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Short communication

Simultaneous determination of 12 chemical constituents in the traditional Chinese Medicinal Prescription Xiao-Yao-San-Jia-Wei by HPLC coupled with photodiode array detection

Hongmin Zhang [b,c], Shiwei Chen [d], Feng Qin [a], Xi Huang [a,c,*], Ping Ren [a,**], Xinqi Gu [c]

[a] Laboratory of Ethnopharmacology and Institute of Integrated Traditional Medicine and Western Medicine, Xiangya Hospital, Central South University, Changsha 410008, PR China
[b] Henan Eye Institute and Department of Ophthalmology, Henan Provincial People's Hospital, Zhengzhou 450003, PR China
[c] Laboratory of Ethnopharmacology and Department of Integrated Chinese and Western Medicine, West China Hospital, Sichuan University, Chengdu 610041, PR China
[d] Henan Food and Drug Administration, Zhengzhou 450012, PR China

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ABSTRACT

An HPLC-photodiode array (PDA) detection method was established for the simultaneous determination of 12 components in Xiao-Yao-San-Jia-Wei (XYSJW): geniposide, puerarin, paeoniflorin, ferulic acid, liquiritin, hesperidin, naringin, paeonol, daidzein, glycyrrhizic acid, honokiol, and magnolol. These were separated in less than 70 min using a Waters Symmetry Shield RP 18 column with gradient elution using (A) acetonitrile, (B) water, and (C) acetic acid at a flow rate of 1 ml/min, and with a PDA detector. All calibration curves showed good linear regression ($r^2 > 0.9992$) within the test ranges. The method was validated for specificity, accuracy, precision, and limits of detection. The proposed method enables in a single run the simultaneous identification and determination for quality control of 12 multi-structural components of XYSJW forming the basis of its therapeutic effect.

1. Introduction

Xiao-Yao-San-Jia-Wei (XYSJW) is an effective Chinese Medicinal Prescription used in the treatment of functional dyspepsia associated with the syndrome of liver stagnation and spleen deficiency and the symptoms of abdominal distention, hiccup, sultriness, poor appetite, dry mouth, and bitter taste in the mouth [1,2]. The preparation is developed from Xiao-Yao-San, which is a classic Chinese formula that has been used in China for approximately one thousand years. Many papers have described the relationship between functional dyspepsia and gastric motility and depression and that XYSJW could improve gastrointestinal movement and had an antidepressant-like effect [3–5].

XYSJW contains the following 14 Chinese herbs: Radix Bupleuri, Radix Angelicae sinensis, Radix Paeoniae alba, Rhizoma Atractylodis macrocephalae, Poria, Rhizoma Zingiberis recens, Radix Glycyrrhizae, Herba Menthae, Cortex Moutan, Fructus Gardeniae, Cortex Magnoliae officinalis, Fructus Aurantii, Radix Puerariae, and Fructus Jujubae. However, it is not clear which of the chemical constituents of XYSJW is medicinally effective.

It is widely accepted that one or even a few marker constituents in a traditional Chinese medicine (TCM) does/do not reflect its overall efficacy. The combined action of multiple constituents is considered to be crucial for the therapeutic effect of a TCM [6]. Furthermore, the bioactive components in a TCM prescription must be absorbable components [7–9]. 12 constituents in XYSJW are as follows: ferulic acid from *Radix Angelicae sinensis*, puerarin and daidzein from *Radix Puerariae*, honokiol and magnolol from *Cortex Magnoliae officinalis*, liquiritin and glycyrrhizic acid from *Radix Glycyrrhizae*, hesperidin and naringin from *Fructus Aurantii*, paeonol from *Cortex Moutan*, paeoniflorin from *Radix Paeoniae alba*, and geniposide from *Fructus Gardeniae* (Fig. 1). Recent pharmacological studies have indicated that ferulic acid, honokiol, and magnolol could improve gastrointestinal motility [10,11]; puerarin has a protective effect on stress-induced gastric mucosal injury [12]; honokiol, magnolol, liquiritin, glycyrrhizic acid, and paeonol have antidepressant-like effect [13–16], and that naringin has an anti-ulcer effect on gastric lesions [17]. The above data demonstrate that the multiple absorbed constituents in XYSJW are responsible for its therapeutic effect.

