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Total Flavonoid Content, the Antioxidant Capacity, Fingerprinting and Quantitative Analysis of Fupenzi (*Rubus chingii* Hu.)

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Abstract

Objective: In this study, one of the objectives was to investigate the total flavonoid contents of Fupenzi (*R. chingii* Hu.) obtained from different regions of China and to evaluate their anatioxidant activities. And the second objective of this study was to develop a validated HPLC method for chromatographic fingerprints of the samples extracts of Fupenzi. Method: The total flavonoid contents were determined by a colorimetric method and the antioxidant activity was determined spectrophotometrically by DPPH and ABTS radical scavenging assays. The chromatographic fingerprint was developed by high-performance liquid chromatography coupled with diode array detection for the control of Fupenzi. Results: A significant correlation between antioxidant activity and the total flavonoid content was observed for the DPPH assay (r^2 = 0.758, ρ = 0.004) and the ABTS assay (r^2 = 0.788, ρ = 0.002). Under the optimized chromatographic conditions, the validated method was successfully applied to assessment of chemical fingerprinting of 12 batches of FPZ collected from different regions of China. Comparisons of the chromatograms showed that 15 characteristic peaks could be selected as markers for identification and evaluation of Fupenzi. In addition, the proposed method was also successfully applied to simultaneous determination of five compounds (including puerarin, rutin, hyperin, quercetin and kaempferol) in these samples. Conclusions: The qualitative and quantitative analysis described in this paper could be used for identification and evaluation of Fupenzi.

Keywords

Flavonoid, Antioxidant Capacity, Fingerprinting, Quantitative Analysis, Fupenzi (*Rubus chingii* Hu.)

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1. Introduction

As a member of the *Rosaceae* family grown as a perennial crop, raspberry is widely grown in China and is popular for its flavour and attractive red color. In addition to being a favorite food, its unripe fruits are usually used as a key member of many ancient prescriptions against aging [1]. Modern pharmacological research showed that scavenging free radicals in the body by natural antioxidants was an effective way for anti-aging. Berries, such as blackberry, raspberry and strawberry, contain high levels of anthocyanins, flavonoids and phenolic acids and are considered a good source of natural antioxidant [2] [3], which might provided protection against various human disease caused by oxidative stress. Therefore, it is very significant to evaluate the antioxidant activity of different kinds of berries. However, to the best of our knowledge, there is no report on evaluation of the antioxidant capacity of Fupenzi (*R. chingii* Hu.).

Since the entire pattern of compounds characterizes the chemical composition of the herbs, the chromatographic fingerprint represents a comprehensive qualitative methodology, in which the entire chromatogram is evaluated during data analysis to discriminate between different species of the same herbal family [4]-[7]. Nowadays, chemical fingerprinting has been internationally accepted as an efficient technique for the assessment of not only herbal medicines, but also botanical food, including the leaves of *R suavissimus* S. Lee [8] and the fruits of *R. chingii* Hu. [9]. However, there were not enough common peaks (only 7 common peaks) selected as characteristic peaks for the identification of the fruits of *R. chingii* Hu. and there was lack of the combination of the constituents contributed to antioxidant activity with chemical fingerprinting.

Taking all these factors into considerations, the objective of this study was to investigate the total flavonoid contents of Fupenzi obtained from different regions of China and to evaluate their antioxidant activities. And the second objective of this study was to develop a validated HPLC method for chromatographic fingerprints of the sample extracts of Fupenzi.

2. Experimental

2.1. Chemicals and Reagents

Twelve batches of Fupenzi samples were collected from different regions of China (1-Hangzhou (Zhejiang), 2-Jinhua (Zhejiang), 3-Zhengzhou (Henna), 4-Nanjing (Jiangsu), 5-Guangzhou (Guangdong), 6-Guilin (Guangxi), 7-Nanning (Guangxi), 8-Chengdu (Sichuang), 9-Fuzhou (Fujian), 10-Zhangzhou (Fujian), 11-Changchun (Jilin), 12-shengyang (Jilin)). Rutin, hyperin, quercetin and kaempferol were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis[3-ethylbenzothiazoline-6-sulphonic acid] diammonium salt) were purchased from Sigma chemical Co. (Sigma, USA). Acetonitrile and methanol were of HPLC grade (Shield Fine Chemicals Company, Tianjin, China). Water for HPLC analysis was purified by a Milli-Q water purification system (Shanghai, China). Other reagents were all of analytical grade.

