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ORIGINAL ARTICLES

Identification and determination of the major constituents in traditional Chinese medicine Longdan Xiegan Pill by HPLC-DAD-ESI-MS

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Abstract: A novel and sensitive HPLC-UV method has been developed for the simultaneous determination of twelve major compounds in Longdan Xiegan Pill. The chemical profile of the twelve compounds, including geniposidic acid (1), geniposide(2), gentiopicroside(3), liquiritin(4), crocin(5), baicalin(6), wogonoside(7), baicalein(8), glycyrrhizic acid (9), wogonin (10), oroxylin A (11) and aristolochic acid A (12), was acquired using high-performance liquid chromatography-diode array detector coupled with an electrospray tandem mass spectrometer (HPLC-DAD-ESI-MS). The analysis was performed on a Dikma Platisil ODS C_{18} column (250 mm × 4.6 mm, 5 μm) with a gradient solvent system of acetonitrile-0.1% aqueous formic acid. The validation was carried out and the linearities ($r > 0.9996$), repeatability (RSD < 1.8%), intra- and inter-day precision (RSD < 1.3%), and recoveries (ranging from 96.6% to 103.4%) were acceptable. The limits of detection (LOD) of these compounds ranged from 0.29 to 4.17 ng. Aristolochic acid A, which is the toxic ingredient, was not detected in all the batches of Longdan Xiegan Pill. Furthermore, hierarchical cluster analysis was used to evaluate the variation of the herbal prescription. The proposed method is simple, effective and suitable for the quality control of this traditional Chinese medicine (TCM).

Keywords: Longdan Xiegan Pill; high-performance liquid chromatography-diode array detector coupled with an electrospray tandem mass spectrometer (HPLC-DAD-ESI-MS); qualitative evaluation; aristolochic acid A; hierarchical cluster analysis

1 Introduction

Traditional Chinese medicines (TCMs), which have been used to prevent and cure diseases in China for centuries, are becoming more and more popular around the world during the last decade. Particular attention has been focused on their efficacy and safety. Systematic research on TCMs has centered on identification of chemical components, pharmaceutical activity, processing methods, and quality control. Great progress has been made in the quality control of TCMs, stemming mainly from modern separation and characterization techniques. Quality control is one of the problems for the application and development of TCMs, which was recognized by the World Health Organization in the document entitled "General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines".

Longdan Xiegan Pill (LXP) is one of the most popular traditional Chinese medicine prescriptions for treatment of jaundice, cystitis, conjunctival congestion, earache, scrotum and extremitas inferior eczema as well in Chinese traditional medication [1]. The chemical components of some ingredient herbs in LXP were iridoidal glycosides, flavonoids, pigments, triterpenoids, and volatile oils, organic acids, amino acids, and inorganic compounds [2-5]. LXP consists of 10 medicinal materials including *Radix Gentianae, Radix Scutellariae, Fructus Gardeniae, Radix Glycytthizae, Rhizoma Alismatis, Radix Angelicae Sinensis, Radix Rehmanniae, Semen Plantaginis, Radix Bupleuri and Caulis Akebiae.*

However, many cases of Longdan Xiegan Pill inducing nephropathy have been reported in the recent ten years [6-8]. It was reported that *Caulis aristolochiae* manshuriensis (Chinese name: Guanmutong) which contains the aristolochic acid A (AA) is the toxic ingredient in Longdan Xiegan Pill [9-12]. AA has drawn extensive attention since the first Belgian reported case of nephropathy in which non-nephrotoxicity herbal *Stephania tetrandra* was inadvertently replaced by Guanmutong containing AA in the 1990s [13]. Since 2000, the USA (FDA., 2001) as well as many other countries such as UK (MHRA, 2003), Canada (Canada, 2002), the Netherlands (Martena *et al.*, 2007), Australia (TGA, 2001) and New Zealand (Medsafe, 2003) has issued warnings and limited or prohibited the imports and sales of herbs containing or suspected of containing AA, including Longdan Xiegan Pill and Guanmutong. To prevent further cases of aristolochic acid related nephropathy, the

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government of China has also called for manufacturers of Longdan Xiegan Pill to change Guanmutong back into Mutong (Chinese Pharmacopoeia Committee, 2002). Due to aliasing application of Mutong and Guanmutong in Longdan Xiegan Pill, AA, which has been characterized as a carcinogen and nephrotoxin, must be detected. Till now, the safety of Longdan Xiegan Pill in the present market has not been investigated in the literature available.

