· Research Paper ·

Simultaneous Analysis of Thirteen Bioactive Components in *Evodia rutaecarpa* and Its Varieties by HPLC-DAD-MS

XU Hai-yu[1,3], ZHANG Tie-jun[2], XIAO Xue-feng[1], ZHAO Ping[1], LIU Chang-xiao[3*], XU Jun[2]

1. Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China
2. Tianjin Engineering Laboratory of Quality Control Techniques for TCM, Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China
3. The National Laboratory of Pharmacodynamics and Pharmacokinetics, Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China

Abstract: **Objective** To control the quality of *Evodia rutaecarpa* better. **Methods** An HPLC-DAD-MS/MS method was established for the rapid and efficient identification of bioactive constituents and for simultaneous quantitative analysis of four bioactive ingredients including evodiamine, rutaecarpine, dehydroevodiamine, and evodin in *E. rutaecarpa*, which was applied to evaluating eight samples of *E. rutaecarpa* and its varieties from different areas. **Results** Thirteen potentially bioactive constituents including one flavonoid glycoside, one limonin, four indoloquinazoline alkaloids, and seven quinolone alkaloids were identified in all samples and the contents of dehydroevodiamine, evodine, evodiamine, and rutaecarpine varied widely from 0.10% to 0.51%, 0.49% to 3.12%, 0.07% to 1.56%, and 0.10% to 0.69%, respectively. **Conclusion** This method is found to be convenient, fast, accurate, and it is facilitated to improve the quality control standard of *E. rutaecarpa* and related products.

Key words: dehydroevodiamine; *Evodia rutaecarpa*; evodiamine; evodin; HPLC-DAD-MS; rutaecarpine
DOI: 10.3969/j.issn.1674-6384.2010.02.003

Introduction

Evodia rutaecarpa (Juss.) Benth. called Wuzhuyu in Chinese is a commonly used traditional Chinese medicinal herb. This herb has long been used for the treatment of headache, abdominal pain, migraine, chill limbs, diarrhea, nausea, hyperbaropathy, dysmenorrheal, and postpartum hemorrhage (Tang and Eisenbrand, 1992). Recently phytochemical and pharmacological studies indicated that limonins, indoloquinazoline, and quinolone alkaloids were major biologically active constituents (Chen, Chiou, and Chou, 2002; Wang and Liang, 2004). They have the characteristic of multi-constituents and multi-targets which bring synergistic actions together responsible for the therapeutic effect. Indoloquinazoline alkaloids, such as evodiamine, rutaecarpine, and dehydroevodiamine, have extensive pharmacologic actions such as anti-inflammatory (Woo *et al*, 2001), analgesic activity (Matsuda *et al*, 1997), protective effect on gastric mucosa (Lin *et al*, 1999), and anticancer (Zhang *et al*, 2004). Quinoline alkaloids also have been reported to have antibacterial activity against *Helicobacter pylori* (Hamasaki *et al*, 2000) and inhibition of angiotension Ⅱ receptor (Lee *et al*, 1998). And limonins possess anti-inflammatory, analgesic (Matsuda *et al*, 1998), anticancer (Poulose, Harris, and Patil, 2006), and antimalarial activities (Roy and Saraf, 2006).

Currently, the quality of *E. rutaecarpa* is mainly evaluated by two strategies. One strategy is to determine single or a few mark compounds by thin-layer chromatography (TCL) (Zhu, 2003; Liu, Luo, and Feng, 1999), high-performance liquid chromatography (HPLC) (Bao *et al*, 2007), high performance capillary electrophoresis (HPCE)

* Corresponding author: Liu CX Address: The National Laboratory of Pharmacodynamics and Pharmacokinetics, Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China Tel/Fax: +86-22-2300 6863 E-mail: liuchangxiao@163.com
Received: April 2, 2010; Revised: April 30, 2010; Accepted: May 13, 2010

(Li, Hon, and Wang, 2000). However, the contents of single or a few mark compounds can not accurately reflect the quality of Chinese materia medica (CMM) due to multiple constituents involved in the therapeutic effect. The other strategy is based on the chromatographic fingerprinting technology. For example, Zhou *et al* (2008) established the chromatographic fingerprint of water soluble components of *E. rutaecarpa* using HPLC. This strategy has been gradually applied for the quality control standards of more and more CMM and related products in China. Nevertheless, this strategy is a "blind analysis", lacking the chemical information about the ingredients, so it could not reflect the pharmaceutical activity of CMM and related products.

