Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 1097–1100

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

GC–MS analysis of liposoluble constituents from the stems of *Cynomorium songaricum*

Yu-Bi Zhou[a,b,c], Run-Rong Ye[a], Xue-Feng Lu[a], Peng-Cheng Lin[c], Shi-Bing Yang[a,b], Peng-Peng Yue[a,b], Chang-Xian Zhang[a,b], Min Peng[a,b,*]

[a] *Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China*
[b] *Graduate School of Chinese Academy of Sciences, Beijing, China*
[c] *Pharmacy Department of Qinghai University for Nationalities, Xining, China*

ARTICLE INFO

Article history:
Received 9 October 2008
Received in revised form 27 January 2009
Accepted 30 January 2009
Available online 7 February 2009

Keywords:
Liposoluble constituents
GC–MS
Cynomorium songaricum
Geographic origin
Host

ABSTRACT

In order to evaluate the differences and similarities between the liposoluble constituents in *Cynomorium songaricum* populations, stem liposoluble constituents in five populations of *C. songaricum* collected from three different geographic regions and four different hosts were obtained by solvent extraction and analyzed by GC–MS. Cluster analysis of the percentage composition of 80 compounds showed differences in chemical composition which were related to the geographic origin rather than the host. Hexadecanoic acid was the most abundant compound in the essential oils of *C. songaricum* from hosts *Nitraria sibirica* and *Nitraria tanguticum*. Whereas (*Z*)-9-octadecenoic acid was accumulated in the oils of *C. songaricum* from *Zygophyllum xanthoxylum* and *Peganum harmala*. Four of the five populations had characteristic components, which were specific to each population.

1. Introduction

Cynomorium songaricum Rupr., known as "Suoyang" in China, is a root-parasitic plant species distributed in northwest China. This species is known to parasitize the plant roots of *Nitraria* spp. including *Nitraria sibirica* and *Nitraria tanguticum*, but is also known to parasitize the roots of other plants such as *Zygophyllum xanthoxylum* (Bunge) Maxim. and *Peganum harmala* L. The stem of *C. songarcum* is used in traditional Chinese medicinal materials, and is generally used to increase sexual capability, as a laxative, and to treat lumbar weakness [1]. This herb has other potential activities including: anti-hypoxic [2], antianoxic and is used as an antiepileptic agent [3]. The herb extract has also been shown to inhibit HIV-1 protease [4], improve immunity [5] and improve physical endurance and antioxidant status [6] in laboratory experiments.

As a medicinal material, the chemical composition of the plant is very important. Earlier studies have identified the presence of organic acids [7], flavonoids [7,8], triterpenes [4,7–10], steroids [7,8], volatiles [11], amino acids [12], glucosides [7,8,13,14], tannins [15], inorganic ions [16,17], and lignanoids [18] in the stem of *C. songaricum*. However, the liposoluble composition of *C. songaricum* has not been comprehensively analyzed.

In this study, five populations of *C. songaricum* were collected from three different regions (Alxa zuoqi in Inner Mongolia, Guazhou county in Gansu Province, Golmud in Qinghai Province) and four different hosts (*N. sibirica*, *N. tanguticum*, *Z. xanthoxylum* and *P. harmala*) between 2005 and 2006. The liposoluble constituents of these samples were comprehensively analyzed by GC–MS, and the relationships between the types of liposoluble compounds, the regions and their hosts were determined.

2. Experimental

2.1. Chemicals and reagents

Petroleum ether (30–60 °C, analytically pure) was obtained from the Dong Fang Hong Chemical Plant of Lin Bo (Zibo, China). *n*-Hexane, dichloromethane, methanol, boron trifluoride and ethyl ether (all analytically pure) were purchased from TianJin BASF Chemical Trade Co. Ltd. (Tianjin, China).

2.2. Plant specimens

Stems of *C. songaricum* were collected from four different hosts and three different sampling sites in the northwest of China in the spring of 2005 and 2006 (Table 1). The plants investigated

* Corresponding author at: Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China. Tel.: +86 971 6143898; fax: +86 971 6143282.
E-mail address: pengm@nwipb.ac.cn (M. Peng).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.01.038

Table 1
Samples of Cynomorium songaricum.