* Corresponding author at: Laboratory of Ethnopharmacology and Institute of Integrated Traditional Medicine and Western Medicine, Xiangya Hospital, Central South University, Changsha 410008, PR China. Tel.: +86 731 4327222 fax: +86 731 4328386.
** Corresponding author. Tel.: +86 731 4327222; fax: +86 731 4328386.
E-mail addresses: tcmhuangx59@163.com (X. Huang), renping3@163.com (P. Ren).

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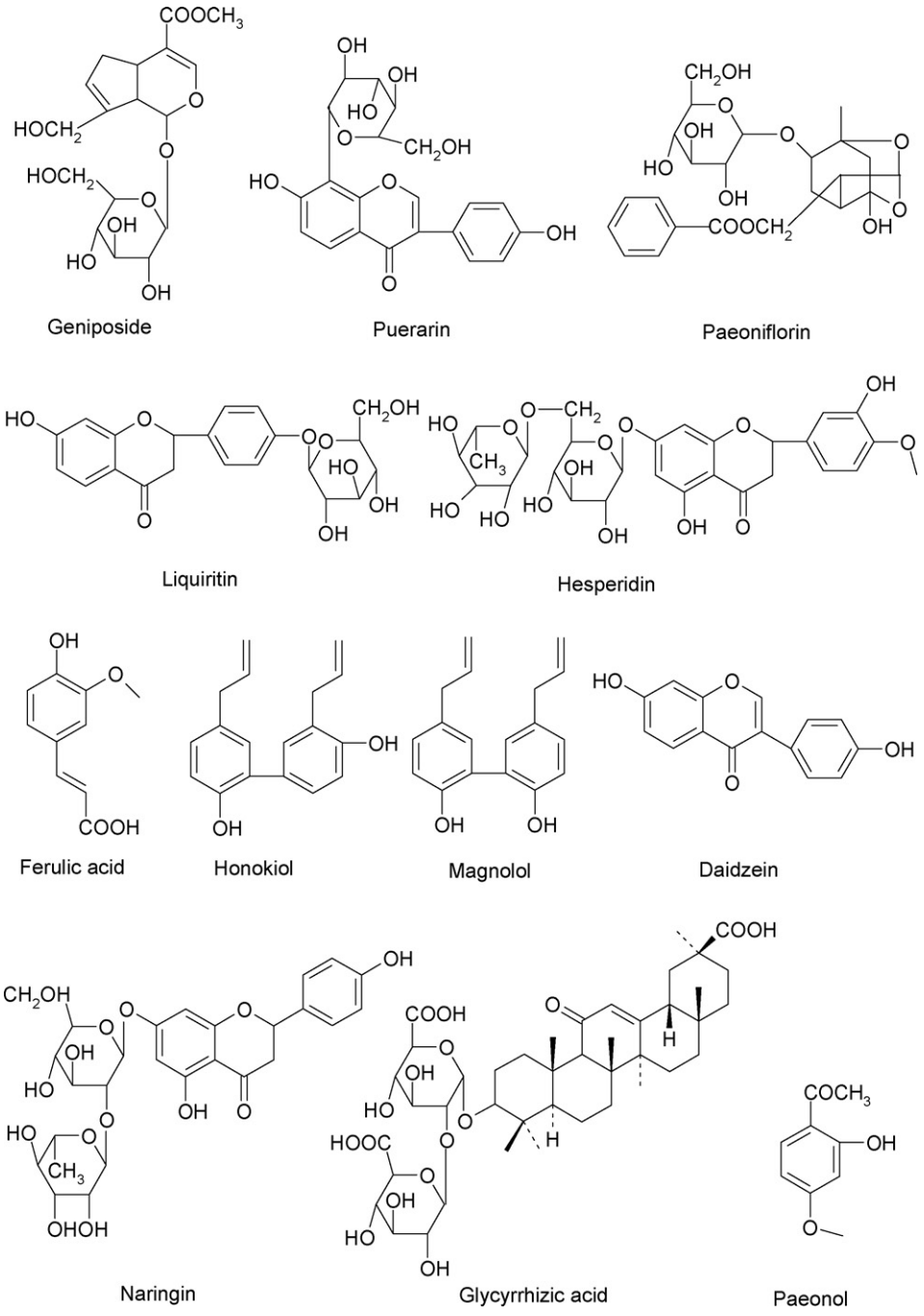