To solve the aforementioned problem, it is necessary for qualitative and quantitative analysis of bioactive components as many as possible to evaluate the quality of *E. rutaecarpa* reasonably. HPLC coupled with ultraviolet (UV) diode array detection (DAD) and electrospray ionization mass spectrometry (ESI-MS) which is a simple, rapid, and accurate method for separation and could offer molecular mass information of the constituents, has become one of effective and important methods of quality control in TCM. The aim of this study was to develop an HPLC–DAD–MS/MS method that is capable of separating, identifying, and characterizing 13 compounds and quantitative analysis of four bioactive ingredients in one chromatographic run and its application to the constituents in commercial products of *E. rutaecarpa*.

Material and methods

Instrumentation and chromatographic condition

HPLC-DAD-MS/MS analyses were carried out using a Finnegan HPLC instrument (Finnegan mat, San jose, CA) consisting of an autosampler (Thermo Finnegan, San Jose, USA), a Finnegan Surveyor with DAD detector (Thermo Finnegan, San Jose, USA) and a Finnegan Surveyor MSQ (Thermo Finnegan, San Jose, USA), equipped with a Z-spray electrospray ionization (ESI) source operating in both negative and positive mode. Xcalibur software (version 3.1, Thermo Finnegan, San Jose, USA) was used to control the instruments, and for data acquisition and processing.

Sample solutions were separated on an Accurasil C_{18} column (250 mm × 4.6 mm, 5μm, Serial no: 065676A, Ameritech Limited Company, USA) at room temperature. A linear gradient elution of A (CH_3CN-H_2O-HCOOH, 80∶20∶0.13) and B (H_2O-CH_3COOH, 100∶0.2) was used. A gradient programmer was used according to the following profile: 0–30 min, 25%–60% A; 30–60 min linear increase to 100% A; 60–120 min, maintaining the constant ratio of 100% A. The solvent flow rate was 0.4 mL/min and 20 μL of sample solution was injected in each run. The effluent was introduced into a DAD detector (scanning range 200–600 nm) and subsequently into a Z-spray ESI source (Key operating parameters included a spray voltage of 4.5 kV, a sheath gas flow of 35 arbs, an auxiliary gas flow of 6 arbs, a capillary temperature of 300 ℃ in positive mode or a spray voltage of 4.5 kV, a sheath gas flow of 35 arbs, an auxiliary gas flow of 10 arbs, a capillary temperature of 260 ℃ in negative mode).

Reagents and materials

HPLC-grade acetonitrile was obtained from Fisher Scientific Company Inc. (Fair Lawn, New Jersey, USA). Formic acid was purchased from Tianhe chemical company Inc. (Tianjin, China). Deionized water (Milli-Q water system, Millipore Bedford, MA, USA) was used in the preparation of the samples and buffer solution. Evodiamine (Batch no: 110802-200505), rutaecarpine (Batch no: 110801-200505), dehydroevodiamine (Batch no: 100012-200805) and evodin (Batch no: 110800-200404) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). A total of eight samples of *E. rutaecarpa* and its varieties were collected (or purchased) from different areas in China. These samples were identified carefully by Prof. ZHANG Tie-jun in Tianjin Institute of Pharmaceutical Research based on morphological characteristics. These samples included *E. rutaecarpa* from Hunan (Batch no: 20080911), Guangxi (Batch no: 20090311), Zhejiang (Batch no: 20081108), *E. rutaecarpa* var. *officinalis* from Zhejang (Batch no: 20080316), Guizhou (Batch no: 20090219), and *E. rutaecarpa* var. *bodinieri* from Sichuan (Batch no: 20090126), Zhejiang (Batch no: 20080926), Jiangxi (Batch no: 20090322). Voucher samples were deposited in the Museum of Tianjin Institute of Chinese Medicine, Tianjin Institute of Pharmaceutical Research.

Preparation of standard solutions

The mixed standard stock solutions of four

reference standards (evodiamine, rutaecarpine, dehydroevodiamine, and evodin) were prepared by dissolving them in methanol. They were then diluted to five different concentrations for construction of calibration plots in the ranges of 3.84–76.8, 4.08–81.60, 1.68–33.6, and 23.16–463.2 μg/mL, respectively. Further dilution with the lowest concentrations in the calibration curves were carried out to afford a series of standard solutions for evaluating the limits of detection (LOD) and the limits of quantity (LOQ) of the compounds. The stock and working solutions were stored at 4 ℃.