2.2. Preparation of Sample Solutions

Grind the fruit samples of Fupenzi into powder by use of a pestle and mortar. 3.0 g pulverized powder was accurately weighed and ultrasonically extracted with 40 mL of 95% ethanol for 50 min in a calibrated flask at 40˚C, and then filtered through a 0.22 μm membrane. The filtrate was evaporated to dryness at 35˚C and the residue was dissolved into 10 mL methanol. The solution was filtrated through a syringe filter (0.22 μm) and aliquots (20 μL) were subjected to HPLC before analysis.

2.3. Preparation of Standard Solutions

A standard stock solution containing the five components (rutin, hyperin, quercetin and kaempferol) was prepared in methanol and stored away from light at 4˚C. Working standard solutions containing the four compounds were prepared by appropriate dilution of the stock solution.

2.4. Determination of the Total Flavonoid Content

The total flavonoid content (TFC) was determined using a colorimetric method [10] with a little modification. 0.1 mL properly diluted sample of the extract was mixed with 0.3 mL of a 5% $NaNO_2$ solution. The mixture was

allowed to stay at room temperature for 6 min; 0.3 mL of a 10% $AlCl_3$ solution was added for 6 min followed by the addition of a 2 mL 4% NaOH solution. The double-distilled water was added to reach a final volume of 10 ml. The solution was mixed and kept at room temperature for 15 min. Absorbance was measured immediately against the prepared blank at 510 nm using a spectrophotometer (UV-2550, Shimadzu, equipped with 10 mm quartz cuvettes). Quantitative measurements were performed, based on a standard calibration curve of six points from 5.0 to 80 $mg \cdot L^{-1}$ of rutin in methanol ($y = 0.0105x - 0.0031$, $R^2 = 0.9965$, y and x represent the absorbance and concentration, respectively). The total phenolic content was expressed as rutin equivalents in $mg \cdot g^{-1}$ of extract.

2.5. DPPH Assay

DPPH radical-scavenging capacity of raspberry extracts was evaluated according to the method of Chen and Ho [11], with a little modification. Briefly, 0.05 mL properly diluted sample of the extract was added to 5.0 mL methanol solution of DPPH radical (final concentration was 0.5 $mmol \cdot L^{-1}$). The mixture was shaken vigorously for 1 min by vortexing and left to stand at room temperature in the dark for 30 min. Thereafter, the absorbance of the sample was measured using the UV-vis spectrophotometer at 517 nm against methanol blank. A negative control was taken after adding DPPH solution to 0.05 mL methanol. The ascorbic acid was used as a reference compound [$y = 14.032x + 0.4534$, $R^2 = 0.9975$, y and x represent the scavenging rate and concentration (0.006 - 5.6 $mg \cdot L^{-1}$), respectively]. The ascorbic acid equivalent antioxidant capacity was expressed as mg ascorbic acid corresponding to one g dry weight.

2.6 ABTS Assay

The ABTS radical scavenging test was used to determine the antioxidant activity [12]. ABTS radical was obtained by reaction between ABTS and potassium persulfate. Blank sample was prepared from the daily solution by adding 100 mL PBS buffer (5 $mmol \cdot L^{-1}$, pH7.4), which gives an absorbance of 0.7 ± 0.02. The radical scavenging activity was assessed by mixing 5.0 mL ABTS solution with 0.05 mL properly diluted sample of the extract. The reactive mixture was allowed to stand at room temperature for 6 min and the absorbance was recorded at 734 nm. The ascorbic acid was used as standard ($y = 22.821x + 1.3393$, $R^2 = 0.9978$, y and x represent the scavenging rate and concentration (0.005 - 4.0 $mg \cdot L^{-1}$), respectively). The ascorbic acid equivalent antioxidant capacity was calculated like DPPH assay.