Population	Plants (*n*)	Collection site	Host	Latitude (N)	Longitude (E)	Altitude (m)	Date of collection
P1	4	Golmud	*N. tanguticum*	36°11′47″	97°24′36″	2814	21 May 2006
P2	4	Guazhou (Anxi)[a]	*N. tanguticum*	40°15′06″	96°11′55″	1347	2 May 2006
P3	4	Alxa zuoqi I	*N. sibirica*	39°36′27″	103°06′10″	1238	27 April 2006
P4	2	Alxa zuoqi II	*Z. xanthoxylon*	39°11′12″	105°39′43″	1282	26 April 2006
P5	2	Alxa zuoqi III	*P. harmala*	39°39′10″	103°06′38″	1245	18 May 2005

[a] Anxi is Guazhou's original name.

were identified by one of the authors (Professor X.L.), and voucher specimens were deposited in the herbarium of the Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China (NWIPB).

2.3. Sample preparation

Following desiccation and comminution, powdered samples were obtained. The powdered crude drug (20 g in each sample) was extracted with petroleum ether (100 ml) for 48 h. The petroleum ether extracts were filtered and allowed to evaporate by placing the beakers in water at 40 °C. Prior to complete evaporation of the solvent, when only 1.5 ml remained in the beakers, the samples were transferred to centrifuge tubes and further evaporated to 0.5 ml by placing the centrifuge tubes in water at 40 °C. The dissoluble organic substances contained in the five populations were then divided into three portions: the non-polar fraction, the weak polar fraction and the polar fraction by silica gel–alumina (3:1) column separation using *n*-hexane, dichloromethane and methanol as the eluting reagent, respectively. The non-polar fraction and the weak polar fraction were analyzed using GC–MS. The polar fraction was analyzed using GC–MS after methyl esterification using BF_3–CH_3OH (1:4) for 24 h and extraction with ethyl ether. Following column chromatography, the liposoluble composition of *C. songaricum* was comprehensively analyzed.

2.4. GC–MS conditions

A HP 6890 gas chromatograph equipped with a HP 7683 auto injector and a HP 5973 MSD (Agilent Technologies, Palo Alto, CA, USA) were used for GC–MS analysis. Gas chromatographic separation was carried out with a HP-5 capillary column (30 m × 0.25 mm I.D., film thickness: 0.25 μm).

Samples (1 μl) were injected manually in the pulsed splitless mode. The pulse time was 1.5 min, the pressure was 200 kPa, the injector temperature was 250 °C and the helium carrier gas flow-rate was 1.2 ml/min. The column temperature was increased from 80 °C to 290 °C at 4 °C/min. Constant temperature time was 30 min. MSD ion source was electron ionization, and the ion source temperature was 230 °C. All mass spectra were recorded at 70 eV.

2.5. Identification

The compounds were identified by comparing their retention indices and mass spectra with those of the NIST02L mass spectral library (National Institute of Standards and Technology) provided by the software of the GC–MS system. Compound concentrations were calculated from the GC peak areas of the total ion current (TIC). The key components found in the non-polar polar fractions included stigmast-3,5-diene in population 1 (P1), P3 and P5, and *n*-heptacosane in P2 and P4. The key components in the weak polar fractions included stigmast-3,5-dien-7-one in P1, P4 and P5, γ-sitosterol in P2 and β-sitosterol in P3. The key components in the polar fractions included hexadecanoic acid in P1, P2 and P3, and (*Z*)-9-octadecenoic acid in P4 and P5.

2.6. Statistics

The percentage composition of essential oil samples was used to determine the relationships between the different populations of *C. songaricum* by cluster analysis using NTSYS software [19]. Euclidean distance was selected as a measure of similarity, and the unweighed pair-group method with arithmetic average (UPGMA) was used for cluster definition. The method achieved rapid cluster analysis of the percentage composition of essential oil samples.

