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Short communication

Quality evaluation of *Evodia rutaecarpa* (Juss.) Benth by high performance liquid chromatography with photodiode-array detection

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ABSTRACT

A simple, sensitive and accurate HPLC-DAD method was developed for simultaneous determination of wuchuyuamide-I, quercetin, limonin, evodiamine and rutaecarpine in *Evodia rutaecarpa* that has been widely used as one of the traditional Chinese medicines (TCMs). Chromatographic separations were performed on a reverse-phase C_{18} column with the gradient elution of acetonitrile–water and the simultaneous detection at five wavelengths. Good linear behaviors over the investigated concentration ranges were observed with the values of *r* higher than 0.999 for all the analytes. The recoveries measured at three levels varied from 98.77 to 102.36%. The validated method was successfully applied for the simultaneous determination of the five chemical constituents in 36 batches of samples collected from different regions or time that were investigated and authenticated as *E. rutaecarpa* (Juss.) Benth. Hierarchical clustering analysis (HCA) and principal components analysis (PCA) were performed to differentiate and classify the samples based on the contents of the five characteristic constituents. The total contents of evodiamine and rutaecarpine in different samples were calculated and the blending method proposed was demonstrated to be very useful in saving resources and in guiding rational herb use.

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1. Introduction

The dried, unripe fruit of *Evodia rutaecarpa* (Juss.) Benth., *E. rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang or *E. rutaecarpa* (Juss.) Benth. var. *bodinieri* (Dode) Huang called Wuzhuyu in Chinese has been used as one of the traditional Chinese medicines (TCMs) for more than 2000 years and is officially listed in the Chinese Pharmacopoeia [1]. The active chemical constituents of Wuzhuyu were divided into fractions of alkaloids, essential oils, limonoids, carboxylic acids and flavonoids [2–6]. Anti-inflammatory [7,8], anti-nociceptive [9,10], anthelmintic [11,12], antidiarrheal [13], anti-anoxic [14,15] and antibacterial effects [16] of the extracts of Wuchuyu have been reported.

Alkaloids are the major active compounds in Wuzhuyu. Pharmacological and clinical studies indicated that evodiamine and rutaecarpine were bioactive compounds with reported anti-polysarcous [17], cardiotonic [18–20], central stimulative [21], vasodilatory [22,23], antithrombotic and bronchoconstrictive activities [24]. Recent studies have shown that rutaecarpine could modulate drug metabolizing enzymes [25–28], inhibit platelet aggregation [29], relax internal anal sphincter [30] and prevent ultraviolet A-induced reactive oxygen species generation [31]. Several studies demonstrated that evodiamine had anticancer activity and induction of apoptosis in several types of cancer cells [32–36]. Furthermore, nine quinolone alkaloids with the inhibitory activity against nuclear factor of activated T cells have been reported [37]. Quercetin, which is a widely distributed flavanoid, was demonstrated to have strong anti-active oxygen effect [38], protective effect on cardiomyocyte injured by hydrogen peroxide [39], inhibitory effect on transplantation tumor of breast carcinoma cell line MCF-7 in nude mice [40] and vasodilatation effect in the isolated rat thoracic aorta [41]. It was reported that limonin had the capability of inhibiting SMMC-7721 cells proliferation [42].

It is well known that a few markers or pharmacologically active compounds are generally used as standards in the quality control of TCM. Thin layer chromatography (TLC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE), liquid chromatography/mass spectrometry (LC/MS) and ion-pair high performance liquid chromatography have been applied for the determination of indoloquinazoline and quinolone alkaloids in *E. rutaecarpa* [43–50]. Gas chromatography/mass spectrometry (GC/MS) has been used to detect the volatile compounds in *Evodia* species [51]. Headspace solid-phase microextraction (HS-SPME)

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combined with GC/MS was also optimized and applied to detect the volatile organic compounds in *E. rutaecarpa* species fruit [52].

In general, herbs collected from different regions or at different time are discrepant in the types and quantities of chemical constituents, which influence their therapeutic effects. Thirty-six batches of *E. rutaecarpa* were collected and the contents of the five markers were simultaneously determined in our study. The HPLC method developed could be responsible for the quality control of *E. rutaecarpa*. Hierarchical clustering analysis (HCA) and principal components analysis (PCA) were performed to evaluate and classify the samples according to the contents of the five chemical constituents. The total contents of evodiamine and rutaecarpine in different samples were analyzed to provide information for using *E. rutaecarpa* reasonably.