Fig. 1. The structures of 12 constituents in Xiao-Yao-San-Jia-Wei.

Therefore, quantitative determination of the above mentioned 12 components in XYSJW is considered to be necessary for quality control and also for determination of the chemical constituent basis of the therapeutic effect. In order to determine the 12 constituents, a convenient and reliable method that is capable of rapidly and simultaneously separating and identifying these 12 active components in XYSJW is urgently needed.

High-performance liquid chromatography (HPLC) and its coupling with other techniques, particularly photodiode array (PDA) detection, is a convenient, widely used, and powerful approach for the rapid identification of the constituents in botanic extracts and TCM [18]. Therefore, in the present study, we focused on quantitative determination of the effective components in XYSJW and investigated HPLC-PDA coupled methods for the simultaneous determination of 12 constituents.

2. Experimental

2.1. Materials and reagents

2.1.1. Materials

The fourteen Chinese herbs that comprise XYSJW were purchased from a TCM dispensary store in the West China Hospital (Chengdu, China) and identified. Voucher specimens (No. 200505) were deposited at the Laboratory of Ethnopharmacology in Xiangya Hospital.

Table 1
Regression data and LODs for the 12 components determined ($n = 6$).

Components	Regression equation	Correlation coefficient (r^2)	Linear range (μg/ml)	LOD (μg/ml)
Geniposide	$y = 1632x - 2187$	0.9996	8.62–275.8	1.08
Puerarin	$y = 4420x - 1211$	0.9999	1.96–62.6	0.24
Paeoniflorin	$y = 1244x - 3705$	0.9992	5.47–175.1	2.74
Ferulic acid	$y = 6027x - 6.4$	0.9999	0.34–10.8	0.19
Liquiritin	$y = 2532x - 286$	0.9999	0.68–21.6	0.17
Hesperidin	$y = 1880x - 1306$	0.9992	2.09–67.0	0.52
Naringin	$y = 1834x - 1216$	0.9999	7.26–232.2	1.81
Paeonol	$y = 5148x - 2395$	0.9998	4.30–137.5	1.07
Daidzein	$y = 9848x - 179$	0.9998	0.4–12.8	0.20
Glycyrrhizic acid	$y = 644x - 760$	0.9999	6.96–222.6	0.87
Honokiol	$y = 3155x + 142$	0.9992	0.32–10.2	0.16
Magnolol	$y = 1589x - 159$	0.9996	0.88–28.3	0.44

In the regression equation $y = ax + b$, x refers to the concentration (μg/ml), y indicates the peak area, and r^2 is the correlation coefficient of the equation. LOD, limit of detection.

2.1.2. Reagents

Authentic standards of geniposide, puerarin, paeoniflorin, ferulic acid, liquiritin, hesperidin, naringin, paeonol, daidzein, glycyrrhizic acid, honokiol and magnolol were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade acetonitrile was purchased from Tedia Company Inc. (OH, USA). Water for HPLC analysis was purified using an ultrapure water purification system (Chengdu Ultrapure Engineering Ltd., Chengdu, China). All other reagents were of analytical grade.