2.7. HPLC Conditions

The HPLC system Dionex P680 series (Dionex, USA), equipped with the Chromeleon software (Dionex) and comprised a binary pump, an online vacuum degasser, a manual sampler, a thermostated column compartment and a diode array detection (DAD), was used for the chromatographic analysis. The chromatographic analysis was carried out on a Kromasil C_{18} (250 mm × 4.6 mm, 5.0 µm) column. The mobile phase was composed of acetonitrile (A) and 1% formic acid solution (B) with gradient elution (0 - 5 min, 5% - 20% A; 5 - 20 min, 20% - 45% A; 20 - 25 min, 45% - 90% A; 25 - 30 min, 90% - 100% A, 30 - 40 min, 100% A). The solvent flow rate was 1.0 $mL \cdot min^{-1}$, the elute was continuously monitored using a DAD detector at a wavelength of 360 nm and the column temperature was maintained at 30˚C. Aliquots of 20 µL were injected into HPLC system for analysis.

2.8. Statistical Analysis

Data analysis was performed using professional software (Analysis System for Chromatographic Fingerprint and Data of Traditional Chinese Medicine version 2004, the Pharmacopoeia Commission of the People's Republic of China). This software allowed calculation of the correlation coefficients of the sample from different technologies with the reference chromatogram. The hierarchical clustering analysis (HCA) and principal component analysis (PCA) was performed using SPSS for Windows v.18.0 software (SPSS Corporation, Stanford, USA).

3. Results and Discussion

3.1. Total Flavonoid Content, DPPH and ABTS Radical Scavenging Activities

The content of total flavonoid was determined by a colorimetric method and the results were summarized in **Ta-**

ble 1. The total flavonoid contents in all samples ranged from 13.17 ± 0.02 to 22.82 ± 0.02 mg rutin g^{-1} d.w. The values for DPPH and ABTS scavenging activities in all samples ranged from 20.51 ± 0.06 to 31.08 ± 0.03 mg ascorbic acid g^{-1} d.w. and from 33.93 ± 0.11 to 54.83 ± 0.12 mg ascorbic acid g^{-1} d.w., respectively (Table 1). A significant correlation between DPPH assay and ABTS assay was observed ($r^2 = 0.821$, $\rho = 0.001$, SPSS for windows 19.0, USA), which suggested the two methods were comparable in the case of Fupenzi (Table 2).

Generally, the total flavonoid contents were positively correlated with the antioxidant activities [13] [14]. In this study a significant correlation between antioxidant activity and the total flavonoid content was also observed for the DPPH assay ($r^2 = 0.758$, $\rho = 0.004$) and the ABTS assay ($r^2 = 0.788$, $\rho = 0.002$) (Table 2).

3.2. HPLC Chromatograms of Fupenzi

With the same sample extracts contributed to antioxidant activities, the HPLC chromatograms of 12 batches of samples were obtained under the optimized chromatographic conditions (Figure 1). As shown in Figure 1, these samples showed similar chromatographic profiles and more than 22 peaks were detected in all the 12 chromatograms. After carefully analyzing the chromatographic profiles of these samples, 15 common peaks with acceptable heights and good resolution were selected as characteristic peaks for fingerprinting analysis (Figure 2). Peak 10 was selected as the reference peak. Relative retention times (RRTs) and relative peak areas (RPAs) of the 15 characteristic peaks were calculated as follows: RRT = retention time of characteristic peak/retention time of marker peak, and RPA = peak area of characteristic peak/peak area of marker peak. The results from the 12 samples indicated that the RPAs of the 15 characteristic peaks varied dramatically (Table 3), but the RRTs were invariable for the Fupenzi (Table 4). The results indicated that the RRTs of the 15 characteristic peaks can serve as characteristic peaks for identification of "unknown" samples. A sample with a similar HPLC chromatographic profile and matched RRT values to the typical fingerprint chromatogram can be authenticated as genuine Fupenzi. However, due to several ingredients, such as climate, season of harvest and regions of cultivation, the same types of components were generally existed in the same variety, but their contents were often different

Table 1. The total flavonoid contents, DPPH and ABTS scavenging activities in the sample extract of 12 raspberries (*R. chingii* Hu.). (n = 6).