In the present work, an efficient high-performance liquid chromatography-diode array detector coupled with an electrospray tandem mass spectrometer (HPLC-DAD-ESI-MS) method was proposed for the identification and quantification of the twelve major compounds in sixteen batches of Longdan Xiegan Pill. At the same time AA, which is recognized as the toxic ingredient in Longdan Xiegan Pill, was detected. Then based on the sample data, hierarchical cluster analysis was utilized for qualitative evaluation on the resemblance and difference of tested samples.

2 Experimental

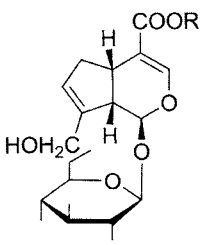
2.1 Chemicals and materials

HPLC-grade acetonitrile and formic acid were purchased from Merck (Darmstadt, Germany). Deionized water was prepared by a Milli-Q_{50} SP Reagent Water System (Bedford, MA, USA) for preparing samples and mobile solution. Other reagents were of analytical grade. All solvents were filtered through 0.22 μm membrane filters before analysis.

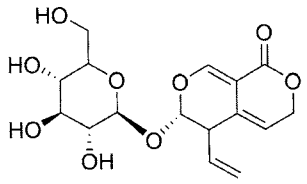
The reference standards of geniposide, gentiopicroside, liquiritin, baicalin, baicalein, wogonin, and aristolochic acid A were obtained from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); geniposidic acid was purchased from Shanghai Xibao Medical Science Co., Ltd. (Shanghai, China); glycyrrhizic acid and wogonoside were purchased from Shanghai Ronghe Medical Science Co., Ltd. (Shanghai, China); crocin was purchased from Chengdu Man Si Te Medical Science Co., Ltd. (Chengdu, China); wogonin and oroxylin A were purchased from Shanghai Yousi Medical Science Co., Ltd. (Shanghai, China). The purities of all the standards were not less than 98% (Figure 1). Sixteen batches of LXP were collected from different pharmaceutical companies in China (Table 1).

2.2 Standard solutions and sample preparation

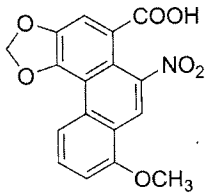
Each accurately weighed standard was dissolved in methanol, respectively, and then a mixed methanolic stock solution of standards was prepared. A set of standard solutions were prepared by appropriate dilution of the stock solution with methanol, in order to make the calibration curve. All the solutions were stored at 4 ℃ in refrigerator.



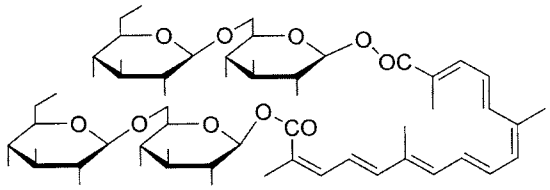
R=H, Geniposidic acid (1)
R=CH_3, Geniposide (2)



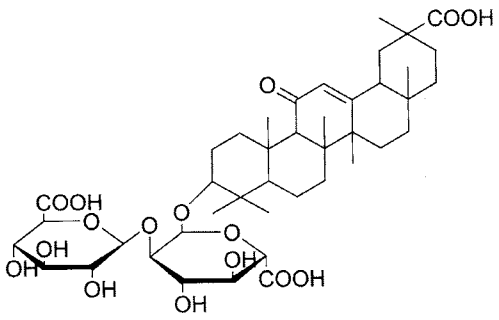
Gentiopicroside (3)



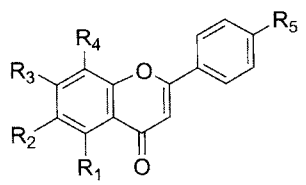
Aristolochic acid A (12)



Crocin (5)



Glycyrrhizic acid (9)