2. Experimental

2.1. Chemicals and reagents

Wuchuyuamide-I, quercetin, limonin, evodiamine and rutaecarpine standards were isolated and identified in our laboratory whose structures (shown in Fig. 1) were confirmed on the basis of spectroscopic analysis (^{1}H NMR, ^{13}C NMR, ESI-MS, UV). The purities were 98.73, 99.05, 99.24, 99.35 and 99.06% respectively by normalization of the peak areas. HPLC-grade acetonitrile was purchased from TEDIA Company, Inc. (Product of Tedia, USA). Ultrapure water was prepared with the Sartorius Arium611UF water purification system (18.2 MΩ, Sartorius, Germany). Other reagent solutions were of analytical grade.

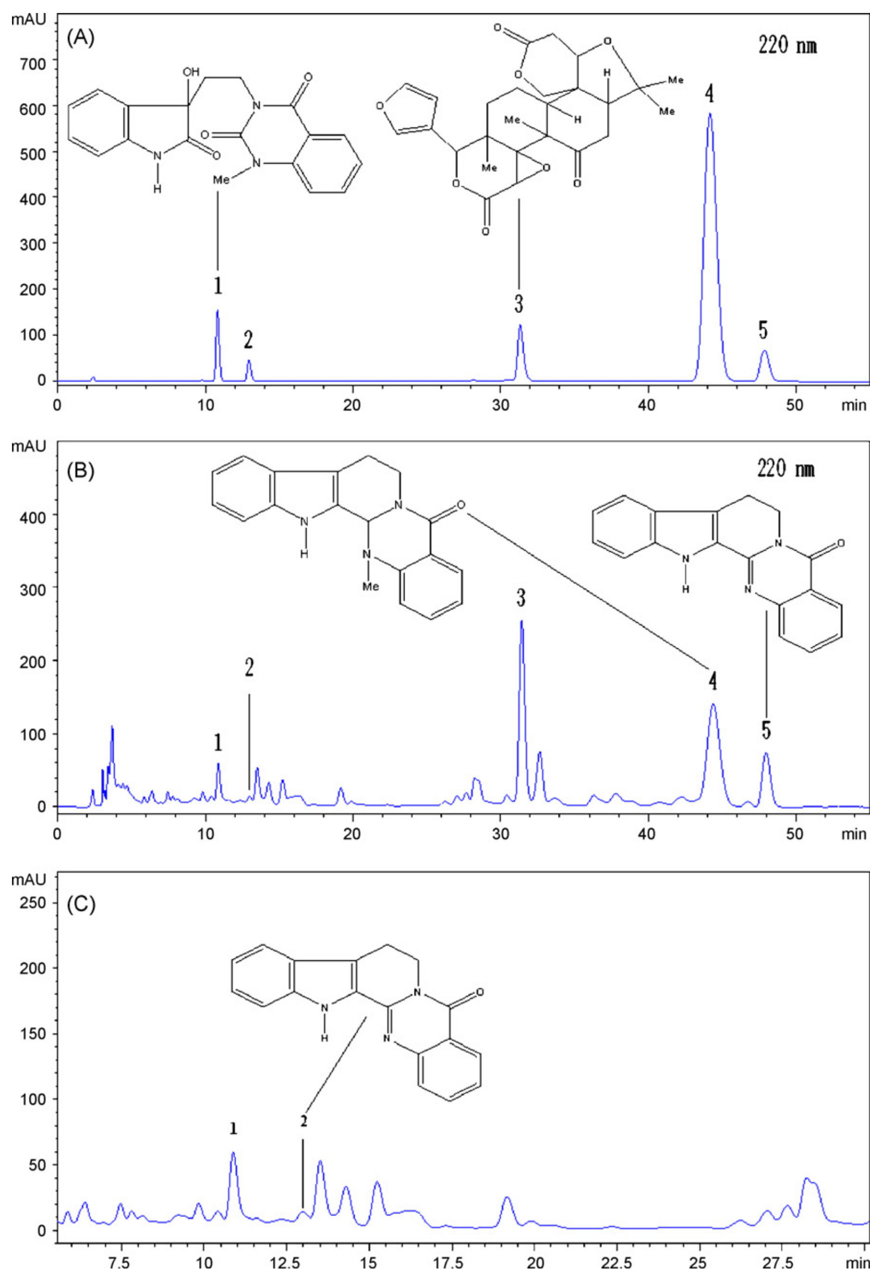

Fig. 1. Representative HPLC chromatograms of mixed standards and the extract of *E. rutaecarpa* at 220 nm. (A) Mixed standards of the five chemical constituents; (B) extract of *E. rutaecarpa* (sample no. 36); (C) enlarged chromatogram of sample solution from 6 to 30 min. Peaks: (1) wuchuyuamide-I; (2) quercetin; (3) limonin; (4) evodiamine; (5) rutaecarpine.