Preparation of samples

According to *Pharmacopoeia of the People's Republic of China* (Pharmacopoeia Committee of P. R. China, 2005), the powder of *E. rutaecarpa* which was passed through 50 sieves (0.20 g) was weighed precisely, dipped in 90 mL of ethanol for 1 h and extracted for 40 min in an ultrasonic bath, then brought volume to 100 mL. The extracted solution was centrifuged at 4000 r/min for 10 min. The supernatant was collected and filtered through 0.45 μm filter, and the filtrate was analyzed directly by HPLC-DAD-MS.

Results and discussion

The mobile phase and wavelength optimization of HPLC analysis

In order to obtain chromatograms with good separation and strong total ion current (TIC), CH_3CN-CH_3COOH (100∶0.13) / H_2O-CH_3COOH (100∶0.2) were found to be the optimal mobile phase in both HPLC and MS analyses. CH_3CN is better than CH_3OH in separation of the major constituents in *E. rutaecarpa*. The addition of CH_3COOH has a substantial effect on selectivity and efficiency. In order to avoid baseline drift, we added different ratio of CH_3COOH both in water phase and in CH_3CN phase because the UV absorption of CH_3COOH is still conspicuous and the same amount of CH_3COOH have different absorption value in the same volume of two solutions at 225 nm.

The wavelength of monitoring was selected as 225 nm since it is suitable to detect all constituents and the maximum absorption of evodiamine. Thirteen peaks of *E. rutaecarpa* were detected under the current HPLC condition. The peaks were characterized by the retention times and UV spectra. The representative HPLC-DAD chromatograms of the extract of *E. rutaecarpa* were presented in Fig. 1a.

Tandem mass spectrometry of authentic compounds

A method of HPLC-DAD-MS/MS was used to obtain MS fragmentation patterns of constituents from *E. rutaecarpa*. In the full scan mass spectra, most of the authentic compounds exhibited $[M + H]^+$ ions and $[M + HCOOH_2]^+$ in positive mode (Fig. 1b). However, no ion peak attributed to evodine was found in the MS analysis both in positive mode and in negative mode *via* direct injection of its standard solution, but Peak 9 whose retention time was 48.2 min was found in the DAD analysis. MS, MS/MS, and UV data were summarized in Table 1.

Among those 13 compounds, there were one flavonoids glycoside, one limonin, four indoloquinazoline

Table 1 Characterization of compounds in the extract of *E. rutaecarpa* by HPLC-DAD-MS/MS

Peak no.	t_R / min	Compound	λ_{max} / nm	MS/MS	Fragment ion
1	21.35	isorhamnetin-3-*O*-β-*D*-galactoside	226, 286, 324	477, 314	$[gal]^-$
2	25.68	dehydroevodiamine	227, 247, 313, 367	302, 287	CH_3
3	32.97	evodianinine	229, 271	346, 300	CH_3COOH
4	46.10	skimmianine	232, 281, 324, 341	260, 244	CH_3
5	48.10	evodine	230, 328		
6	53.89	evodiamine	225, 267	304, 161	C_7H_3ON
7	57.03	rutaecarpine	234, 330, 343, 359	288, 161	C_7H_3ON
8	72.33	1-methyl-2-nonyl-4(1H)-quinolone	235, 322	286, 173	C_8H_{15}
9	77.66	1-methyl-2-[(*Z*)-6-undecenyl]-4(1H)-quinolone	234, 322	312, 173	$C_{10}H_{17}$
10	92.29	1-methyl-2-undecyl-4(1H)-quinolone	234, 322	342, 173	$C_{12}H_{25}$
11	93.50	1-methyl-2-[(6*Z*,9*Z*)-6,9-pentadecenyl]-4(1H)-quinolone	234, 322	314, 173	$C_{10}H_{19}$
12	101.33	evocarpine	234, 322	340, 173	$C_{12}H_{23}$
13	106.59	1-methyl-2-[(*Z*)-10-pentadecadienyl]-4(1H)-quinolone	234, 322	366, 173	$C_{14}H_{25}$