R_1=H, R_2=H, R_3=OH, R_4=H, R_5=Glc,	Liquiritin (4)
R_1=OH, R_2=OH, R_3=GlcA, R_4=H, R_5=H,	Baicalin (6)
R_1=OH, R_2=H, R_3=GlcA, R_4=OCH_3, R_5=H,	Wogonoside (7)
R_1=H, R_2=OH, R_3=OH, R_4=H, R_5=H,	Baicalein (8)
R_1=OH, R_2=H, R_3=OH, R_4=OH, R_5=H,	Wogonin (10)
R_1=OH, R_2=OCH_3, R_3=OH, R_4=H, R_5=H,	Oroxylin A (11)

Figure 1 Structures of the twelve major constituents in LXP

Table 1 Summary of the tested samples of LXP

No.	Sample	Source	Batch No.	Dosage form
1	Tongren Tang	Beijing, China	8083078	Water-honeyed pill
2	Tongren Tang	Beijing, China	8083109	
3	Tongren Tang	Beijing, China	8083143	
4	Tongren Tang	Beijing, China	9083021	
5	Tongren Tang	Beijing, China	9083088	
6	He Nan Xing Yuan	Henan, China	0805260	
7	Shan Xi Wan Hui	Shanxi, China	080501	
8	He Nan Bai Nian Kang Xin	Henan, China	20080601	
9	Luo Yang Shun Shi	Henan, China	20090401	
10	Luo Yang Shun Shi	Henan, China	20081101	
11	Shi Jia Zhuang Hai Tian	Hebei, China	20080501	
12	Yao Du	Hebei, China	080501	
13	Shan Xi Xiang Ju	Shanxi, China	080302	
14	Guang Zhou Zhong Yi	Guangdong, China	L00004	
15	Liao Ning Jin Dan	Liaoning, China	20080111	Big honeyed pill
16	Ha Yao Shi Yi Tang	Heilongjiang, China	0810114	

The water-honeyed pills of LXP were powdered to a homogeneous size by a mortar, and sieved through a No.40 mesh sieve. 2 g of pulverized samples and 4 g of big honeyed pills (Batch No. 20080111 and 0810114) were accurately weighed, transferred into 25 mL volumetric flask, ultrasonically extracted at room temperature with 75% methanol for 1 hour, and then made up to volume. The obtained solution was filtered through a 0.22 μm syringe filter.

2.3 Analytical method

An Agilent-1100 HPLC system with diode array detector was coupled with an LC/MSD Trap XCT electrospray ion mass spectrometer (Agilent Corporation, MA, USA) equipped with quaternary pump, vacuum degasser, autosampler, column heater-cooler (Agilent Corporation, MA, USA). The chromatographic separation was performed on a Dikma ODS C_{18} column (250 mm × 4.6 mm, 5 μm) with the column temperature set at 25 ℃. The mobile phase consisted of acetonitrile (A) and 0.1% (v/v) formic acid (B) with a linear gradient: 0 – 10 min, 5% – 20% A; 10 – 25 min, 20% – 30% A; 25 – 40 min, 30% – 50% A; 40 – 50 min, 50% – 70% A. The flow rate was 1.0 mL/min, and the injection volume was 10 μL. The analytes were monitored at 254 nm. By solvent splitting, 0.2 mL/min portion of the column effluent was delivered into the ion source of the mass spectrometer.

LC-MS detection was performed directly after UV-DAD measurements. Analyses were performed using an LC/MSD Trap XCT mass spectrometer (Agilent Corporation, MA, USA) equipped with an ESI source. The ESI-MS spectra were acquired both in positive and negative ion modes. The MS conditions were as follows: collision energy (Ampl), 1.0 V; collision gas, He; drying gas N_2, 8 L/min; temperature, 350 ℃; pressure of nebulizer, 30 psi; HV voltage, 3.5 kV; scan range, 100 – 1 200 u; target mass, 350 u; smart parameter setting, active. Data acquisition was performed using Chemstation software (Agilent Corporation, MA, USA).

3 Results and discussion

3.1 Optimization of the chromatographic conditions and extraction

Because of the existence of acidic ingredients in LXP extraction, a small amount of acid was added into the mobile phase which could inhibit the ionization of these components to improve the peak shape and restrain the peak tailing. Zero%, 0.1% and 0.2% aqueous formic acid and acetic acid solutions were compared. The results showed that all compounds could be baseline separated when 0.1% aqueous formic acid solution was selected.

DAD detection was employed at wavelength range of 190 – 400 nm to investigate the UV spectra of the twelve reference compounds. It was found that 254 nm was the best wavelength for the detection because almost all the investigated constituents had the maximum absorption there (Figure 2B, C, and D).