2.2. Apparatus and chromatographic conditions

Analysis was performed using a Waters 2695 Alliance HPLC system (Waters Corp., Milford, MA, USA), consisting of a quaternary pump solvent management system, an on-line degasser, and an autosampler. The raw data were detected by 2996 PDA, acquired, and processed with Empower™ Software. The analytical column used was a Waters Symmetry Shield RP 18 column (150 mm × 3.9 mm i.d., particle size 5 μm) preceded by a Waters Symmetry Shield RP 18 guard column (20 mm × 3.9 mm i.d., particle size 5 μm). The mobile phase was composed of A-acetonitrile, B-water, and C-acetic acid (the amount of acetic acid is kept constant at 0.5% during the entire method) with gradient elution (0–5 min, 5–10% A; 5–20 min, 10–15% A; 20–40 min, 15–20% A; 40–50 min, 20–30% A; 50–70 min, 30–60% A). The flow rate of the mobile phase was 1.0 ml/min, and the temperature was maintained at 20 °C. The components were quantified based on peak areas at the maximum wavelength in their UV spectrum.

2.3. Preparation of standard solutions

A standard stock solutions of each of the 12 components were directly prepared in methanol. Working standard solutions containing the 12 compounds were prepared and diluted with methanol to appropriate concentrations for establishment of calibration curves. The standard stock solutions and working solutions were all prepared in dark brown calibrated flasks and stored at 4 °C. The linearity of the responses was determined for six concentrations. Empower software was used to prepare the standard curves

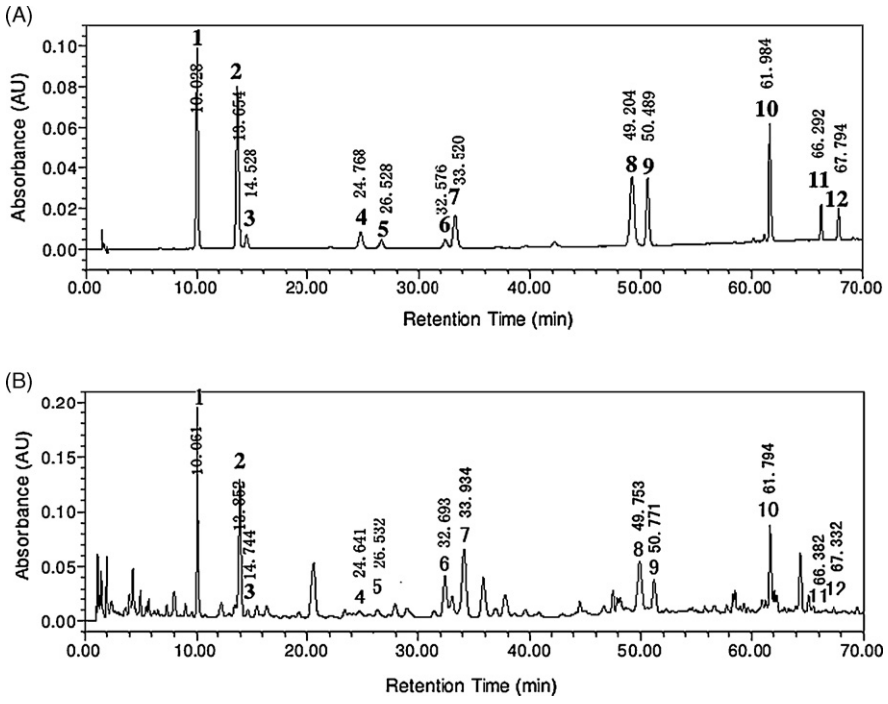

Fig. 2. Typical chromatograms of the standard mixture (A) and Xiao-Yao-San-Jia-Wei (B) at 254 nm. (1) Geniposide; (2) puerarin; (3) paeoniflorin; (4) ferulic acid; (5) liquiritin; (6) hesperidin; (7) naringin; (8) paeonol; (9) daidzein; (10) glycyrrhizic acid; (11) honokiol; (12) magnolol.

Table 2
The precision data of the proposed HPLC method.