alkaloids, and seven quinolone alkaloids. Those compounds in the samples were identified by comparing the online UV, PDA, and MS information with authentic standards or literature data (Liao *et al*, 2008; Qiu, Luan, and Cheng 2005; Zhang *et al*, 2005; Zhou *et al*, 2006; Wang and Liang, 2004; Luo *et al*, 2005). The abundant protonated molecular ions $[M + H]^+$ were found in the ESI-MS spectra for all alkaloids. Indoloquinazoline alkaloids included dehydroevodiamine, evodianinine, evodiamine, and rutaecarpine. Moreover, compound **6** was representtative of indoloquinazoline alkaloid and its MS spectrum showed a strong protonated molecular ion $[M + H]^+$ at *m/z* 304 and the characteristic fragment ion at *m/z* 161 were noted in the MS/MS spectrum (Li, Hong, and Wang, 2000). Dehydroevodiamine, evodiamine, and rutaecarpine were also identical with authentic standards (Fig. 1c). Quinolone alkaloids including skimmianine, 1-methyl-2-nonyl-4(1H)-quinolone, 1-methyl-2-[(*Z*)-6-undecenyl]-4(1H)-quinolone, 1-methyl-2-[(*Z*)-10-pentadecenyl]-4(1H)-quinolone, 1-methyl-2-undecyl-4(1H)-quinolone, evocarpine, and 1-methyl-2-[(6*Z*,9*Z*)-6,9-pentadecadienyl]-4(1H)-quinolone had the characteristic fragment ion at *m/z* 173 because they had the same quinolone fragment in agreement with the reported HPLC-MS profiles (Wang and Liang, 2004; Luo *et al*, 2005) and they also behaved the UV absorption spectrum similarly in DAD. Compound **1** was flavonoids glycoside and its characteristic fragmentation was studied in negative mode which had the characteristic fragmentation $[gal]^-$ from the molecular peaks in the MS/MS. Thirteen authentic compounds included quercetin-3-*O*-β-*D*-galactoside, dehydroevodiamine, evodianinine, skimmianine, evodine, evodiamine, rutaecarpine, 1-methyl-2-nonyl-4(1H)-quinolone, 1-methyl-2-[(*Z*)-6-undecenyl]-4(1H)-quinolone, 1-methyl-2-[(*Z*)-10-pentadecenyl]-4(1H)-quinolone, 1-methyl-2-undecyl-4(1H)-quinolone, evocarpine, and 1-methyl-2[(6*Z*,9*Z*)-6,9-pentade-cadienyl]-4(1H)-quinolone, as shown in Fig. 2.

Method validation of quantification

Linearity, LOD, and LOQ All calibration graphs were plotted based on linear regression analysis of the integrated peak areas (*Y*) in DAD at 225 nm *vs* concentrations (*X*) of the four markers in the standard solution at five different concentrations. Results showed a good linear relationship between the peak

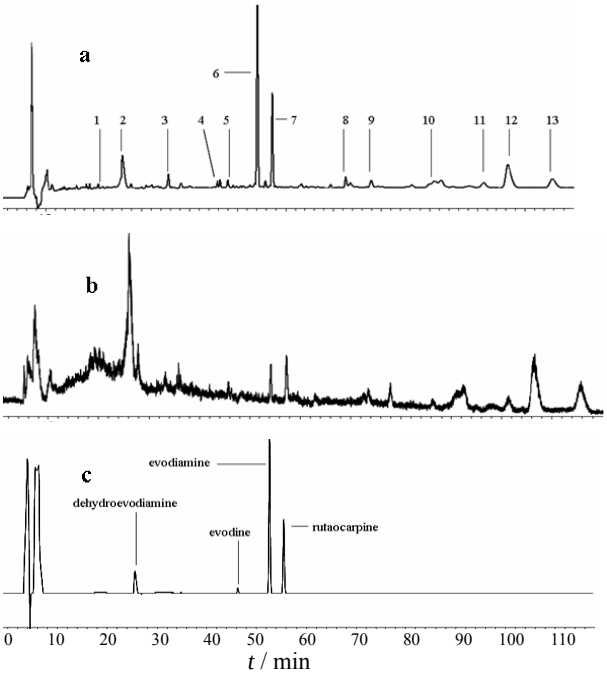
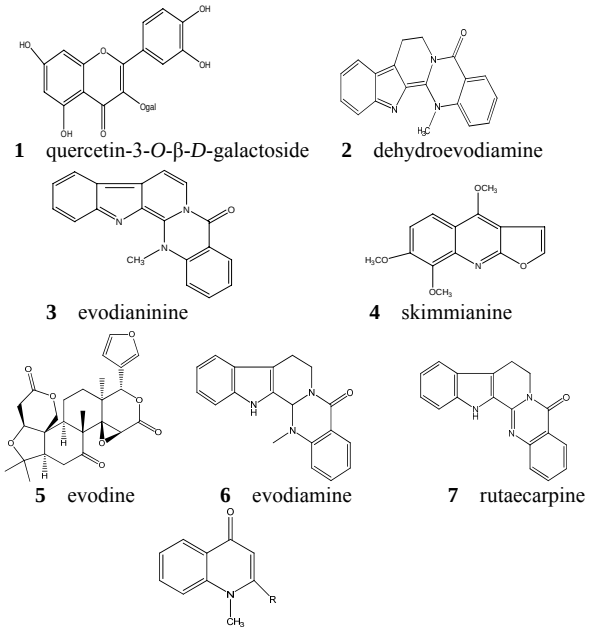