Prior to sample analysis the extraction procedure was optimized. 2.0 g samples were extracted with water, 5% methanol, 30% methanol, 50% methanol, 75% methanol, methanol and ethanol to analyze the effect of the solvent on extraction efficiency. Investigating the dependence of the yield on the extraction solvents, it was found that using 25 mL 75% methanol was the best result. Investigating the dependence of the yield on the duration of the extraction (15, 30, 60 and 90 min), it was found that all the investigated compounds were almost completely extracted when 60 min extraction was used.

3.2 Identification of the bioactive markers in LXP

In the HPLC-ESI/MS spectra, most of investigated compounds exhibited their quasi-molecular ions $[2M+H]^+$, $[M+H]^+$, $[M+Na]^+$ in positive ion mode and $[2M-H]^-$ or $[M-H]^-$ in negative ion mode. Fragment ions obtained by the loss of hexose $[M-162]^-$, H_2O $[M-18]^+$ and CO, could also be observed in the MS^n spectra. On the basis of the MS and UV spectra and comparison of the chromatographic retention times with those of authentic standards, the 11

compounds were identified in 16 batches of LXP. All the investigated compounds showed typical fragmentation patterns as previously reported (Table 2) [14-21].

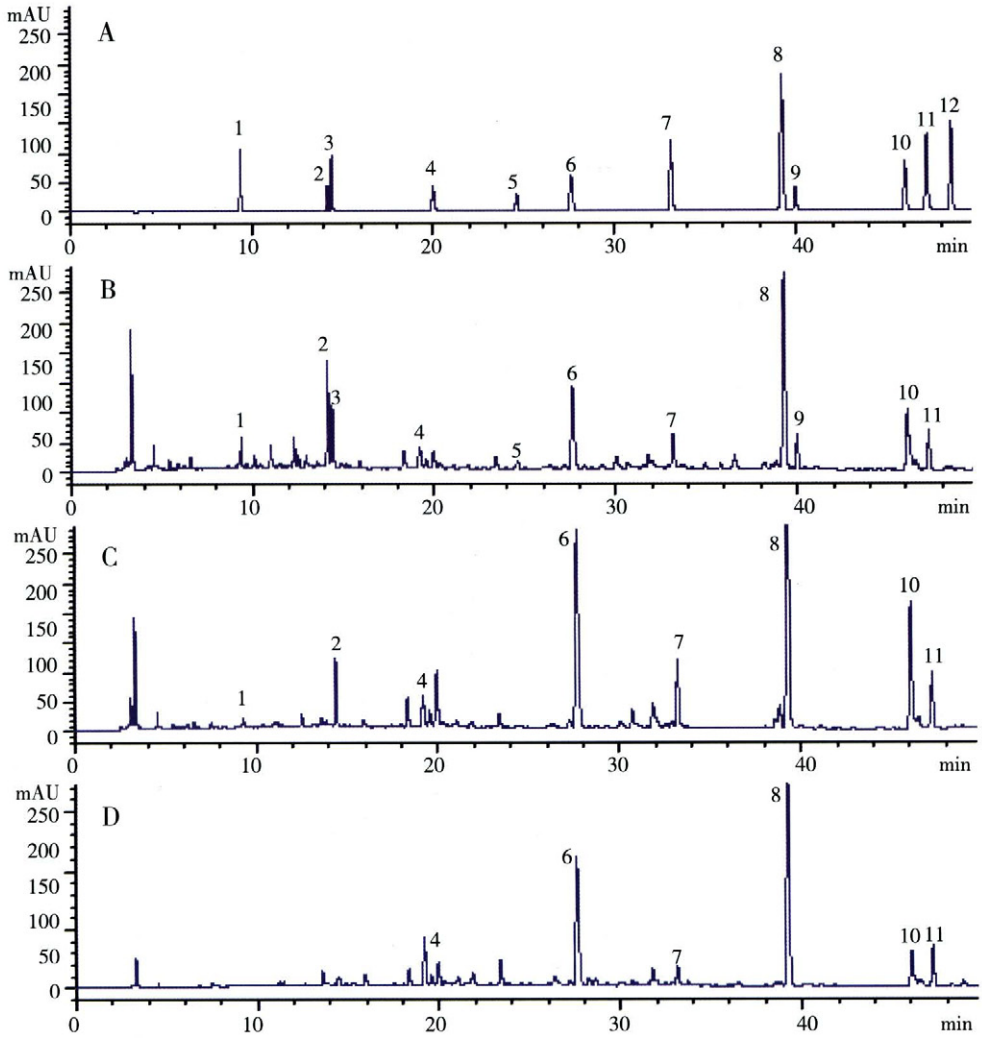


Figure 2 HPLC-DAD chromatograms. (A) HPLC-UV chromatograms of twelve major mixed standards in LXP; (B) HPLC-UV chromatogram of LXP with the detection at 254 nm; (C) HPLC-UV chromatogram of LXP with the detection at 280 nm; (D) HPLC-UV chromatogram of LXP with the detection at 320 nm: geniposidic acid(1), geniposide(2), gentiopicroside(3), liquiritin(4), crocin(5), baicalin(6), wogonoside(7), baicalein(8), glycyrrhizic acid(9), wogonin(10), oroxylin A(11), and aristolochic acid A (12).

Table 2 Chromatographic, UV and mass spectral data of the 12 compounds analyzed by HPLC-DAD-ESI-MS^n

No.	t_R (min)	(+)ESI-MS^n (m/z)		(−)ESI-MS^n (m/z)		λ_{max} (nm)	Identification