Components	Nominal Concentration (μg/ml)	Precision			
		Intra-day (n = 5)		Inter-day (n = 3)	
		Mean ± SD (μg/ml)	R.S.D. (%)	Mean ± SD (μg/ml)	R.S.D. (%)
Geniposide	17.24	16.53 ± 0.99	5.99	15.58 ± 0.75	4.81
	34.48	34.65 ± 0.72	2.08	34.60 ± 0.78	2.25
	137.90	142.46 ± 3.70	2.60	143.04 ± 3.50	2.45
Puerarin	3.91	3.98 ± 0.26	6.53	3.83 ± 0.25	6.53
	7.82	8.11 ± 0.29	3.58	8.01 ± 0.22	2.75
	31.29	31.74 ± 2.35	7.40	30.50 ± 1.82	5.97
Paeoniflorin	10.94	12.06 ± 0.56	4.64	11.49 ± 0.68	5.92
	21.88	22.26 ± 0.60	2.70	22.13 ± 0.90	4.07
	87.53	83.38 ± 2.95	3.54	86.26 ± 4.07	4.72
Ferulic acid	0.68	0.64 ± 0.04	6.25	0.62 ± 0.06	9.68
	1.35	1.36 ± 0.04	2.94	1.35 ± 0.02	1.48
	5.40	5.51 ± 0.08	1.45	5.40 ± 0.21	3.89
Liquiritin	1.35	1.38 ± 0.07	5.07	1.33 ± 0.06	4.51
	2.70	2.72 ± 0.05	1.84	2.72 ± 0.05	1.84
	10.80	10.77 ± 0.44	4.09	10.48 ± 0.20	1.91
Hesperidin	4.19	4.64 ± 0.11	2.37	4.66 ± 0.14	3.00
	8.37	8.40 ± 0.18	2.14	8.41 ± 0.27	3.21
	33.48	32.42 ± 0.79	2.44	32.16 ± 0.45	1.40
Naringin	14.52	14.78 ± 0.74	5.01	14.74 ± 0.18	1.22
	29.03	29.40 ± 0.78	2.65	29.85 ± 0.42	1.41
	116.12	115.99 ± 5.45	4.70	111.25 ± 5.13	4.61
Paeonol	8.59	8.83 ± 0.67	7.59	8.92 ± 0.08	0.90
	17.19	17.18 ± 0.50	2.91	17.05 ± 0.48	2.82
	68.74	67.42 ± 1.67	2.48	68.71 ± 0.90	1.31
Daidzein	0.80	0.78 ± 0.03	3.85	0.79 ± 0.05	6.33
	1.60	1.60 ± 0.03	1.88	1.60 ± 0.04	2.50
	6.40	6.32 ± 0.16	2.53	6.34 ± 0.23	3.63
Glycyrrhizic acid	13.92	13.51 ± 0.82	6.07	13.59 ± 0.19	1.40
	27.83	28.16 ± 0.68	2.41	28.02 ± 0.71	2.53
	111.32	111.61 ± 0.54	0.48	110.81 ± 1.93	1.74
Honokiol	0.64	0.65 ± 0.04	6.15	0.64 ± 0.04	6.25
	1.28	1.28 ± 0.04	3.13	1.28 ± 0.05	3.91
	5.10	5.11 ± 0.17	3.33	5.04 ± 0.29	5.75
Magnolol	1.77	1.74 ± 0.11	6.32	1.73 ± 0.17	9.83
	3.54	3.55 ± 0.07	1.97	3.54 ± 0.09	2.54
	14.15	14.19 ± 0.31	2.18	14.04 ± 0.65	4.63

from the peak area of each compound. The contents of these constituents in the test samples were calculated using the regression parameters obtained from the standard curves.