Samples	Total flavonoids content (mg rutin g^{-1} d.w.)	DPPH (mg ascorbic acid g^{-1} d.w.)	ABTS (mg ascorbic acid g^{-1} d.w.)
Hangzhou	14.87 ± 0.02	20.51 ± 0.06	33.93 ± 0.11
Jinhua	17.08 ± 0.03	25.65 ± 0.03	48.67 ± 0.06
Zhengzhou	19.50 ± 0.03	31.08 ± 0.03	54.26 ± 0.12
Nanjing	17.40 ± 0.03	27.01 ± 0.03	47.86 ± 0.07
Guangzhou	17.50 ± 0.03	24.68 ± 0.05	42.93 ± 0.06
Guilin	19.60 ± 0.02	25.94 ± 0.05	41.62 ± 0.12
Nanning	13.17 ± 0.02	21.98 ± 0.03	37.99 ± 0.07
Chengdu	19.16 ± 0.02	26.70 ± 0.05	49.51 ± 0.06
Fuzhou	18.27 ± 0.02	27.97 ± 0.03	42.05 ± 0.06
Zhangzhou	15.52 ± 0.05	21.69 ± 0.05	37.41 ± 0.07
Changchun	22.82 ± 0.02	26.90 ± 0.06	54.83 ± 0.12
Shenyang	14.88 ± 0.02	21.11 ± 0.08	40.55 ± 0.13

Table 2. Pair-wise correlations among total flavonoid content, DPPH and ABTS.

	DPPH	ABTS
Total flavonoid content	0.758*	0.788*
DPPH		0.821*

*Was significant at 0.01 probability level.

Table 3. Relative peak areas (RPAs) of 15 characteristic peaks in HPLC fingerprints of 12 samples of FPZs.

Samples	Peaks														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	3.921	1.579	1.500	1.303	1.789	41.961	3.711	2.711	0.224	1.000	0.553	1.921	2.474	5.513	0.645
2	2.421	0.871	1.374	0.807	2.158	30.474	4.965	3.760	0.193	1.000	0.327	0.620	0.854	3.222	0.304
3	5.307	1.973	4.347	3.000	5.520	66.107	8.347	3.640	0.107	1.000	0.360	1.293	1.400	4.253	0.387
4	4.155	0.613	0.735	0.826	1.555	32.981	3.432	2.594	0.226	1.000	0.303	0.858	1.052	2.574	0.226
5	2.514	1.140	0.444	0.720	0.252	29.591	0.633	0.962	0.227	1.000	0.066	0.266	0.451	1.066	0.014
6	4.965	1.451	0.876	1.496	0.779	43.496	2.257	1.646	0.381	1.000	0.221	0.531	0.938	2.885	0.257
7	4.289	1.505	1.011	0.847	1.242	39.211	3.326	1.868	0.332	1.000	0.295	0.679	0.868	2.337	0.189
8	6.397	0.446	0.306	0.066	0.653	2.562	1.289	4.041	0.281	1.000	0.322	1.149	1.397	3.066	0.264
9	3.249	0.633	1.096	0.847	2.667	31.701	3.808	2.322	0.175	1.000	0.424	1.153	1.345	3.520	0.333
10	6.510	1.657	2.294	1.618	1.931	59.206	11.049	5.814	0.324	1.000	0.324	1.431	1.667	4.235	0.343
11	0.267	1.168	0.168	1.534	------	69.351	0.603	0.588	0.672	1.000	0.214	0.550	1.015	3.115	0.252
12	5.491	1.613	0.943	1.406	0.425	51.981	1.698	2.807	0.245	1.000	0.052	0.297	0.425	0.679	0.042
Mean	4.124	1.221	1.258	1.206	1.581	41.552	3.760	2.729	0.282	1.000	0.288	0.896	1.157	3.039	0.271
RSD (%)	181.00	48.79	113.02	72.15	148.99	1849.35	313.75	144.42	14.36	0.00	13.91	50.08	55.88	133.11	16.27

Table 4. Relative retention times (RRTs) of 15 characteristic peaks in HPLC fingerprints of 12 samples of FPZs.