		MS	MS^n	MS	MS^n		
1	9.3	–	–	373 $[M-H]^-$	211,123	247	Geniposidic acid
2	14.1	389 $[M+H]^+$	249	775 $[2M-H]^-$, 347 $[M-H]^-$	225,123	240	Geniposide
3	14.4	357 $[M+H]^+$	195,177,149	–	–	204,243,275	Gentiopicroside
4	20.0	–	–	835 $[2M-H]^-$, 417 $[M-H]^-$	255,135	220	Liquiritin
5	24.6	1000 $[M+Na]^+$	675,583,347	976 $[M-H]^-$	651,327,283,234	230	Crocin
6	27.6	447 $[M+H]^+$	271,253,225	891 $[2M-H]^-$, 445 $[M-H]^-$	651	217,277,316	Baicalin
7	33.1	461 $[M+H]^+$	285,270,240,391	919 $[2M-H]^-$, 459 $[M-H]^-$	739,283	274	Wogonoside
8	39.2	271 $[M+H]^+$	241	–	–	247,274,323	Baicalein
9	39.9	823 $[M+H]^+$	669,454,408,390,189	–	–	249	Glycyrrhizic acid
10	46.0	–	–	283 $[M-H]^-$	268, 239,223,212	277	Wogonin
11	47.2	–	–	283 $[M-H]^-$	268,239,223	227,270,317	Oroxylin A
12	48.5	342 $[M+H]^+$	324,298,296	340 $[M-H]^-$	–	241	Aristolochic acid A

The molecular weight of AA was 341, with the fragment ion $[M-H]^-$ at *m/z* 340 and the fragment ion $[M+H]^+$ at *m/z* 342 in the mass spectra. The major fragment ions in the MS^2 spectra of AA were at *m/z* 324 by losing an H_2O unit ($[M+H-H_2O]^+$), *m/z* 298 by losing a CO_2 unit ($[M+H-CO_2]^+$) and *m/z* 296 by losing an NO_2 unit ($[M+H-NO_2]^+$) [22]. But AA in all samples was not detected by extracting its molecularion and fragment ions.

3.3 Validation of the quantitative analysis

3.3.1 Linearity, limit of detection and limit of quantification

The linear calibration curves were constructed with at least six different concentrations of chemical markers. Each concentration was analyzed in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were measured on the basis of the signal-to-noise ratio of 3 and 10 as criteria, respectively. Good linear correlation and high sensitivity under these chromatographic conditions were confirmed by the correlation coefficients ($r > 0.9996$), LOD was 0.29 – 4.17 ng, and LOQ was 0.97 – 12.50 ng (Table 3).