2.4. Preparation of sample solutions

12.6 g of Radix Bupleuri, Radix Angelicae sinensis, Radix Paeoniae alba, Rhizoma Atractylodis macrocephalae, Poria, Rhizoma Zingiberis recens, Radix Glycyrrhizae, Herba Menthae, Cortex Moutan, Fructus Gardeniae, Cortex Magnoliae officinalis, Fructus Aurantii, Radix Puerariae, and Fructus Jujubae at the weight ratio of 1:1:1:0.6:1:1:0.6:0.6:0.6:0.6:1.2:1.2:1.6:0.6 were crushed into small pieces. The mixture was soaked with 100.8 ml of pure methanol for 30 min and extracted for 2 h at 64 °C by microwave. The extraction procedure was repeated three times. The decoctions collected from the three successive extractions were mixed and passed through filter paper. The filtrates were concentrated to 63 ml using a rotary evaporator (Büchi, Switzerland). The final concentration is equal to 0.2 g crude drug/ml methanol. The methanolic extract was sealed in sterile bottles and retained in a refrigerator (4 °C) until used. The sample injection volume for HPLC analysis was 5 μl. The samples were filtered through 0.45 μm film before HPLC analysis.

2.5. Recovery test

In order to evaluate the accuracy of the proposed methods, a recovery test was performed by adding known amounts of reference standard solutions to the sample of XYSJW before extraction, followed by analysis using the proposed method. Three concentrations of accurately determined amounts of the 12 standard substances were used to spike the XYSJW crude mixture, and then extracted and analyzed as described in the above paragraph. The percentage of recovery was calculated according to the formula: recovery (%) = (total amount after spiking − original amount in sample)/spiked amount × 100%.

3. Results and discussion

3.1. Chromatography

A common limitation of multiple component analysis is the low sensitivity of detection for many analytes under the selected single monitoring wavelength. However, with a PDA detector simultaneous detection at more than one wavelength is possible. In this study, an HPLC method was successfully developed to analyze 12

Table 3
The contents of 12 components in XYSJW ($n = 3$).

Components	Contents (ng/mg)	R.S.D. (%)
Geniposide	2236 ± 70	3.13
Puerarin	443.4 ± 4.5	1.02
Paeoniflorin	1095 ± 13.4	1.23
Ferulic acid	20.3 ± 0.24	1.18
Liquiritin	44.4 ± 0.52	1.16
Hesperidin	1249 ± 29.7	2.38
Naringin	2787 ± 85.9	3.08
Paeonol	882 ± 9.9	1.13
Daidzein	63.4 ± 0.96	1.52
Glycyrrhizic acid	1553 ± 9.7	0.62
Honokiol	7.67 ± 0.05	0.63
Magnolol	10.4 ± 0.07	0.71

components in XYSJW. The selected monitoring wavelengths for these components were their maximum absorption wavelengths.

With the PDA, UV spectra of the bioactive constituents could be compared with those of the authentic standards. The desired compound from XYSJW was identified by comparing both the retention times and UV spectra with those of the authentic standard. The analyte was further confirmed by spiking the actual sample with the standard. The excellent agreement between standard and sample spectra found in all analyzed samples of XYSJW indicates that under the proposed analytical conditions, the 12 marker constituents were sufficiently resolved, that they were separated successfully by gradient elution in less than 70 min, and that there was no interference from other components in the matrix. Typical chromatograms of the authentic standards and XYSJW recorded at 254 nm are depicted in Fig. 2.

3.2. Method validation

The assay linearity was determined by the analysis of six different concentrations of the standard solutions. Table 1 shows the regression data and LODs (S/N = 3) of the components determined. All calibration curves showed good linear regression ($r^2 > 0.9992$) within test ranges.

The relative standard deviation (R.S.D.) was considered to be a measurement of precision and accuracy. Intra- and inter-day precisions were determined by assaying standard solutions at three concentrations during a single day and on five different days, respectively. As shown in Table 2, the overall intra- and inter-day variations was less than 5% for all 12 analytes. These results demonstrated that the developed method is reproducible with good precision. The accuracy tests were carried out using a recovery test. Recovery of all 12 tested bioactive compounds was within the range of 96.4–100.5%, with an R.S.D. of between 0.40% and 3.6% ($n = 3$). These values indicated that the method is acceptable.