Table 1
Collected information and contents of the five markers of the samples ($n = 3$)

Sample no.	Sources	Acquisition time	Contents (%) ± S.D.				
			Wuchuyuamide-I	Quercetin	Limonin	Evodiamine	Rutaecarpine
1	Guangxi	September 2007	0.0167 ± 0.00021	0.0087 ± 0.00035	0.8682 ± 0.00221	0.0407 ± 0.00035	0.0589 ± 0.00022
2	Zhonghua culativation base, zhangshu, Jiangxi	September 2007	0.0259 ± 0.00031	0.0074 ± 0.00038	1.2157 ± 0.00342	0.0366 ± 0.00025	0.0827 ± 0.00033
3	Linxiang, Hunan	September 2007	0.0438 ± 0.00028	0.0223 ± 0.00049	0.3892 ± 0.00048	0.7957 ± 0.00065	0.0346 ± 0.00035
4	Shiqian, Tongren, Guizhou	October 2007	0.0563 ± 0.00022	0.0271 ± 0.00029	0.3814 ± 0.00098	0.5680 ± 0.00033	0.4958 ± 0.00045
5	Guanling, Tongren, Guizhou	September 2007	0.0392 ± 0.00036	0.0211 ± 0.00034	0.6541 ± 0.00046	0.4646 ± 0.00046	0.3948 ± 0.00261
6	Xinwo, Panan, Zhejiang	September 2007	0.0730 ± 0.00029	0.0058 ± 0.00031	0.9684 ± 0.00127	0.0767 ± 0.00038	0.0918 ± 0.00046
7	Xingyi, Guizhou	September 2007	0.0453 ± 0.00049	0.0825 ± 0.00044	0.9812 ± 0.00068	0.7041 ± 0.00046	0.6236 ± 0.00311
8	Nanchang, Jiangxi	September 2007	0.0251 ± 0.00053	0.0045 ± 0.00056	1.6383 ± 0.00276	0.0695 ± 0.00126	0.1279 ± 0.00043
9	Zhejiang	November 2007	0.0287 ± 0.00044	0.0134 ± 0.00071	0.8085 ± 0.00034	0.1935 ± 0.00036	0.1846 ± 0.00089
10	Jiangkou, Guizhou	September 2007	0.0388 ± 0.00021	0.0088 ± 0.00072	0.7215 ± 0.00265	0.0516 ± 0.00076	0.1276 ± 0.00083
11	Zhangshu, Jiangxi	September 2007	0.0059 ± 0.00048	0.0251 ± 0.00066	0.6148 ± 0.00371	0.0403 ± 0.00068	0.0409 ± 0.00021
12	Liuzhi, Guizhou	August 2007	0.0263 ± 0.00095	0.0124 ± 0.00089	0.6221 ± 0.00065	0.1212 ± 0.00082	0.1664 ± 0.00047
13	Shengjian, Guangzhou	September 2007	0.0179 ± 0.00039	0.0050 ± 0.00092	0.7511 ± 0.00042	0.0294 ± 0.00046	0.0765 ± 0.00083
14	Shiqian, Tongren, Guizhou	September 2007	0.0107 ± 0.00076	0.0053 ± 0.00068	0.4379 ± 0.00075	0.0475 ± 0.00061	0.0708 ± 0.00025
15	Hunan	November 2007	0.0127 ± 0.00054	0.0061 ± 0.00058	1.1930 ± 0.00154	0.0254 ± 0.00055	0.1480 ± 0.00421
16	Daozhen, Zunyi, Guizhou	September 2007	0.0289 ± 0.00091	0.0245 ± 0.00048	0.5956 ± 0.00066	0.1437 ± 0.00066	0.2085 ± 0.00123
17	Taizhou, Zhejiang	September 2007	0.0429 ± 0.00077	0.0053 ± 0.00039	0.9688 ± 0.00189	0.0675 ± 0.00092	0.0783 ± 0.00052