Table 3 Linear regression data, LOD and LOQ of the 12 compounds

Analyte	Linear regression data			LOD (ng)	LOQ (ng)
	Regressive equation	Test range (μg/mL)	*r*		
Geniposidic acid	$y = 6.86x - 10.38$	2.07 – 310.50	0.9999	1.24	4.96
Geniposide	$y = 7.79x + 1.08$	2.51 – 376.50	1.000	1.07	3.73
Gentiopicroside	$y = 10.61x + 14.45$	2.47 – 370.50	0.9996	1.05	2.89
Liquiritin	$y = 5.93x + 0.05$	1.47 – 220.50	1.000	4.17	12.50
Crocin	$y = 5.89x - 3.77$	1.39 – 208.50	0.9999	1.77	3.54
Baicalin	$y = 13.45x - 0.53$	2.46 – 369.00	1.000	1.05	3.14
Wogonoside	$y = 17.66x + 0.73$	2.43 – 364.50	1.000	1.03	2.58
Baicalein	$y = 27.65x + 3.99$	2.44 – 366.00	0.9999	0.41	1.46
Glycyrrhizic acid	$y = 7.84x - 2.90$	2.44 – 366.00	1.000	1.04	3.63
Wogonin	$y = 25.18x + 32.98$	2.90 – 435.00	1.000	0.29	0.97
Oroxylin A	$y = 21.97x - 13.49$	2.54 – 381.00	1.000	0.48	1.27
Aristolochic acid A	$y = 40.04x - 22.50$	2.10 – 315.00	1.000	1.05	2.10

3.3.2 Precision and repeatability

The mixture standard solution was analyzed for six times under the optimal conditions both within 1 day for intra-day variation and on 3 successive days for inter-day variation to evaluate the precision and accuracy. The intra- and inter-day precisions were within 0.7% and 1.3%, respectively. In order to check the repeatability, five different solutions made from the same sample (S4) were determined. The RSD of repeatability was less than 1.8%. These results indicated that the developed method had acceptable precision and repeatability (Table 4).

Table 4 Statistical results of precision and repeatability of the 12 compounds

Compound	Precision		Repeatability	
	Intra-day (RSD, %)	Inter-day (RSD, %)	Content (mg/g)	RSD (%)
Geniposidic acid	0.4	1.0	0.83	1.1
Geniposide	0.4	0.9	1.66	1.3
Gentiopicroside	0.3	0.9	0.70	1.8
Liquiritin	0.3	0.6	0.36	1.2
Crocin	0.6	1.1	0.26	0.7
Baicalin	0.3	0.3	1.66	1.2
Wogonoside	0.2	0.7	0.10	1.8
Baicalein	0.7	0.8	2.91	1.3
Glycyrrhizic acid	0.3	0.7	0.77	0.8
Wogonin	0.7	1.3	0.90	0.8
Oroxylin A	0.4	0.6	0.60	0.9
Aristolochic acid A	0.5	0.6	–	–

3.3.3 Accuracy

In order to evaluate the recovery of this method, three different concentration levels (approximately equivalent to 0.8, 1.0 and 1.2 times of the concentration of the matrix) of the reference standards were added into the sample S4 (about 50% of the sample in triplicate). The solutions were extracted and quantified as described before. The results showed that the assay was satisfactory with the mean recovery from 95.6% to 103.8% with RSD less than 1.9% for the 12 components (Table 5).

3.4 Sample analysis

The described method was applied to analyze the twelve compounds in 16 batches of LXP. The variations of their contents were great (Table 6). Among them, crocin which comes from *Fructus Gardeniae* was even hardly detected in a few samples probably because the content of this bioactive marker was also affected by the year of the plant cultivation, harvest time, climate and environment. The content of AA was under LOD in all the batches of Longdan Xiegan Pill.

To further explore the relationship between different companies, hierarchical cluster analysis was performed, which was a multivariate analysis technique that is used to sort samples into groups. In our study, the hierarchical cluster analysis of samples was performed using SPSS 16.0 software (Chicago, IL, USA). The between-groups linkage method as the amalgamation rule and the squared Euclidean distance as metric were applied to establish clusters. Figure 3