Samples	Peaks														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.394	0.424	0.452	0.470	0.487	0.515	0.585	0.785	0.843	1.000	1.488	1.573	1.592	1.616	1.653
2	0.396	0.425	0.453	0.472	0.488	0.515	0.586	0.786	0.843	1.000	1.489	1.574	1.596	1.616	1.654
3	0.394	0.424	0.453	0.471	0.487	0.514	0.586	0.785	0.843	1.000	1.487	1.572	1.593	1.615	1.652
4	0.394	0.423	0.453	0.471	0.488	0.514	0.586	0.786	0.843	1.000	1.489	1.574	1.596	1.618	1.654
5	0.393	0.421	0.452	0.472	0.489	0.513	0.570	0.786	0.843	1.000	1.481	1.565	1.586	1.607	1.644
6	0.396	0.424	0.454	0.472	0.489	0.515	0.587	0.786	0.844	1.000	1.486	1.570	1.591	1.612	1.650
7	0.394	0.423	0.453	0.472	0.488	0.513	0.585	0.786	0.844	1.000	1.488	1.572	1.594	1.615	1.652
8	0.394	0.423	0.452	0.459	0.470	0.487	0.567	0.785	0.843	1.000	1.492	1.576	1.597	1.619	1.656
9	0.394	0.424	0.452	0.470	0.487	0.513	0.586	0.785	0.844	1.000	1.488	1.573	1.594	1.616	1.653
10	0.396	0.425	0.454	0.471	0.488	0.514	0.592	0.785	0.843	1.000	1.491	1.576	1.598	1.619	1.657
11	0.395	0.423	0.453	0.471	------	0.513	0.598	0.785	0.843	1.000	1.487	1.572	1.593	1.615	1.652
12	0.395	0.423	0.452	0.470	0.487	0.510	0.584	0.785	0.843	1.000	1.492	1.577	1.598	1.619	1.657
Mean	0.395	0.424	0.453	0.470	0.446	0.511	0.584	0.785	0.843	1.000	1.488	1.573	1.594	1.616	1.653
RSD (%)	0.10	0.11	0.08	0.36	0.14	0.78	0.84	0.05	0.05	0.00	0.30	0.32	0.34	0.34	0.35

[15].

Due to similarity in appearance, it is impossible to divide these samples from the physical appearances. According to the RRTs and RPAs of 15 characteristic peaks, hierarchical clustering analysis (HCA) was perforemed by SPSS software (SPSS for windows 19.0, SPSS Inc., USA) and the results were shown in **Figure 3**. It is clear that the samples could be divided into three clusters: with samples 1, 2, 4, 5, 6, 7 and 9 in cluster one, samples 3, 10, 11 and 12 in cluster two and sample 8 in cluster three. The rescaled distance between group three and group one, group two are 25, while the rescaled distance between group one and group two are 10, which could demonstrate that the quality of group one and group two are more similar than with group three.