18	Kaiyang, Guizhou	September 2007	0.0187 ± 0.00086	0.0192 ± 0.00072	0.1842 ± 0.00025	0.1204 ± 0.00014	0.1604 ± 0.00035
19	Anhui	November 2007	0.0328 ± 0.00043	0.0687 ± 0.00064	0.8070 ± 0.00036	0.0567 ± 0.00035	0.5214 ± 0.00042
20	Haozhou, Anhui	September 2007	0.0311 ± 0.00091	0.0343 ± 0.00034	0.3959 ± 0.00056	0.3219 ± 0.00046	0.3091 ± 0.00036
21	Liuzhi, Guizhou	September 2007	0.0245 ± 0.00066	0.0591 ± 0.00039	0.7045 ± 0.00067	0.1480 ± 0.00021	0.1622 ± 0.00054
22	Shiquan, Shanxi	September 2007	0.0319 ± 0.00047	0.1210 ± 0.00046	0.5912 ± 0.00075	0.3854 ± 0.00054	0.3808 ± 0.00254
23	Xiangtan, Hunan	November 2007	0.0361 ± 0.00059	0.0076 ± 0.00072	0.5108 ± 0.00056	0.0053 ± 0.00062	0.0222 ± 0.00057
24	Jiangkou, Guizhou	September 2007	0.0174 ± 0.00098	0.0338 ± 0.00059	0.4591 ± 0.00036	0.3253 ± 0.00046	0.2736 ± 0.00042
25	Guangzhou	September 2007	0.0262 ± 0.00074	0.1412 ± 0.00041	0.3208 ± 0.00021	0.0285 ± 0.00057	0.0774 ± 0.00021
26	Haozhou, Anhui	October 2007	0.0363 ± 0.00038	0.2144 ± 0.00069	0.1186 ± 0.00026	0.4643 ± 0.00088	0.3367 ± 0.00215
27	Aba, Sichuan	September 2007	0.0215 ± 0.00067	0.1694 ± 0.00092	0.3433 ± 0.00046	0.0585 ± 0.00036	0.0951 ± 0.00031
28	Fenggang, Zunyi, Guizhou	November 2007	0.0401 ± 0.00077	0.0768 ± 0.00023	2.0507 ± 0.00167	0.1877 ± 0.00026	0.2557 ± 0.00124
29	Songtao, Guizhou	September 2007	0.0464 ± 0.00081	0.0905 ± 0.00014	2.3036 ± 0.00254	0.1682 ± 0.00051	0.2048 ± 0.00143
30	Xian, Shanxi	September 2007	0.0339 ± 0.00033	0.1771 ± 0.00046	1.3690 ± 0.00042	0.0161 ± 0.00011	0.0641 ± 0.00053
31	Yuping, Guizhou	September 2007	0.0148 ± 0.00045	0.0186 ± 0.00043	0.9007 ± 0.00024	0.0119 ± 0.00086	0.0269 ± 0.00026
32	Guizhou	September 2007	0.0207 ± 0.00068	0.0329 ± 0.00036	0.6971 ± 0.00045	0.0343 ± 0.00023	0.0803 ± 0.00026
33	Liuzhi, Guizhou	November 2007	0.0241 ± 0.00049	0.0708 ± 0.00032	0.3527 ± 0.00087	0.2114 ± 0.00024	0.2746 ± 0.00164
34	Jiangxi	September 2007	0.0244 ± 0.00037	0.0976 ± 0.00034	0.6402 ± 0.00074	0.1275 ± 0.00061	0.1989 ± 0.00041
35	Guangxi	October 2007	0.0226 ± 0.00087	0.0482 ± 0.00039	0.7014 ± 0.00042	0.0237 ± 0.00044	0.0816 ± 0.00022
36	Tongren, Guizhou	October 2007	0.0292 ± 0.00069	0.0349 ± 0.00038	1.0211 ± 0.00145	0.1720 ± 0.00072	0.1597 ± 0.00231