Table 5 Statistical results of recovery of the 12 compounds

Compound	Original (mg)	Spiked (mg)	Found (mg)	Recovery (%)	Mean (%)	RSD (%)
Geniposidic acid	0.84	0.65	1.51	103.1	103.4	0.3
	0.84	0.86	1.73	103.4		
	0.85	1.05	1.94	103.8		
Geniposide	1.68	1.35	3.06	102.2	102.5	0.8
	1.66	1.66	3.35	101.8		
	1.67	2.00	3.74	103.5		
Gentiopicroside	0.72	0.58	1.28	96.6	97.6	1.0
	0.71	0.70	1.40	98.6		
	0.71	0.87	1.56	97.7		
Liquiritin	0.38	0.30	0.67	96.7	96.6	0.9
	0.37	0.38	0.74	97.4		
	0.38	0.45	0.81	95.6		
Crocin	0.26	0.21	0.47	100.0	102.2	1.9
	0.26	0.26	0.53	103.8		
	0.27	0.34	0.62	102.9		
Baicalin	2.04	1.65	3.73	102.4	102.7	0.4
	2.02	2.04	4.11	102.5		
	2.03	2.47	4.58	103.2		
Wogonoside	0.11	0.088	0.20	102.3	100.8	1.3
	0.11	0.11	0.22	100.0		
	0.11	0.13	0.24	100.0		
Baicalein	2.92	2.34	5.33	103.0	103.2	0.3
	2.91	2.90	5.90	103.1		
	2.93	3.59	6.65	103.6		
Glycyrrhizic acid	0.78	0.60	1.36	96.7	97.4	1.1
	0.78	0.79	1.56	98.7		
	0.79	0.96	1.72	96.9		
Wogonin	0.92	0.75	1.69	102.7	102.9	0.3
	0.91	0.92	1.86	103.3		
	0.92	1.08	2.03	102.8		
Oroxylin A	0.61	0.50	1.12	102.0	102.1	0.6
	0.61	0.66	1.28	101.5		
	0.62	0.75	1.39	102.7		
Aristolochic acid A	0	0.40	0.40	100.0	100.6	1.0
	0	0.50	0.50	100.0		
	0	0.60	0.61	101.7		

shows the resulting dendrogram, which is divided into two main clusters. Cluster Ⅰ was formed by the sample S1 – S5 and S12. The remaining 10 samples from 9 companies belonged to cluster Ⅱ. Cluster Ⅰ was branched into two subgroups, which indicated that the internal quality of samples in the same company was much similar to each other. Cluster Ⅱ was also branched into two subgroups, which indicated that same dosage form was much similar to each other. Therefore, the supply and quality of medicinal substances and the quality standard of preparations should be regulated in the future to ensure the safety of LXP.

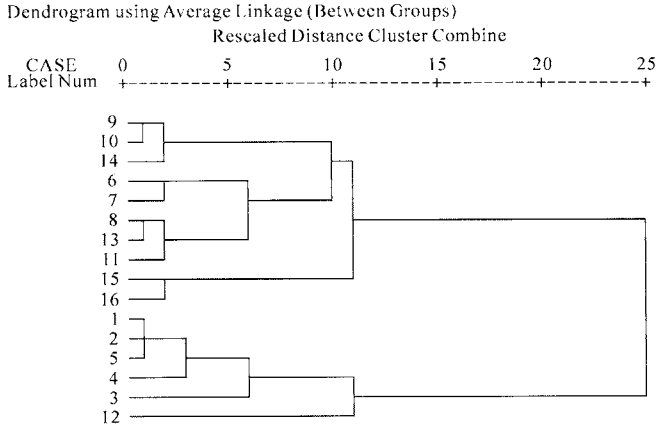


Figure 3 Dendrograms of hierarchical cluster analysis for the 16 tested samples of LXP. The hierarchical clustering was done by SPSS software. Between-groups linkage method was applied, and Squared Euclidean distance was selected as measurement.

4 Conclusion

Traditional Chinese medicine has been used for thousands of years in China and the adverse effects of this remedy have been said to be rare. However, with its increasing popularity in western countries, an increasing number of adverse effects have also been observed. Some of these adverse effects were due to the incorrect identification of plant material.