3. Results and discussion

From the soak extraction, clear to light yellow liposoluble constituents were obtained in yields of 0.135–0.304% (Table 2). The dissoluble organic substances were divided into three portions: the non-polar fraction (8.06–18.42%), the weak polar fraction (5.39–26.53%) and the polar fraction (65.36–78.77%). The liposoluble constituents contained in *C. songaricum* were mainly found in the polar fraction in yields of 65.37–78.78%. The extract contained more non-polar fraction than the weak polar fraction in population 1 (P1) and P2, and the reverse was observed in P3, P4 and P5.

A total of 80 compounds were identified in the stem extracts, including 22 non-polar compounds, 37 weak polar compounds and 21 polar compounds, which amounted to a total percentage of 97.66–98.19%. The identified components in the stem extracts and their percentages in the respective fractions are shown in Table 2. Typical non-polar fraction, weak polar fraction and polar fraction chromatograms of *C. songaricum* are shown in Fig. 1.

70 compounds were found in all analyzed samples (five populations). However, there were characteristic components which were observed in some populations but not in others. These characteristic components were only found in the weak polar fractions, and included urs-12-en-3-one (0.23%) and 4,4-dimethylcholest-7-en-3-one (0.17%) in P1, stigmast-4-en-3-one (0.37%) in P2, β-sitosterol (6.96%) and 22,23-dihydrostigmasterol (1.36%) in P3, and stigmast-3,5,22-trien-7-one (0.62%) in P5. Based on the number of chemical components, P3 with 76 components contained the most complex oil. P4 and P5 with 73 components contained the simplest oil.

In terms of percentage amounts, hexadecanoic acid and (*Z*)-9-octadecenoic acid were the two major compounds found in the liposoluble constituents. In the volatile oil, hexadecanoic acid and 9-octadecenoic acid were identified as the two main compounds [12]. The chemical compounds of parasitic plants are often affected by distinct hosts [20]. Hexadecanoic acid (21.89–39.79%) was the most abundant liposoluble constituent in P1, P2 and P3. However, the most abundant component in P4 and P5 was (*Z*)-9-octadecenoic acid (27.08% and 29.16%, respectively). *N. sibirica* and *N. tanguticum* were host to *C. songaricum* (P1, P2 and P3). *Z. xanthoxylon* and *P. harmala* were host to P4 and P5, respectively.

The results showed that *n*-alkanes were the main grouped components in the non-polar fraction. Aldehydes, methyl esters and ethyl esters, alkyl-2-ketones and steroids (including four phytosterols: campesterol, γ-sitosterol, β-sitosterol, 22,23-dihydrostigmasterol) were the major grouped components in the weak polar fraction. Phytosterols have cholesterol-lowering properties [21]. Campesterol was found in P2 and P3 and γ-

Table 2
Chemical composition of the non-polar fraction, the weak polar fraction and the polar fraction of liposoluble constituents from the stems of *Cynomorium songaricum*.