2.2. Plant materials

Thirty-six batches of samples were authenticated as *E. rutaecarpa* (Juss.) Benth (Table 1). Voucher specimens were stored at the Research Center for Quality Control of Natural Medicine, Guizhou Normal University. Then they were stored in sealed bottles before use in order to avoid moisture and chemical changes.

2.3. Standard solution preparation

The reference compounds were weighed accurately and dissolved in methanol in a 25-ml volumetric flask to make a stock solution (0.1089, 0.3168, 0.7623, 0.2178 and 0.2871 mg/ml). Working standard solutions were prepared from the stock solution by further dilution with the appropriate volume of methanol. These solutions were stored away from light at 4 °C.

2.4. Sample solution preparation

Pulverized sample (60 mesh, 1.0 g) was weighed accurately into a 150-ml round bottom flask and then extracted for three times at 70 °C (1 h each) under reflux with 30 ml chloroform and 2 ml concentrated ammonia solution. The extracts were combined and filtered through analytical filter paper. The filter liquor was evaporated at 70 °C to dryness. The dry extract was dissolved in 10 ml methanol and then filtrated through a 0.45-μm membrane filter for analysis.

2.5. Instrumentation and chromatographic conditions

An Agilent 1100 series HPLC instrument equipped with a quaternary pump, a diode-array detector, an autosampler, a column compartment and a ChemStation for LC 3D software was used. Chromatographic separations were carried out on a ZORBAX SB-C18 column (250 mm × 4.6 mm I.D., 5 μm) protected by a ZORBAX SB-C18 guard column (4.0 mm × 3.0 mm I.D., 5 μm). The mobile phase consisted of acetonitrile (A) and water (B). The gradient program was as follow: 0–5 min, linear gradient 25–29% A; 5–20 min, linear gradient 29–35% A; 20–25 min, linear gradient 35–42% A; 25–50 min, linear gradient 42% A. The flow rate program was as follow: 0–25 min, 1.0 ml/min; 25–40 min, 1.0–0.6 ml/min; 40–46 min, 0.6 ml/min; 46–46.01 min, 0.6–1.1 ml/min; 46.01–50 min, 1.1 ml/min. The volume injected was 10 μl. The column temperature was maintained

Table 2
Linear regression data, LOD and LOQ of investigated compounds

Analytes	Linear regression data[a]			LOD (ng)	LOQ (ng)
	Regressive equation	r	Linear range (μg)		
Wuchuyuamide-I	$Y = 4032.5X + 92.018$	0.9999	0.0544–4.356	0.039	0.121
Quercetin	$Y = 701.26X + 13.899$	0.9999	0.1593–12.74	1.187	3.710
Limonin	$Y = 723.53X + 139.740$	0.9999	0.3816–30.53	1.985	6.204
Evodiamine	$Y = 12794X - 1643.800$	0.9998	0.1089–8.712	0.077	0.256
Rutaecarpine	$Y = 3310.8X + 248.430$	0.9993	0.1436–11.48	0.792	2.399

All the analytes showed good linearity ($r > 0.999$) in the concentration ranges.
[a] In the linear regression data, Y refers to the peak area, X is the concentration, and r is the correlation coefficient of the equation.

Table 3
Precision, repeatability and stability of the HPLC method for determination of the five markers

Analytes	Precision								Repeatability		Stability	
	Intra-day ($n = 6$)				Inter-day ($n = 6$)				Mean (%)	R.S.D. (%)	Mean (%)	R.S.D. (%)
	Mean (%)[a]	R.S.D. (%)	Average peak area[b]	R.S.D. (%)	Mean (%)[a]	R.S.D. (%)	Average peak area[b]	R.S.D. (%)				
Wuchuyuamide-I	0.029	1.28	4406.3	1.06	0.031	2.67	4538.4	3.47	0.030	0.21	0.031	0.21
Quercetin	0.036	1.56	612.0	1.43	0.037	2.98	629.7	2.96	0.035	1.84	0.035	1.84
Limonin	1.048	1.45	5533.8	1.19	1.050	1.98	5573.6	1.06	1.049	2.26	1.048	2.25
Evodiamine	0.177	1.58	27113.0	1.57	0.178	2.06	27539.9	0.77	0.179	2.90	0.178	2.90
Rutaecarpine	0.162	0.73	9603.9	0.85	0.161	1.88	9803.7	1.57	0.161	2.35	0.162	2.35

R.S.D. (%) = (S.D./mean) × 100. Considering the results, the method was deemed to be accurate and reproducible. The analytes were found to be stable during the tested period.
[a] Tested by sample solution.
[b] Tested by standard mixture solution.

at 25 °C. Detection was made simultaneously at five different wavelengths, i.e., 220, 255, 215, 225 and 345 nm at the absorption maxima of the five markers.

3. Results and discussion

3.1. Optimization of extraction method

The efficiency of extraction procedure was evaluated by using different solvents, i.e., ethanol, methanol, ethylacetate, and chloroform. The best solvent was found to be chloroform, which enabled less interfering peaks and provided the highest values in the contents of the five markers.

A method involving four-factor-three-level orthogonal array design (OAD) including the components of volume of chloroform (30, 40, and 50 ml), times of reflux (once, twice, and three times) and duration of extraction (1, 2, and 3 h) was developed for the optimization of the extraction. The results demonstrated that the established extraction method was adequate and appropriate for the analysis.