Table 6 Contents of the 12 compounds in the 16 samples ($n = 3$)

Sample No.	Content of each compound (mg/g)											
	Geniposidic acid	Geniposide	Gentiopicroside	Liquiritin	Crocin	Baicalin	Wogonoside	Baicalein	Glycyrrhizic acid	Wogonin	Oroxylin A	Aristolochic acid A
S1	0.85±0.01	1.86±0.01	0.65±0.01	0.30±0.01	0.220±0.010	0.59±0.01	0.14±0.01	2.21±0.03	0.74±0.01	0.73±0.02	0.550±0.010	–
S2	0.72±0.02	1.73±0.02	0.51±0.01	0.33±0.01	0.220±0.010	0.20±0.01	0.04±0.002	1.93±0.02	0.57±0.01	0.76±0.01	0.490±0.020	–
S3	0.70±0.03	1.85±0.02	0.93±0.01	0.30±0.01	0.320±0.020	1.56±0.02	0.34±0.01	1.55±0.02	0.80±0.01	0.61±0.01	0.400±0.010	–
S4	0.83±0.01	1.66±0.02	0.70±0.02	0.36±0.01	0.260±0.010	1.66±0.02	0.10±0.02	2.91±0.03	0.77±0.01	0.90±0.02	0.600±0.010	–
S5	0.61±0.02	1.26±0.01	0.40±0.02	0.32±0.01	0.200±0.010	0.94±0.01	0.24±0.01	2.34±0.01	0.61±0.01	0.87±0.01	0.430±0.010	–
S6	0.31±0.01	0.63±0.03	0.83±0.04	0.41±0.02	–	0.25±0.02	0.18±0.01	0.08±0.005	0.39±0.03	0.16±0.01	0.120±0.010	–
S7	0.47±0.01	1.54±0.03	1.13±0.02	0.25±0.01	–	1.11±0.06	0.36±0.04	0.25±0.06	0.24±0.05	0.17±0.01	0.160±0.010	–
S8	0.72±0.01	2.55±0.04	1.18±0.02	0.28±0.01	0.090±0.001	2.03±0.07	0.46±0.02	0.47±0.02	0.42±0.01	0.58±0.02	0.200±0.010	–
S9	1.30±0.02	1.92±0.02	1.33±0.02	0.30±0.01	0.070±0.003	3.77±0.03	0.81±0.01	0.61±0.01	0.89±0.02	0.26±0.02	0.190±0.010	–
S10	1.49±0.01	2.52±0.02	1.72±0.01	0.31±0.01	0.040±0.002	4.30±0.04	0.87±0.02	0.71±0.01	1.26±0.01	0.26±0.01	0.170±0.020	–
S11	1.39±0.01	3.46±0.04	0.73±0.01	0.23±0.01	–	2.59±0.03	0.46±0.02	0.77±0.03	0.58±0.02	0.48±0.02	0.180±0.010	–
S12	1.01±0.02	1.31±0.02	3.43±0.02	0.31±0.01	0.090±0.001	1.69±0.01	0.34±0.01	1.69±0.01	1.57±0.01	0.55±0.02	0.180±0.010	–
S13	0.81±0.02	1.86±0.02	1.12±0.02	0.17±0.01	0.050±0.001	1.99±0.01	0.42±0.01	0.24±0.01	0.27±0.01	0.13±0.01	0.080±0.003	–
S14	1.27±0.02	1.35±0.02	3.02±0.02	0.21±0.01	–	3.85±0.03	0.86±0.01	0.55±0.02	0.94±0.01	0.38±0.01	0.140±0.010	–
S15	2.35±0.01	0.95±0.02	0.01±0.004	0.08±0.002	0.030±0.001	1.14±0.02	0.26±0.01	0.22±0.02	0.25±0.01	0.12±0.01	0.090±0.002	–
S16	3.20±0.01	0.56±0.02	0.72±0.03	0.10±0.01	0.030±0.001	1.17±0.01	0.25±0.01	0.31±0.02	0.40±0.01	0.15±0.01	0.050±0.001	–

In practical application, Mutong is often substituted by nephrotoxic and carcinogenic Guanmutong by mistake. In Guanmutong, the content of AA is found at a high level, while in Mutong the AA is not found. We suggest that all herbs should undergo quality controls and toxicological studies as strict as conventional drugs.

The proposed HPLC-DAD-ESI-MS method makes it possible to evaluate the quality of the commonly used TCM LXP through a simultaneous determination of multi-components. This method has been successfully applied to simultaneously identify and quantify 11 compounds in 16 batches LXP samples. Additionally, the method was validated for good linearity, limit of detection, accuracy and precision. The HPLC assay can be utilized as a suitable quality control method for the determination of the major biologically active ingredients in LXP.

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