No. compound[a]	Percent composition[b]				
	P1	P2	P3	P4	P5
Non-polar fraction					
n-Heptadecane	0.41	0.09	0.10	T	0.01
Pristane	0.16	0.04	0.06	T	T
n-Octadecane	0.56	0.18	0.89	T	0.02
Phytane	0.45	0.10	0.23	T	0.01
n-Nonadecane	0.53	0.20	0.34	0.01	0.02
n-Eicosane	0.50	0.48	1.05	0.03	0.11
n-Heneicosane	0.70	0.45	0.36	0.04	0.07
n-Docosane	0.64	0.53	0.59	0.13	0.18
n-Tricosane	0.47	0.87	0.56	0.18	0.22
n-Tetracosane	0.53	0.77	0.64	0.25	0.34
n-Pentacosane	0.84	1.33	0.84	0.39	0.53
n-Hexacosane	0.65	0.90	0.72	0.35	0.44
n-Heptacosane	0.97	2.23	0.83	0.55	0.60
n-Octacosane	0.84	0.78	0.58	0.27	0.28
n-Nonacosane	1.02	1.21	0.57	0.41	0.27
n-Triacontane	0.42	0.51	0.28	0.11	0.11
Stigmast-3,5-diene	6.60	0.75	2.78	5.19	4.24
n-Hentriacontane	0.62	0.54	0.32	0.08	0.08
n-Dotriacontane	0.38	0.30	0.11	0.03	0.04
n-Tritriacontane	0.40	0.29	0.15	0.02	0.03
n-Tetratriacontane	0.34	0.22	0.17	0.01	0.03
n-Pentatriacontane	0.39	0.22	0.13	0.01	0.03
Identified components (%)	94.63	91.33	92.04	95.15	93.17
Weak polar fraction					
Octanoic acid, ethyl ester	0.02	T	T	T	0.01
Decanal	0.02	T	T	T	0.01
2-Acetyl-3,4,5,6-tetrahydropyridine	0.26	T	0.02	0.03	0.15
(*E*)-2-Decenal	0.10	T	0.02	0.04	0.13
(*Z*,*E*)-2,4-Decadienal	0.13	0.01	0.04	0.13	0.06
(*E*,*E*)-2,4-Decadienal	0.22	0.01	0.09	0.20	0.09
2-Undecenal	0.08	T	0.01	0.02	0.11
2,6-Bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione	0.04	T	T	0.01	0.01
Benzyl benzoate	0.04	T	0.01	T	0.02
9-Oxononanoic acid, ethyl ester	0.03	0.01	T	0.05	0.06
Nor-pristanone	0.02	0.01	0.01	0.08	0.01
Hexadecanoic acid, methyl ester	0.02	T	0.01	0.02	0.02
Hexadecanoic acid, ethyl ester	0.03	T	0.09	0.04	0.03
9,12-Octadecadienoic acid, methyl ester	0.02	0.01	0.02	0.05	0.02
9-Octadecenoic acid, methyl ester	0.08	0.02	0.02	0.08	0.05
9,12-Octadecadienoic acid, ethyl ester	0.07	0.01	0.16	0.15	0.03
9-Octadecenoic acid, ethyl ester	0.16	0.01	0.31	0.19	0.09
2-Heneicosanone	0.02	0.01	T	0.02	0.03
Eicosanoic acid, methyl ester	0.02	T	0.01	0.02	0.02
4,8,12-Trimethyltridecan-4-olide	0.04	0.01	0.02	0.02	T
Docosenoic acid, methyl ester	0.02	T	T	0.01	0.01
Ethyl 13-docosenoate	0.01	T	0.02	0.02	0.02
2-Pentacosanone	0.02	T	T	0.01	0.02
Tetracosanoic acid, methyl ester	0.02	T	T	0.03	0.02