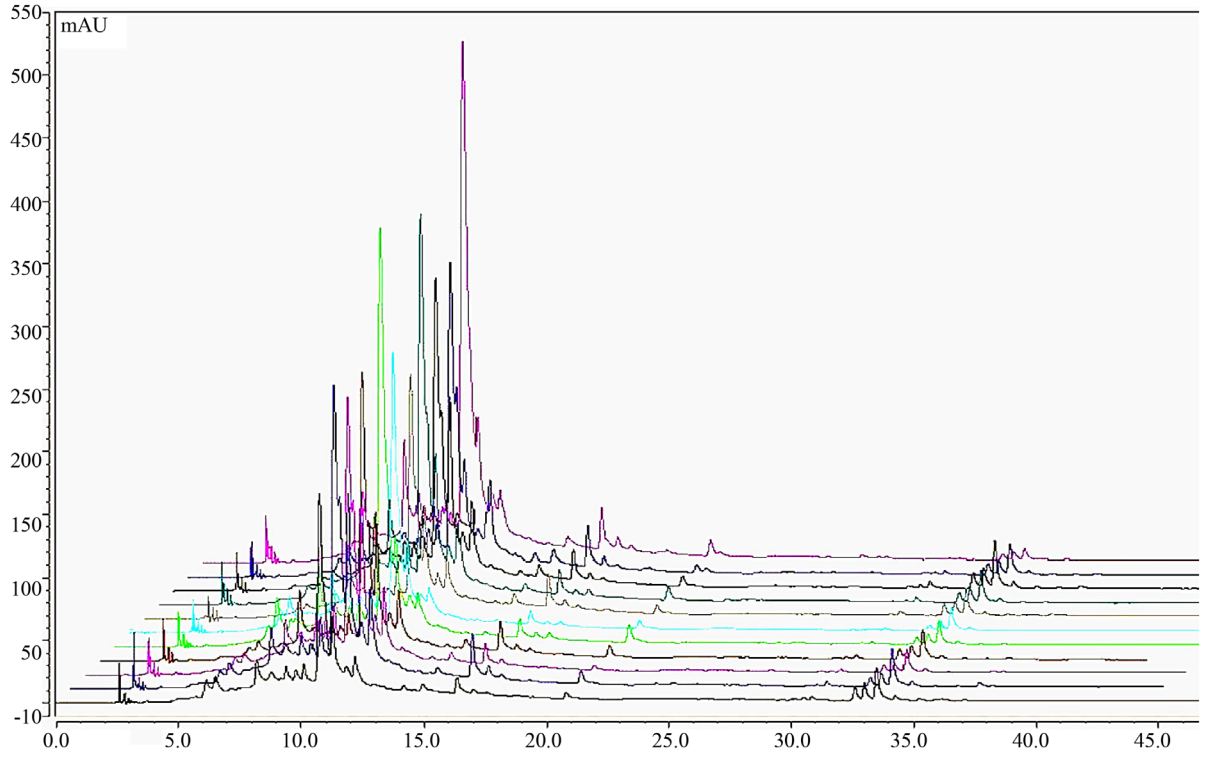

Figure 1. Overlaid HPLC chromatograms of extracts of Samples. From down to up: Hangzhou, Jinhua, Zhengzhou, Nanjing, Guangzhou, Guilin, Nanning, Chengdu, Fuzhou, Zhangzhou, Changchun and Shenyang.

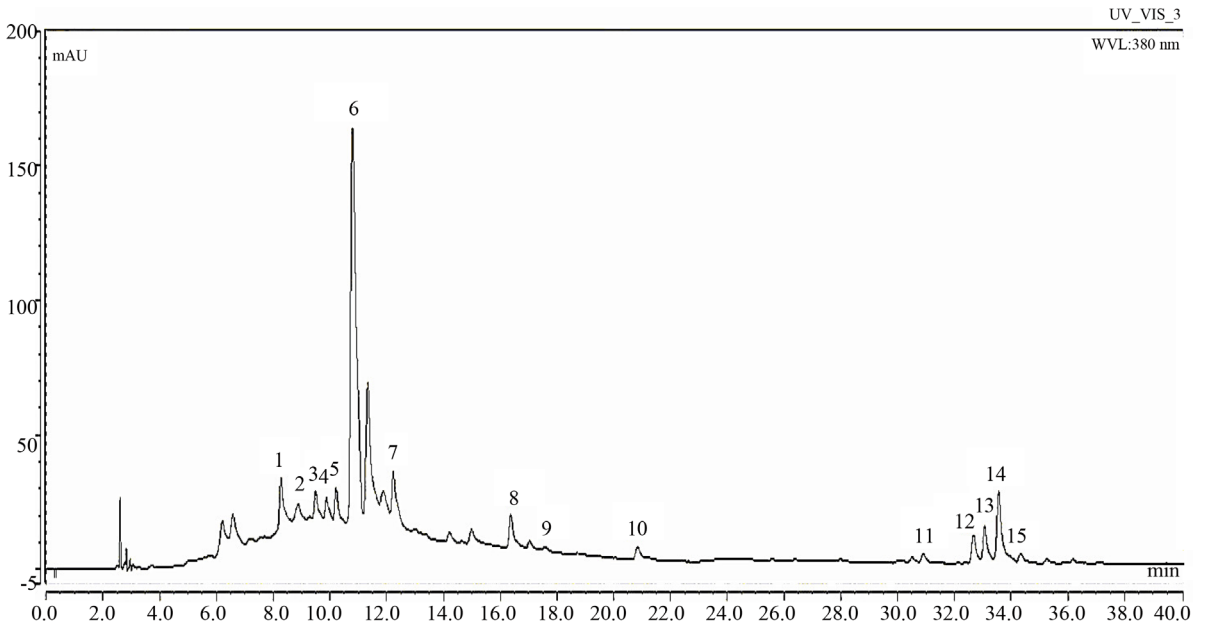

Figure 2. 15 characteristic peaks of fingerprinting. 1, 2, 3, 4, 7, 8, 11, 12, 13, 14, 15: unknown compounds, 5: rutin, 6: hyperin, 9: quercetin and 10: kaempferol.