3.2. Optimization of the chromatographic conditions

The optimization of the chromatographic conditions was performed by using the solution of sample no. 36. The investigated compounds were tested and compared by using different analytical

Table 4
Recovery of the five chemical constituents

Analytes	Sample	Concentration			Recovery (%)	Mean recovery (%) ± R.S.D.
		Original (mg)	Added (mg)	Found (mg)		
Wuchuyuamide-I	S1[a]	0.3081	0.2426	0.5503	99.83	100.71 ± 1.08
	S2[b]	0.3022	0.3003	0.6083	101.93	
	S3[c]	0.3029	0.3581	0.6623	100.38	
Quercetin	S1	0.3716	0.2950	0.6741	102.57	102.36 ± 1.10
	S2	0.3592	0.3644	0.7277	101.14	
	S3	0.3517	0.4338	0.8000	103.36	
Limonin	S1	10.8481	8.5306	19.4219	100.51	100.28 ± 0.51
	S2	10.6388	10.5378	21.2445	100.64	
	S3	10.6643	12.6725	23.2982	99.70	
Evodiamine	S1	1.8276	1.4892	3.3056	99.25	99.05 ± 0.54
	S2	1.7940	1.7870	3.5714	99.46	
	S3	1.7925	2.1593	3.9183	98.45	
Rutaecarpine	S1	1.7001	1.3514	3.0246	98.02	98.77 ± 1.64
	S2	1.6689	1.6776	3.3071	97.66	
	S3	1.6674	2.0038	3.6837	100.63	

Recovery (%) = ((found − original)/added) × 100. The results indicated that the developed method was reliable and accurate for the measurement of the five analytes.
[a] The samples added known amounts of standards at low level (80% of the known amounts).
[b] The samples added known amounts of standards at medium level (same as the known amounts).
[c] The samples added known amounts of standards at high level (120% of the known amounts).

columns (ZORBAX SB-C18 or ZORBAX RX-C18) with different compositions of mobile phases (acetonitrile–water or methanol–water) and different gradient elution programs. The results showed that ZORBAX SB-C18 column with gradient elution of acetonitrile–water could efficiently separate the investigated markers (Fig. 1). According to the UV spectra of the five markers recorded by DAD in the range from 200 to 400 nm, 220, 255, 215, 225 and 345 nm were selected for monitoring wuchuyuamide-I, quercetin, limonin, evodiamine and rutaecarpine, respectively.

3.3. Validation of the HPLC method

3.3.1. Calibration curves, LOD and LOQ

The stock solution containing the five markers was prepared and diluted to appropriate concentration ranges for the establishment of calibration curves. The calibration graphs were plotted after linear regression (Table 2) of the peak areas versus the corresponding concentrations.

LOD and LOQ were determined at signal-to-noise ratios (S/N) of about 3 and 10, respectively. The data were summarized in Table 2.

3.3.2. Precision, repeatability and stability

Precision was evaluated with both mixed standards solution and sample solution under the selected optimal conditions six times in 1 day for inter-day variation and twice a day on 3 consecutive days for intra-day variation. Repeatability was confirmed with six different working solutions prepared from sample no. 36 and one of them was injected into the apparatus every 2 h within 10 h to evaluate the stability of the solution. All the results were expressed as relative standard deviations (R.S.D.s) which were shown in Table 3.

3.3.3. Recovery

The recovery was performed by adding known amounts of the five standards at low (80% of the known amounts), medium (same as the known amounts) and high (120% of the known amounts) levels. The spiked samples were then extracted, processed, and quantified in accordance with the methods mentioned above. The results were shown in Table 4.

3.3.4. Robustness

Method robustness was tested on ZORBAX SB-C18 column (250 mm × 4.6 mm I.D., 5 μm) and Diamonsil C18 column (250 mm × 4.6 mm I.D., 5 μm). The same sample solution was separately analyzed and the contents of the five characteristic constituents were calculated. The mean contents of the five compounds were 0.0294, 0.0359, 1.0231, 0.1698 and 0.1579% for ZORBAX SB-C18 column and 0.0289, 0.0362, 1.0228, 0.1697 and 0.1576% for Diamonsil C18 column. No significant difference existed between the results from the two columns by *t*-test ($P > 0.05$), which indicated that the developed method was capable of producing results with acceptable performance.

3.4. Sample analysis

The established analytical method was then applied to determine the five markers in 36 batches of *E. rutaecarpa*. The contents were listed in Table 1.

3.5. Assessment of peak purity

Peak purity was assessed by Agilent ChemStation for LC 3D software. The purity factors of the five characteristic constituents were 992.517, 995.885, 997.140, 993.741 and 991.836, respectively.