2-Heptacosanone	0.08	T	T	0.03	0.02
Cholesta-2,5-dien-4-one	0.07	T	0.02	0.19	0.08
Stigmast-2,5,22-triene	0.48	0.02	0.07	0.44	2.31
Homopregnan-5,18-dienol acetate	0.17	T	0.02	0.32	0.12
Urs-12-en-3-one	0.23	–	–	–	–
4,4-Dimethylcholest-7-en-3-one	0.17	–	–	–	–
Stigmast-3,5-dien-7-one	2.20	0.54	3.45	12.36	10.85
β-Sitosterol acetate	0.50	–	0.30	2.94	1.45
Campesterol	–	1.41	3.68	–	–
γ-Sitosterol	–	5.75	4.30	9.03	–
Stigmast-4-en-3-one	–	0.37	–	–	–
Stigmast-3,5,22-trien-7-one	–	–	–	–	0.62
β-Sitosterol	–	–	6.96	–	–
22,23-Dihydrostigmasterol	–	–	1.36	–	–
Identified components (%)	90.85	98.03	97.60	96.72	94.70
Polar fraction[c]					
N-(2-Methylacryloyl)imidazola	0.48	0.18	0.06	0.10	0.20
9-Oxononanoic acid	0.78	0.43	0.92	0.36	0.27
7-(1,1-Dimethylethyl)-3,4-dihydro-1(2H)-naphthalenone	0.11	0.07	0.12	0.53	0.16
Nonanedioic acid	0.09	0.19	0.07	0.15	0.11
10-Oxo-8-decenoic acid	0.58	0.41	0.16	0.20	0.36
Tetradecanoic acid	0.24	0.28	0.16	0.22	0.13
9-Hexadecenoic acid	0.09	0.19	0.19	0.14	0.08
Hexadecanoic acid	38.49	39.79	21.89	13.50	26.20
Heptadecanoic acid	0.02	0.10	0.14	0.07	0.08
9,12-Octadecadienoic acid	1.08	1.81	6.11	7.93	3.96
(*Z*)-9-Octadecenoic acid	15.19	8.58	18.50	27.08	29.16
(*E*)-9-Octadecenoic acid	0.29	0.46	0.31	0.36	0.18
Octadecanoic acid	1.11	2.57	1.34	1.15	1.27
(*E*,*E*)-9,11-Octadecadienoic acid	0.20	0.17	0.44	0.08	0.10
11-Eicosenoic acid	0.65	0.30	0.76	0.77	0.55
Eicosanoic acid	3.71	9.39	5.78	4.49	3.47
(*Z*)-14-Octadecen-1-ol acetate	1.56	1.23	0.50	0.25	0.47
13-Docosenoic acid	0.60	0.40	0.78	1.01	0.87
Docosenoic acid	8.73	10.28	6.95	5.46	5.49
Tricosanoic acid	0.06	0.08	0.09	0.04	0.04
Tetracosanoic acid	2.03	1.74	1.22	1.28	2.67
Heptacosanoic acid	0.09	0.12	0.16	0.19	0.07
Identified components (%)	99.04	98.75	98.85	98.24	99.41
Total identified components (%)	97.74	97.73	97.79	97.66	98.19
Grouped components					
n-Alkanes	11.23	12.10	9.23	2.88	3.42
Aldehydes	0.54	0.02	0.16	0.40	0.39
Methyl esters and ethyl esters	0.53	0.08	0.64	0.66	0.39
Alkyl-2-ketones	0.12	0.01	0.01	0.06	0.07
Steroids	4.99	8.13	20.83	30.38	17.73
Fatty acids	74.05	77.30	65.98	64.49	75.04
Others	8.55	2.36	3.15	1.12	2.95
Oil yield (%, v/w)	0.232	0.135	0.185	0.177	0.304