The stability test was performed with sample solutions placed under 4 °C and these were analyzed at 0, 24, and 48 h. The R.S.D. values of the peak area and retention times were no more than 5.9% and 2.0%, respectively. The solution was therefore considered to be stable for at least 48 h at 4 °C.

3.3. Determination of 12 components in XYSJW

The developed assay was subsequently applied to the simultaneous determination of the twelve major compounds in the XYSJW samples. A representative chromatogram of the extracts is shown in Fig. 2(B) and the quantity of each compound identified is summarized in Table 3. These data indicate that the proposed method is suitable for the simultaneous determination of 12 compounds in XYSJW.

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References

[1] P. Ren, X. Huang, S.Q. Li, S.Y. Xu, M.H. Wan, L. Zhu, W.F. Tang, J. Integr. Tradit. Chin. West. 26 (2006) 398–402.
[2] J.X. Li, Zhejiang J. Integr. Tradit. Chin. West. Med. 14 (2004) 203–204.
[3] K. Haruma, H. Kusunoki, N. Manabe, T. Kamada, M. Sato, M. Ishii, A. Shiotani, J. Hata, Digestion 77 (2008) 48–51.
[4] L.T. Chou, C.Y. Wu, H.P. Chen, C.S. Chang, P.G. Wong, C.W. Ko, G.H. Chen, J. Clin. Gastroenterol. 33 (2001) 127–131.
[5] H.C. Luo, R.Q. Qian, X.Y. Zhao, J. Bi, H. Xin, X. Jiang, K. Xu, S. Yan, Zhongguo Zhong Xi Yi Jie He Za Zhi 26 (2006) 212–214.
[6] T. Xue, R. Roy, Science 300 (2003) 740–741.
[7] W. Jia, J. Chin. Integr. Med. 4 (2006) 441–446.
[8] X. Huang, Chin. J. Exp. Trad. Med. Form. 8 (2002) 55–58.
[9] X.J. Wang, W.J. Sun, N. Zhang, H. Sun, F. Geng, C.Y. Pu, Chin. J. Nat. Med. 5 (2007) 277–280.
[10] O.A. Badary, A.S. Awad, M.A. Sherief, F.M. Hamada, World J. Gastroenterol. 12 (2006) 5363–5367.
[11] W.W. Zhang, Y. Li, X.Q. Wang, F. Tian, H. Cao, M.W. Wang, Q.S. Sun, World J. Gastroenterol. 11 (2005) 4414–4418.
[12] F.W. Wang, J. Li, Z.L. Hu, Y.Y. Xie, Zhongguo Zhong Yao Za Zhi 31 (2006) 504–506.
[13] Q. Xu, L.T. Yi, Y. Pan, X. Wang, Y.C. Li, J.M. Li, C.P. Wang, L.D. Kong, Prog. Neuropsychopharmacol. Biol. Psych. 32 (2008) 715–725.
[14] W. Wang, X. Hu, Z. Zhao, P. Liu, Y. Hu, J. Zhou, D. Zhou, Z. Wang, D. Guo, H. Guo, Prog. Neuropsychopharmacol. Biol. Psych. 32 (2008) 1179–1184.
[15] O. Ainsah, B.M. Nabishah, C.B. Osman, B.A. Khalid, Clin. Exp. Pharmacol. Physiol. 26 (1999) 444–448.
[16] X.J. Mi, S.W. Chen, W.J. Wang, R. Wang, Y.J. Zhang, W.J. Li, Y.L. Li, Pharmacol. Biochem. Behav. 81 (2005) 683–687.
[17] M.J. Martín, E. Marhuenda, C. Pérez-Guerrero, J.M. Franco, Pharmacology 49 (1994) 144–150.
[18] H. Zhang, P. Shen, Y. Cheng, J. Pharm. Biomed. Anal. 34 (2004) 705–713.