Fig. 1 HPLC-PDA-MS/MS analysis of *E. rutaecaroa*

a: HPLC-DAD chromatogram monitored at 225 nm

b: Positive ion mode MS spectra

c: HPLC-DAD chromatogram of four standards monitored at 225 nm



1 quercetin-3-*O*-β-*D*-galactoside **2** dehydroevodiamine

3 evodianinine **4** skimmianine

5 evodine **6** evodiamine **7** rutaecarpine

8 R = $(CH_2)_8CH_3$; 1-methyl-2-nonyl-4(1H)-quinolone

9 R = $(CH_2)_5CH{=}CHC_4H_9$;
1-methyl-2-[(*Z*)-6-undecenyl]-4(1H)-quinolone

10 R = $(CH_2)_{12}CH_3$;
1-methyl-2-[(*Z*)-10-pentadecenyl]-4(1H)-quinolone

11 R = $(CH_2)_{10}CH_3$; 1-methyl-2-undecyl-4(1H)-quinolone

12 R = $(CH_2)_7CH{=}CHC_4H_9$; evocarpine

13 R = $(CH_2)_9CH{=}CHC_4H_9$;
1-methyl-2-[(6*Z*,9*Z*)-6,9-pentadecadienyl]-4-(1H)-quinolone

Fig. 2 Chemical structures of compounds from *E. rutaecarpa*

Table 2 Regression equation, correlation coefficients, linearity ranges, LOD, and LOQ for the markers of *E. rutaecarpa*

Compounds	Regression equation	Linear range / (μg·mL^{-1})	*r*	LOD / (ng·mL^{-1})	LOQ / (ng·mL^{-1})
dehydroevodiamine	$Y = 130\,189\,X + 213\,074$	1.68–33.6	0.9998	31.2	66.7
evodine	$Y = 3084.4\,X - 15\,716$	23.16–463.2	0.9991	302.1	786.2
evodiamine	$Y = 103\,109\,X + 49\,299$	3.84–76.8	0.9999	23.5	57.5
rutaecarpine	$Y = 60\,617\,X + 1556.1$	4.08–81.60	0.9998	36.4	71.5

All the analytes showed good linearity ($r > 0.999$) in the concentration ranges
Y refers to the peak area. *X* is the concentration. *r* is the correlation coefficient of the equation

area and concentration (Table 2).

LOD and LOQ were determined with standard solution on the basis of a signal-to-noise ratio of 3 and 10, respectively. The LOD and the LOQ were in the range of 23.5 to 302.1 ng/mL and 57.5 to 786.2 ng/mL, respectively (Table 2).

Precision The intra-day precision was evaluated by determining a standard mixture solution of the four markers under the optimized condition six times within a day. For inter-day precision, the measurement was conducted two times per day for three consecutive days. As shown in Table 3, the intra- and inter-day relative standard deviations (RSD) were 1.15% to 2.08% and 0.95% to 2.25%, respectively.

Table 3 Precision, repeatability, and recovery of four markers in *E. rutaecarpa* samples ($n = 6$)

Compounds	Intra-day		Inter-day		Repeatability		Recovery	
	Contents / (μg·mL^{-1})	RSD / %	Contents / (μg·mL^{-1})	RSD / %	Contents / (μg·mL^{-1})	RSD / %	Recovery / %	RSD / %
dehydroevodiamine	7.89	2.08	7.83	2.25	22.15	2.14	97.54	2.10
evodine	522.87	1.89	523.21	1.49	142.51	2.83	98.21	2.85
evodiamine	63.32	1.21	63.26	1.42	127.20	1.14	99.89	0.56
rutaecarpine	56.35	1.15	56.28	0.95	81.35	1.07	101.12	0.97

Repeatability Six independently prepared sample solutions of concentrated *E. rutaecarpa* with the same amount were analyzed and the variations within six measurements were calculated for repeatability. The measurements followed those described in sample preparation. RSD (parameters for repeatability) ranged from 1.07% to 2.83% (Table 3), indicating that the conditions used in the quantitative analysis were satisfactory.