[a] Compounds listed in order of elution from HP-5 column.
[b] T (trace): relative content <0.01%; (–) not detected.
[c] In polarity fraction, fatty acids were detected as methyl esters using BF_3–CH_3OH (1:4) as the methyl esterification system.

sitosterol were detected in P2, P3 and P4. β-Sitosterols and 22,23-dihydrostigmasterol were characteristic components of P3. Moreover, the polar fraction mostly contained fatty acids, including a small amount of (*E*, *E*)-9,11-octadecadienoic acid which is a conjugated linoleic acid and is an important nutrient in human nutrition.

The three main grouped components were found in the essential oils of all five populations of *C. songaricum*. The content of these three main grouped components was over 90%. Fatty acids (64.49–77.30%, Table 2) were the major grouped components in all five populations. *n*-Alkanes were the second major grouped components in P1 and P2. Steroids were the second major grouped components in P3, P4 and P5 obtained from Alxa zuoqi. The sec-

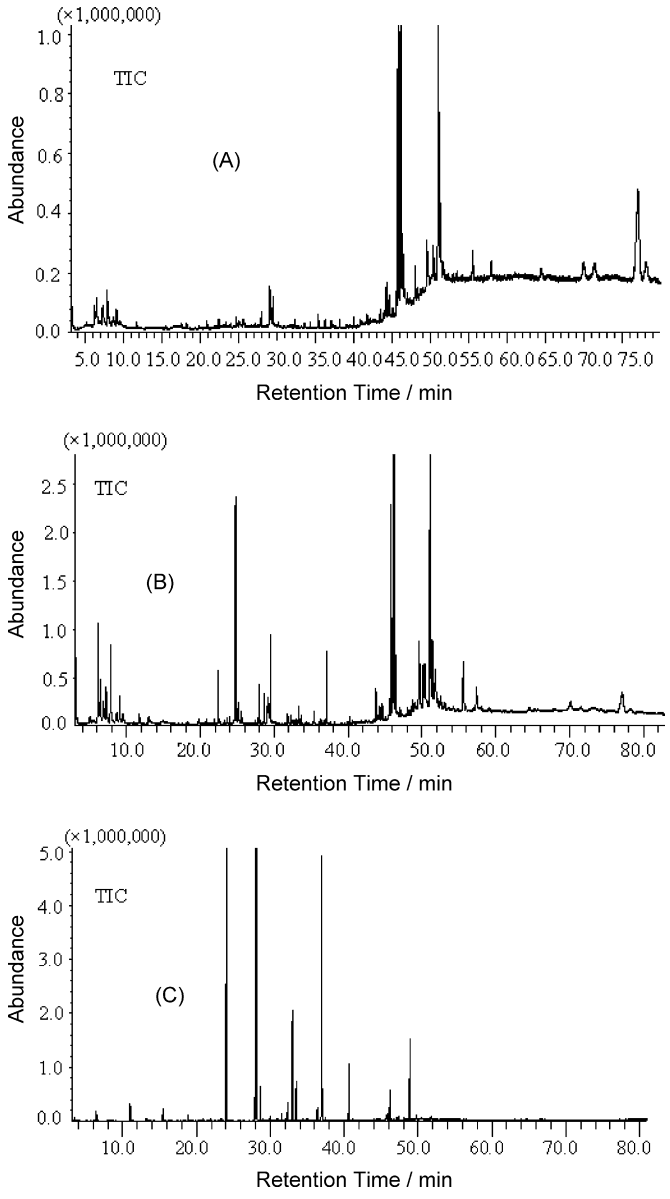

Fig. 1. TIC of the essential oil from the stem of *Cynomorium songaricum* (population 1). (A) Non-polar fraction, (B) weak polar fraction and (C) polar fraction. For chromatographic conditions see Section 2.

ond major grouped components were found to be related to the geographic origin of the populations.

Cluster analysis of all identified components grouped the stem oils from the five populations into two main clusters (Fig. 2). The first cluster was formed by P1 and P2, and *N. tanguticum* was the common host of P1 and P2. The second cluster was composed of P3, P4 and P5 which was parasitic on three different hosts: *N. sibirica*, *Z. xanthoxylon* and *P. harmala*. The habitat of P1 and P2 is gravel desert. However, the habitat of P3, P4 and P5 is sand desert as Alxa zuoqi is located in the Tengger Desert. Although P3, P4 and P5 were collected from three different hosts, they were clustered together in the second cluster. In the second cluster, P4 and P5 obtained from *Z. xanthoxylon* and *P. harmala*, respectively were clustered together. Although samples of P1, P2 and P3 were obtained from the genus *Nitraria*, they were not clustered together. Thus cluster analysis of the percentage composition of 80 compounds showed differences in the chemical composition according to geographic origin. In data reported for *Hyptis suaveolens*, differences in chemical composition were also related to geographic origin [22].

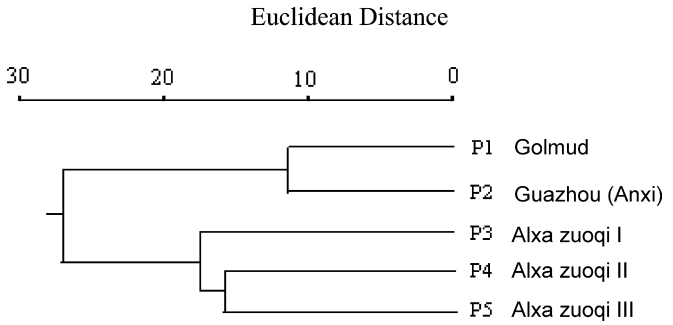

Fig. 2. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from five populations of *Cynomorium songaricum*, based on Euclidean distance and the use of the unweighed pair-group method with arithmetic average (UPGMA).

