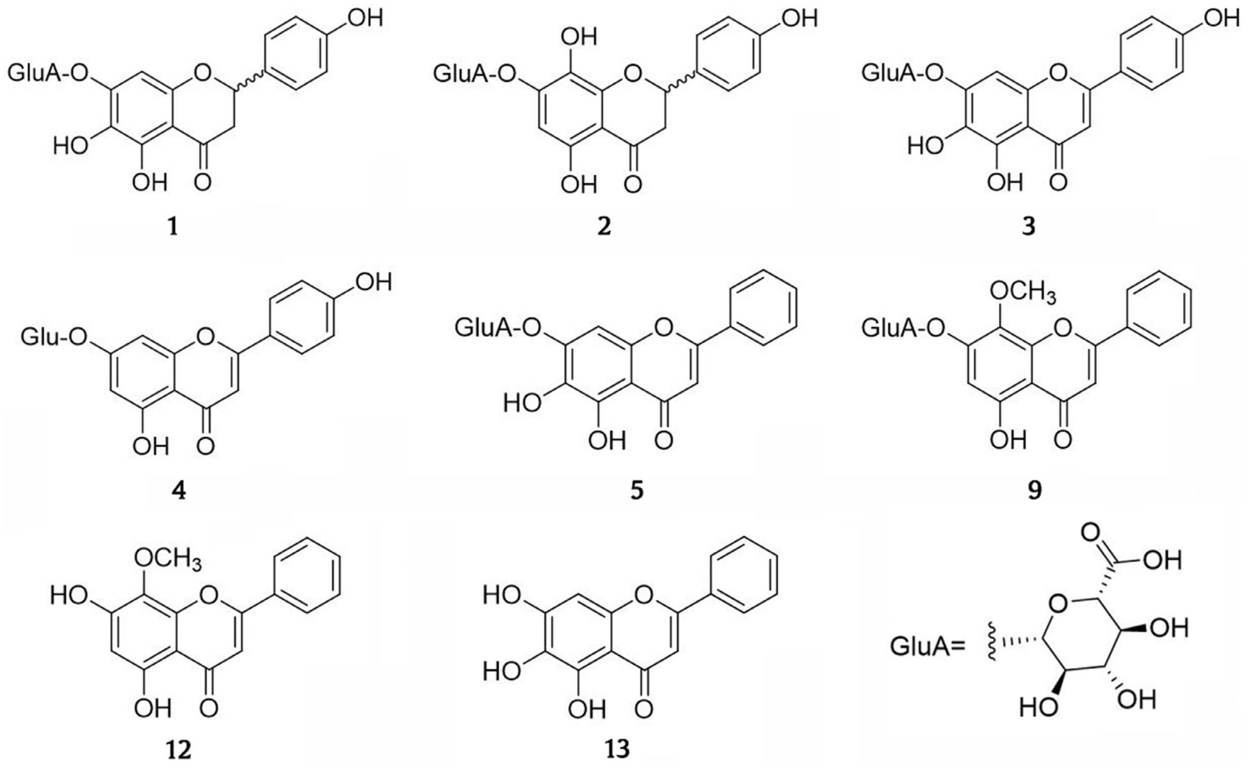

Fig. 5. Structures of compounds **1-5, 9, 12, and 13.**

dry dampness and promote digestion function. Furthermore, The Huangqin Tea is safe when used in daily life, and has been used for thousands of years without reported toxic effects. Therefore, there is the potential of processing the aerial parts of *S. baicalensis* into novel high quality functional food, such as drinks and cakes. However, detailed experiments *in vitro* and *in vivo* and clinical studies are essential for the development of legitimated functional food products in the future.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgments

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