Recovery Recovery studies were carried out by spiking three different concentrations of the mixed standards to the *E. rutaecarpa* sample. The average recoveries were 97.54% for dehydroevodiamine, 98.21% for evodiamine, 99.89% for rutaecarpine, and 101.12% for evodine (Table 3). The results showed that the proposed method was accurate for the determination.

Application We tested the effectiveness of this HPLC-DAD-MS/MS method for quality analysis of 13 bioactive components and simultaneous quantity analysis of four active components in eight samples of *E. rutaecarpa* and its varieties from different areas in China. The results obtained were presented in Table 4. These samples included *E. rutaecarpa*, *E. rutaecarpa* var. *officinalis*, and *E. rutaecarpa* var. *bodinieri*. All samples contained the above 13 active components but the contents of four active components including dehydroevodiamine, evodine, evodiamine, and rutaecarpine varied with different places and varieties by quantity analysis. Evodine in *E. rutaecarpa* var. *bodinieri* was higher than that in the others. The contents of dehydroevodiamine, evodine, evodiamine and rutaecarpine varied widely in the range from 0.10% to 0.51%, 0.49% to 3.12%, 0.07% to 1.56%, and 0.10% to 0.69%, respectively. The total contents of evodiamine and rutaecarpine in all samples were more than 0.15% and exceeded regulation of *Chinese Pharmacopoeia* (Pharmacopoeia Committee of R. P. China, 2005).

Conclusion

A reliable and simple analytical method based on HPLC-DAD-MS/MS has been developed for the analysis of pharmacologically active limonins, indoloquinazoline, and quinolone alkaloids in commercial *E. rutaecarpa* samples. Using this assay, one flavonoid glycoside, one liminon, four indoloquinazoline alkaloids, and seven quinolone alkaloids could be identified by comparing the online PDA and MS information with authentic standards

Table 4 Compounds in eight different brands of *E. rutaecarpa* commercial products

Peak no.	Compounds	*E. rutaecarpa*			*E. rutaecarpa* var. *officinalis*		*E. rutaecarpa* var. *bodinieri*		
		Hunan 20080911	Guangxi 20090311	Zhejiang 20081108	Zhejiang 20080316	Guizhou 20090219	Sichuan 20090126	Zhejiang 20080926	Jiangxi 20090322
1	isorhamnetin-3-*O*-β-*D*-galactoside	+	+	+	+	+	+	+	+
2	dehydroevodiamine	0.10%	0.24%	0.26%	0.35%	0.38%	0.51%	0.33%	0.26%
3	evodianinine	+	+	+	+	+	+	+	+
4	skimmianine	+	+	+	+	+	+	+	+
5	evodine	0.49%	1.83%	1.62%	1.65%	2.62%	2.85%	2.67%	3.12%
6	evodiamine	0.28%	0.74%	0.27%	1.12%	1.56%	0.24%	0.32%	0.07%
7	rutaecarpine	0.15%	0.54%	0.22%	0.54%	0.69%	0.13%	0.22%	0.10%
8	1-methyl-2-nonyl-4(1H)-quinolone	+	+	+	+	+	+	+	+
9	1-methyl-2-[(*Z*)-6-unde-cenyl]-4(1H)-quinolone	+	+	+	+	+	+	+	+
10	1-methyl-2-[(*Z*)-10-pentadecenyl]-4(1)-quinolone	+	+	+	+	+	+	+	+
11	1-methyl-2-undecyl-4 (1H)-quinolone	+	+	+	+	+	+	+	+
12	evocarpine	+	+	+	+	+	+	+	+
13	1-methyl-2-[(6Z,9Z)-6,9-pentadecadienyl]-4-(1H)-quinolone	+	+	+	+	+	+	+	+

+ means the product containing this compound

or literature data. In addition, rutaecarpine, evodiamine, dehydroevodiamine, and evodine could be measured with high precision, accuracy, and sensitivity. If we apply this method to evaluating the commercial products of *E. rutaecarpa*, it would provide the chemical support for the chromatographic fingerprint technology. Moreover, this method is found to be convenient, fast, accurate, and it is facilitated to improve the quality control standard of *E. rutaecarpine* and related products.

References

Bao TD, Dong Y, Yang Q, Zhu XX, 2007. Simultaneous determination of evodiamine, rutaecarpine and evodin in cut crude drug and exact of *Fructus Evodiae* by high-performance liquid chromatography. *Chin J Exp Trad Med Formula* 13: l-3.

Chen CF, Chiou WF, Chou CJ, 2002. Pharmacological effects of *Evodia rutaecarpa* and its bioactive components. *Chin Pharm J (Taipei)* 54: 419-435.