3.3. Quantitative Analysis of Four Active Compounds in *R. chingii* Hu.

Flavonols such as rutin, hyperin, quercetin [16] and kaempferol [17] possess antioxidant activity and have been suggested to play an important role in the protective effects of berries and vegetables [18]. In the chromato-

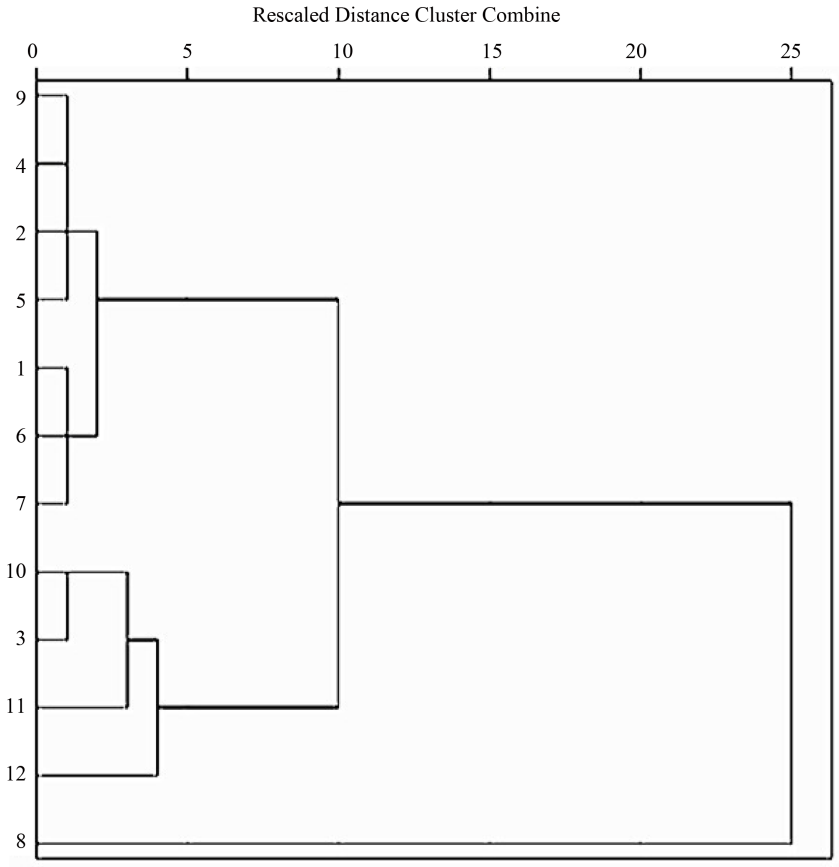

Figure 3. Dendrogram of clustering analysis. 1: Hongzhou; 2: Jinhua; 3: Zhengzhou; 4: Nanjing; 5: Guangzhou; 6: Guilin; 7: Nanning; 8: Chengdu; 9: Fuzhou; 10: Zhangzhou; 11: Changchun and 12: Shenyang.

graphic profile, four peaks (5, 6, 9 and 10) were structurally identified as rutin, hyperin, quercetin and kaempferol, respectively, by comparing their retention times (10.12 min for rutin, 10.80 for hyperin, 17.49 min for quercetin and 20.75 min for kaempferol) with those of standard substance. Therefore, the simultaneous determination of four active components in these samples was investigated by the proposed method.

3.3.1. Calibration Curves, LOD and LLOQ

Calibration curves were constructed by plotting the peak area (y) against the corresponding concentration of the standard solutions (x). The injection concentration, which could be detected at the signal-to-noise ratio of 3 (S/N = 3), was considered to be the limit of detection (LOD). The lower limit of quantification (LLOQ) was the injection concentration corresponding to the peak heights with signal-to-noise ratio of 10 (S/N = 10). The detailed descriptions of the regression curves were presented in **Table 5**.

3.3.2. Precision, Repeatability, Stability and Accuracy

Intra- and inter-day precision and accuracy tests were performed by analyzing standard solutions (rutin, 18.75 $\mu g \cdot mL^{-1}$; hyperin, 12.50 $\mu g \cdot mL^{-1}$; quercetin, 12.50 $\mu g \cdot mL^{-1}$; kaempferol, 12.50 $\mu g \cdot mL^{-1}$) six times a day and once a day for six sequential days, respectively. The injection repeatability was determined by the injection of continuous six times using the same sample, while the analysis repeatability was examined by the injection of six different samples, which were prepared with the same sample preparation procedure. The precision and repeatability of the solution at medium concentration were shown in **Table 6**. For the stability testing, the same real sample was analyzed within 24 h at the room temperature. The stabilities of the solution shown in RSD of retention time and peak area were all within ±3% and no significant difference was observed, indicating that the solution was stable (**Table 6**).