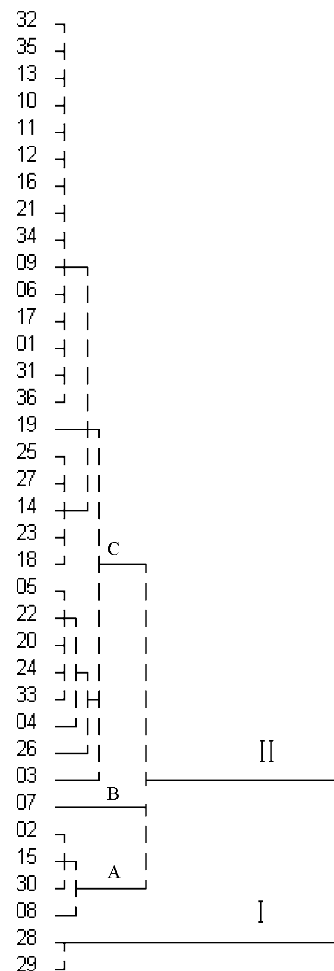

Fig. 2. Dendrogram of HCA for the 36 tested samples of *E. rutaecarpa*. The hierarchical clustering was done by SPSS software. Ward's method was applied, and Squared Euclidean distance was selected as a measurement. Thirty-six batches of *E. rutaecarpa* were divided into two main clusters. Sample nos. 28 and 29 were in cluster I and the other samples were in cluster II, which was divided into three subgroups again. Sample nos. 2, 8, 15 and 30 were in subgroup A, no. 7 was in subgroup B and the others were in subgroup C.

3.6. HCA of the samples

HCA is a statistical method for finding relatively homogeneous clusters of cases based on measured characteristics. It starts with each case in a separate cluster and then combines the clusters sequentially, reducing the number of clusters at each step until only one cluster is left. When there are N cases, this involves $N-1$ clustering steps or fusions. This hierarchical clustering process can be represented as a tree or dendrogram, where each step in the clustering process is illustrated by a joint of the tree [53–55]. The contents of the five chemical constituents were defined as five characteristics in the analysis so as to analyze, differentiate and classify the 36 samples.

Ward's method, which is a very efficient method for the analysis of variance between clusters, was applied, and Square Euclidean distance was selected as a measurement. A dendrogram was generated (Fig. 2), which revealed the relationships among the samples. Thirty-six tested samples of *E. rutaecarpa* were divided into two main clusters. Sample nos. 28 and 29 were in cluster I and the other samples were in cluster II, which was divided into three subgroups again. Sample nos. 2, 8, 15 and 30 were in subgroup A, no. 7 was in subgroup B and the others were in subgroup C. The result indicated

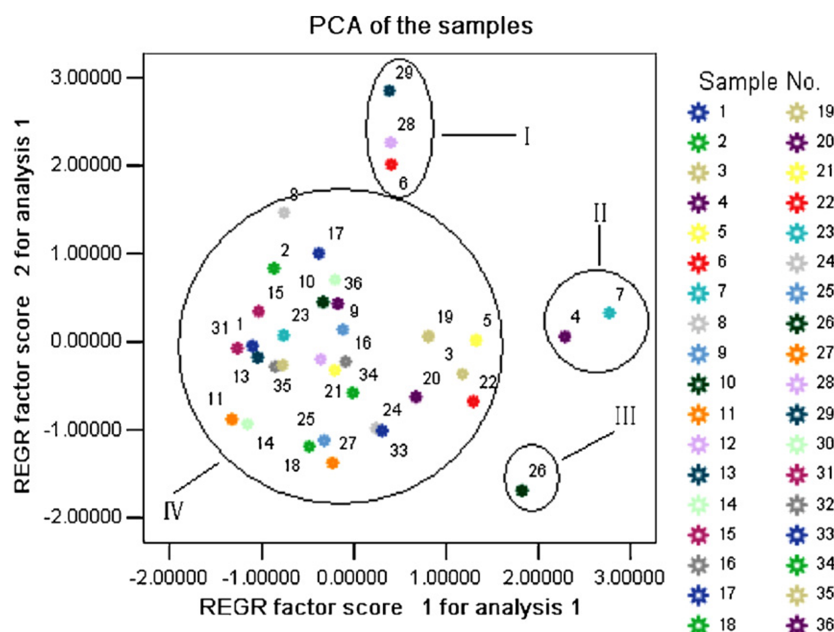

Fig. 3. The scatter plot obtained by PCA of the 36 batches of *E. rutaecarpa*. All the samples were clearly divided into four domains and each sample is represented as a marker. Sample nos. 6, 28 and 29 were in domain I, nos. 4 and 7 were in domain II, no. 26 was in domain III and the others were in domain IV.

that samples which had similar chemical profiles were divided into one group.