Hamasaki N, Ishii E, Tominaga K, Tezuka Y, Nagaoka T, Kadota S, Kuroki T, Yano I, 2000. Highly selective antibacterial activity of novel alkyl quinolone alkaloids from a Chinese herbal medicine, Gosyuyu (Wu-Chu-Yu), against *Helicobacter pylori in vitro*. *Microbiol Immunol* 44: 9-15.

Lee HS, Oh WK, Choi HC, Lee JW, Kang DO, Park CS, Mheen TI, Ahn JS, 1998. Inhibition of angiotensin II receptor binding by quinolone alkaloids from *Evodia rutaecarpa*. *Phytother Res* 12: 212-214.

Lee MC, Chuang WC, Sheu SJ, 1996. Determination of the alkaloids in *Evodiae fructus* by capillary electrophoresis. *J Chromatography A* 755: 113-119.

Liao QF, Xie ZY, Zhang L, Deng YT, Li C, Wei CF, Yao Y, 2008. Mass spectrometric analysis of quinazoline a lkaloid and limonin from *Fructus Evodiae*. *J Chin Med Mater* 31: 673-676.

Lin H, Tsai SC, Chen JJ, Chiao YC, Wang SW, Wang GJ, Chen CF, Wang PS, 1999. Effects of evodiamine on the secretion of testosterone in rat testicular interstitial cells. *Metabolism* 48: 1532-1534.

Liu YX, Luo SR, Feng XZ, 1999. Determination of five alkoids of *Evodiae rutaecarpa* and its preparation by HPTLC fluorescent scanning. *Acta Pharm Sin* 34: 383-386.

Luo XB, Chen B, Yao SZ, 2005. Simultaneous analysis of protoberberine, indolequinoline and quinolone alkaloids in coptis-evodia herb couple and the Chinese herbal preparations by high-performance liquid chromatography-electrospray mass spectrometry. *Talanta* 66: 103-110.

Matsuda H, Wu JX, Tanaka T, Iinuma M, Kubo M, 1997. Antinociceptive activities of 70% methanol extract of *Evodiae Fructus* (fruit of *Evodia rutaecarpa* var. *bodinieri*) and its alkaloidal components. *Biol Pharm Bull* 20: 243-245.

Matsuda H, Yoshikawa M, Iinuma M, Kubo M, 1998. Antinociceptive and anti-inflammatory activities of limonin isolated from the fruits of *Evodia rutaecarpa* var. *bodinieri*. *Planta Med* 64: 339-342.

Pharmacopoeia Committee of P. R. China, 2005. *Pharmacopoeia of the People's Republic of China*. Chemical Industry Press, Beijing, 118.

Poulose SM, Harris ED, Patil BS, 2006. Antiproliferative effects of citrus limonoids against human neuroblastoma and colonic adenocarcinoma cells. *Nutr Cancer* 56: 103-112.

Qiu GL, Luan LJ, Cheng YY, 2005. Simultaneous determination of evodiamine and rutaecarpine in rabbit plasma by HPLC-MS. *Chin J Pharm Anal* 25: 1179-1182.

(Continue on page 131)

cell death in rat cortical neurons: Roles of NMDA receptors and glutathione. *Neurochem Int* 47: 51-57.

Livak KJ, Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}$T method. *Methods* 25: 402-408.

Makino T, Tsubouchi R, Murakami K, Haneda M, Yoshino M, 2006. Generation of reactive oxygen species and induction of apoptosis of HL60 cells by ingredients of traditional herbal medicine, Sho-saiko-to. *Basic Clin Pharmacol Toxicol* 98: 401-405.

Matsui S, Matsumoto H, Sonoda Y, Ando K, Aizu-Yokota E, Sato T, Kasahara T, 2004. Glycyrrhizin and related compounds down-regulate production of inflammatory chemokines IL-8 and eotaxin 1 in a human lung fibroblast cell line. *Int Immuno-pharmacol* 15: 1633-1644.

Matsumoto K, Ueda S, Hashimoto T, Kuriyama K, 1991. Ischemic neuronal injury in the rat hippocampus following transient forebrain ischemia: Evaluation using *in vivo* microdialysis. *Brain Res* 543: 236-242.

Naarala TJ, Leikkanen JJ, Ruotsalainen MH, Savolainen KM, 1995. Lead amplifies glutamate-induced oxidative stress. *Free Radic Biol Med* 19: 689-693.