In addition, the cluster formed by P1 and P2 showed similar high levels of hexadecanoic acid (38.49% and 39.79%, respectively). High levels of 9,12-octadecadienoic acid in P3, P4 and P5 from Alxa zuoqi were found compared with P1 and P2 from Golmud and Guazhou.

4. Conclusions

Chemical polymorphism was found in the liposoluble constituents from the stems of five populations of *C. songaricum*. The liposoluble constituents of *C. songaricum* were influenced by geographic origin more than the host. GC–MS was found to be a robust method for analyzing and identifying liposoluble constituents in *C. songaricum* samples. GC–MS may also be used in the quality control of this herbal medicine.

Acknowledgements

We are grateful to Dr. Yongzhen Tian (Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences) for his assistance in offering partial testing materials as well as help given in the sampling process, and to Qianxiang Meng (Key Laboratory of Gas Geochemistry, Chinese Academy of Sciences) for identifying chemical constituents.

References

[1] Pharmacopoeia PRC, vol. 1, 2005, p. 241.
[2] J. Luo, R. Zhang, Z. Jia, M. Li, J. Wang, J. Hu, Tradit. Chin. Drug Res. Clin. Pharmacol. 18 (2007) 275–279.
[3] Y. Hu, Z. Wang, W. Xiao, J. Shihezi Univ. (Nat. Sci.) 23 (2005) 302–303.
[4] C. Ma, N. Nakamura, H. Miyashiro, M. Hattori, K. Shimotohno, Phytother. Res. 12 (1998) 138–142.
[5] Y. Zhen, Q. Sun, Z. Yan, J. Gansu Coll. TCM 4 (1991) 28–30.
[6] Z. Wang, C. Guo, Y. Fu, D. Li, J. Northwest Norm. Univ. (Nat. Sci.) 4 (2006) 100–102.
[7] J. Tao, P. Tu, China J. Chin. Mater. Med. 24 (1999) 292–295.
[8] S. Zhang, Y. Wang, L. Liu, J. Yu, J. Hu, J. Chin. Pharm. 42 (2007) 975–977.
[9] D. Di, Y. Liu, X. Mao, J. Chen, Chin. Hosp. Pharm. J. 24 (2004) 730–732.
[10] C. Ma, N. Nakamura, M. Hattori, S. Cai, Chin. Pharm. J. 37 (2002) 336–338.
[11] S. Zhang, S. Zhang, China J. Chin. Mater. Med. 2 (1990) 39–41.
[12] S. Zhang, S. Zhang, Chin. Pharm. J. 26 (1991) 649–651.
[13] C. Zhang, X. Xu, C. Li, Phytochemistry 41 (1996) 975–976.
[14] S. Zhang, S. Zhang, J. Hu, China J. Chin. Mater. Med. 26 (2001) 409–411.
[15] Y. Chang, G. Su, C. Yin, J. Zhang, H. Bu, J. Chin. Med. Mater. 28 (2005) 116–118.
[16] G. Xue, Q. Liu, X. Ren, Y. Han, Spectrosc. Spect. Anal. 24 (2004) 1461–1463.
[17] Y. Zhang, D. Wu, C. Li, P. Li, J. Tradit. Chin. Vet. Med. 5 (2004) 8–9.
[18] Z. Jiang, T. Tanaka, M. Sakamoto, T. Jiang, I. Kouno, Chem. Pharm. Bull. 49 (2001) 1036–1038.
[19] F.J. Rohlf, NTSYS-pc (Numerical taxonomy and multivariate analysis system. Version 1.8). Applied Biostatistics Inc., New York, 1992.
[20] Y. Chao, Chem. Bioeng. 3 (2005) 55–56.
[21] R.A. Moreau, B.D. Whitaker, K.B. Hicks, Prog. Lipid Res. 41 (2002) 457–500.
[22] P. Grassi, M. Nunez, K. Varmuza, C. Franz, Flavour Frag. J. 20 (2005) 13–135.