Accuracy was defined as the rate of the calculated value by the standard curve to that of its true value, expressed as recovery rate (%). The mean recoveries and RSD for analytes at different concentrations were shown in **Table 6**.

3.3.3. Determination of Four Active Compounds in Raspberry

Under the same chromatographic conditions, four active components in raspberries were determined by the proposed method. The results were summarized in **Table 7**. These results indicated that the concentrations of analytes existed obvious difference between different samples, and the contents of these components could not be used as a standard of quality control, however, assessing the quality of *R. chingii* Hu. using these four active components alone is recommented to a certain extent.

Table 5. Calibration curves, LOD and LLOQ of four active components (n = 3).

Analytes	Calibration curves	R^2	Linear range (μg·mL^{-1})	LOD (μg·mL^{-1})	LLOQ (μg·mL^{-1})
rutin	$y = 0.4177x - 0.3565$	0.9996	1.17 - 250.00	0.22	0.73
hyperin	$y = 0.5932x - 0.2930$	0.9992	0.39 - 125.00	0.07	0.24
quercetin	$y = 0.8238x - 0.3514$	0.9995	0.20 - 125.00	0.04	0.12
kaempferol	$y = 0.6348x - 0.2658$	0.9993	0.20 - 125.00	0.04	0.12

Table 6. Precision, repeatability and stability of the method (n = 6).

Analytes	Concentration (μg·mL^{-1})	Precision		Repeatability RSD (%)	Stability RSD (%)
		Intra-day RSD (%)	Inter-day RSD (%)		
Rutin	18.75	1.40	1.69	1.48	1.61
Hyperin	12.50	0.43	0.32	0.92	1.01
Quercetin	12.50	1.05	0.00	2.88	2.70
Kaempferol	12.50	2.71	0.51	2.44	2.92

Table 7. Simultaneous determinations of four active components in *R. chingii* Hu. by the proposed method (n = 6).

Samples	Compounds (μg·g^{-1})			
	Rutin	Hyperin	Quercetin	Kaempferol
Hangzhou	13.96 ± 0.28	181.29 ± 0.42	2.12 ± 0.02	4.00 ± 0.06
Jinhua	8.72 ± 0.12	478.57 ± 1.19	4.04 ± 0.06	11.69 ± 0.15
Zhengzhou	32.66 ± 0.45	300.03 ± 7.25	5.64 ± 0.09	7.80 ± 0.15
Nanjing	9.92 ± 0.17	278.60 ± 4.41	3.12 ± 0.04	6.14 ± 0.08
Guangzhou	18.30 ± 0.39	337.28 ± 4.78	2.73 ± 0.05	5.46 ± 0.08
Guilin	ND	508.88 ± 3.02	4.97 ± 0.10	5.68 ± 0.11
Nanning	9.92 ± 0.18	624.16 ± 3.00	3.57 ± 0.04	10.22 ± 0.09
Chengdu	40.30 ± 0.30	317.22 ± 2.79	2.70 ± 0.05	6.56 ± 0.18
Fuzhou	21.39 ± 0.44	418.93 ± 1.27	4.02 ± 0.06	8.75 ± 0.09
Zhangzhou	27.02 ± 0.65	255.34 ± 1.11	2.84 ± 0.04	5.77 ± 0.11
Changchun	36.28 ± 0.69	280.19 ± 2.28	1.77 ± 0.08	3.78 ± 0.03
Shenyang	22.40 ± 0.29	289.50 ± 0.99	2.82 ± 0.06	7.21 ± 0.12

ND, not detected.

4. Conclusion

In this study, the total flavonoid contents and the antioxidant activities of 12 batches of raspberries (*R. chingii* Hu.) collected from different regions of China were investigated in detail. According to their antioxidant activities, a simple and valid chromatographic fingerprint method was developed for analysis of the sample extracts of Fupenzi by HPLC-DAD. The proposed method and 15 characteristic peaks can be used for the rapid identification and evaluation of FPZs and their differentiation from substitutes conveniently.

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