3.7. PCA of the samples

When a number of variables need to be analyzed, data decomposition should be carried out to reduce multidimensional data sets to lower dimensions. Among these techniques, PCA is a useful tool of chemometricians for data compression and information extraction which find combinations of variables or factors that describe major trends in a data set. Mathematically, PCA relies on an eigenvector decomposition of the covariance or correlation matrix of the process variables [56,57]. The contents of the five markers were analyzed as five variables, which were then translated mathematically into two main comprehensive factors to analyze the samples.

The 36 samples were further analyzed and classified by PCA. The scatter plot was shown in Fig. 3, where each sample was represented as a marker. It was noticeable that the samples were clearly clustered into four domains. Sample nos. 6, 28 and 29 were in domain I, no. 4 and no. 7 were in domain II, no. 26 was in domain III and the others were in domain IV. The result was accordant with the one obtained from HCA in general.

3.8. Analysis of the total contents of evodiamine and rutaecarpine in the samples

It is defined in Chinese Pharmacopoeia [1] that the total content of evodiamine and rutaecarpine in *E. rutaecarpa* should not be less than 0.15%, or it would not be used as the raw material and is regarded as substandard herb. Based on this definition, sample nos. 1, 2, 11, 13, 14, 17, 23, 25, 30–32 and 35 should not be put into production, which causes serious waste of the herbs. So, we recommend strongly that authentic and substandard ones be blended in accordance with appropriate proportion to meet the requirement of Chinese Pharmacopoeia. For example, the total contents of evodiamine and rutaecarpine in sample no. 3 and no. 1 are 0.8303 and 0.0996%, respectively. They can be blended in accordance with the ratio of 1:13 to meet the requirement. This approach is very conducive to save resources and to guide rational herb use.

Wuzhuyu plays an important role in pharmacological activity and application in TCM. Its quality control may be difficult due to many factors such as harvest season, climate or geography (soil or minerals) which may influence the composition of the chemical constituents. Several LC methods have been developed for the determination of evodiamine and rutaecarpine [58–60], but the traditional theory emphasizes the importance of multi-compound, multi-ingredient as being responsible for the activity of the herbal drug, in contrast to modern pharmacology and drug development that often focus on a single chemical entity. Therefore, determination of the two ingredients is not enough and it is absolutely necessary to determine as many active ingredients as possible in order to better evaluate the quality of TCM. Although no pharmacological effect of wuchuyuamide-I has been reported so far, it is possible that the compound may play a vital role in comprehensive effect of *E. rutaecarpa*. The determination of wuchuyuamide-I may provide additional information for the overall quality control. Kano et al. [61] developed an LC method for determination of hydroxyevodiamine, evodiamine and rutaecarpine, but the method needs quite a long analysis time of 130 min. Zhang et al. [45] developed an LC–MS determination for evodiamide, evodiamine, hydroxyvodiamine, rutaecarpine and goshuyuamide-I. Zhou et al. [48] developed an LC–MS method for simultaneous determination of dehydroevodiamine, 14-formyldihydrorutaecarpine, evodiamine, rutaecarpine and goshuyuamide. Although the two methods were highly sensitive and selective, their popularities were limited because of the high cost of instrumentation. Zhao et al. [49] developed an LC method for the determination of dehydroevodiamine, wuchuyuamide-I, 5-hydroxyrutaecarpine, 14-formyldihydrorutaecarpine, evodiamine and rutaecarpine, but it took a long analysis time of 70 min and complicated mobile phase consisted of methanol, acetonitrile and phosphoric acid–triethylamine–buffer solution was used.

4. Conclusion

In the present study, the alkaloids of wuchuyuamide-I, evodiamine and rutaecarpine, the flavonoid of quercetin and the

limonoid of limonin were simultaneously determined in *E. rutaecarpa* by the developed HPLC-DAD method. It is the first time that these five chemical constituents were analyzed simultaneously with acceptable performance of linearity, precision, repeatability, accuracy and robustness in an analysis time of 50 min. Furthermore, the optimized method was successfully applied to analyze 36 batches of *E. rutaecarpa*. HCA and PCA were utilized to differentiate and classify the 36 samples based on the contents of the five chemical constituents. Analysis of the total contents of evodiamine and rutaecarpine in different samples were also performed and the blending method was demonstrated to be able to save resources and to guide rational herb use, which may play important role in herbal and medicinal production.

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