Ni J, Ohta H, Matsumoto K, Watanabe H, 1994. Progressive cognitive impairment following chronic cerebral hypoperfusion induced by permanent occlusion of bilateral carotid arteries in rats. *Brain Res* 653: 231-236.

Ni JW, Matsumoto K, Li HB, Murakami Y, Watanabe H, 1995. Neuronal damage and decrease of central acetylcholine level following permanent occlusion bilateral common carotid arteries in rats. *Brain Res* 673: 290-296.

Ohkawa H, Ohishi N, Yagi K, 1979. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95: 351-358.

Pappas BA, de la Torre JC, Davidson CM, Keyes MT, Fortin T, 1996. Chronic reduction of cerebral blood flow in the adult rat: Late emerging CA1 cell loss and memory dysfunction. *Brain Res* 708: 50-58.

Rockwood K, Wentzel C, Hachinski V, Hogan DB, MacKnight C, McDowell I, 2000. Prevalence and outcomes of vascular cognitive impairment. *Neurology* 54: 447-451.

Román GC, 2003. Vascular dementia: Distinguishing characteristics, treatment, and prevention. *J Am Geriatr Soc* 51 (5 Suppl. Dementia): a296-a304.

Sarti C, Pantoni L, Inzitari D, 2002. Cognitive impairment and chronic cerebral hypoperfusion: What can be learned from experimental models. *J Neurol Sci* 203/204: 263-266.

Sattler R, Tymianski M, 2001. Molecular mechanisms of glutamate receptor-mediated excitotoxic neuronal cell death. *Mol Neurobiol* 24: 107-129.

Stewart JT, 2007. Psychiatric and behavioral manifestations of vascular dementia. *Am J Geriatr Cardiol* 16: 165-170.

Stewart ZA, Pietenpol JA, 2001. Signaling and cell cycle checkpoints. *Chem Res Toxicol* 14: 243-263.

Sun Y, Oberley LW, Li Y, 1998. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 34: 497-500.

Yu J, Liu C, Zhang X, Han J, 2005. Acupuncture improved cognitive impairment caused by multi-infarct dementia in rats. *Physiol Behav* 86: 434-441.

Zhou J, Zhang HY, Tang XC, 2001. Huperzine A attenuates cognitive deficits and hippocampal neuronal damage after transient global ischemia in gerbils. *Neurosci Lett* 313: 137-140.

(Continue from page 117)

Roy A, Saraf S, 2006. Limonoids: Overview of significant bioactive triterpenes distributed in plants kingdom. *Biol Pharm Bull* 29: 191-201.

Wang QZ, Liang JY, 2004. Survey on chemical constituents and physiological activities of *Evodia* Forst plants. *Chin Tradit Herb Drugs* 35: a7-a10.

Tang W, Eisenbrand G, 1992. *Evodia rutaecarpa (Juss) Benth*. In: Chinese Drugs of Plant Origin, Springer-Verlag, Berlin, 509-514.

Wang QZ, Liang JY, 2004. Studies on the chemical constituents of *Evodia rutaecarpa* (Juss.) Benth. *Acta Pharm Sinica* 39: 605-608.

Woo HG, Lee CH, Noh MS, Lee JJ, Jung YS, Baik EJ, Moon CH, Lee SH, 2001. Rutaecarpine, a quinazolino-carboline alkaloid, inhibits prostaglandin production in RAW264.7 macrophages. *Planta Med* 67: 505-509.

Zhang XQ, Zhou X, Wang DP, Hao XJ, 2005. Analysis of alkaloids in *Evodia rutaecarpa* from different habitats by liquid chromatography / mass spectrometry. *Chin J Anal Chem* 33: 241-244.

Zhang Y, Zhang QH, Wu LJ, Tashiro S, Onodera S, Ikejima T, Zheng Y, Zhang QH, Wu LJ, 2004. A typical apoptosis in L929 cells induced by evodiamine isolated from *Evodia rutaecarpa*. *J Asian Nat Prod Res* 6: 19-27.

Zhou X, Lei PH, Liu H, 2008. Study on fingerprint of *Evodia rutaecarpa* by high performance liquid chromatography. *China J Trad Chin Med Pharm* 23: 313-316.

Zhou Y, Li SH, Jiang RW, C M, Liu X, Ding LS, Xu HX, But PP, Shaw PC, 2006. Quantitative analyses of indoloquinazoline alkaloids in *Fructus Evodiae* by high-performance liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom* 20: 3111-3118.

Zhu KF, 2003. Determination of rutacarpine in the granules of *Evodiae rutaecarpa*. *Lishizhen Med Mater Med Res* 14: 337-338.