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VOLATILES OF *LYSIMACHIA PARIDIFORMIS* VAR. *STENOPHYLLA*, *LYSIMACHIA FORTUMEI* AND *LYSIMACHIA CHIKUNGENSIS* BY HS-SPME-GC-MS

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Abstract

Background: *Lysimachia paridiformis* Var. *Stenophylla* mainly contain flavonoid constituents. Flavonoids and benzoquinones are the main compounds in *L. fortumei* Maxim. The objective of this paper was to study the volatile compounds of leaves in *L. paridiformis* Var. *Stenophylla*, *L. fortumei* and *L. chikungensis* for the first time.

Materials and Methods: Volatiles were extracted by the manual solid phase micro-extraction (SPME). The volatile constituents were analyzed by an Agilent 6890 N gas chromatograph equipped and coupled with a 5975B mass selective detector spectrometer.

Results: Twenty-nine compounds were identified in the leaves of *L. paridiformis* var. *Stenophylla*, accounting for 89.17% of the total volatile fraction. The main constituents were ethanol (13.58%), and *β*-ionone (8.05%). linalool and *β*-ionone were the main aroma constituents in *L. paridiformis* var. *Stenophylla*. Twenty-one compounds were identified in the leaves of *L. fortumei*, accounting for 94.72% of the total volatile fraction. The main constituents were tricosane (14.72%), docosane (11.02%), tetracosane (10.77%) and pentacosane (9.81%). Thirty-two compounds were identified in the leaves of *L. chikungensis*, accounting for 88.58% of the total volatile fraction. Typical compounds detected in *L. chikungensis* were *cis*-3-hexenyl pentanoate (13.33%), followed by ethanol (12.13%), ethyl palmitate (7.78%), and heneicosane (5.38%).

Conclusion: The results showed that the main composition types were similar in the three plants, but the content was different, which indicated that the similar composition types provided the same medical effect for three plants.

Keywords: *Lysimachia paridiformis* Var. *Stenophylla*, *Lysimachia fortumei*, *Lysimachia chikungensis*, solid phase microextraction, GC-MS, volatiles.

Introduction

Lysimachia paridiformis var. *stenophylla* (Myrsinaceae), the variety of *L. paridiformis* Franch, is much more widely distributed than the type variety. The whole plant was also called “Zhui Fengsan” used in Chinese folk medicine against rheumatic arthralgia, hemiparalysis, pedo-infantile convulsion and bone fracture. Phytochemical research showed that flavonoids as the main components were accumulated in *L. paridiformis* var. *stenophylla* (Li and Xiang, 2010). *L. fortumei* Maxim, as an erect perennial herb distributed in Japan, Korea, southeast China and Taiwan. The whole plants have been used in Chinese folk medicine for activating blood circulation to dissipate blood stasis and dissipating dampness. Phytochemical research showed that flavonoids and benzoquinones are the main compounds (Huang et al., 2007; Fang et al., 1989). *L. chikungensis*, an endemic plant in China, is a perennial plant and is mainly distributed in rock crevices and grassy mountain slopes in Henan and Hubei Province in China, and is grown in Guizhou Province.

Extraction methods for volatiles include solvent extraction, steam distillation, pressing, chromatography, grease separation and carbon dioxide extraction. These methods generally need organic solvent and have long time for extraction (Jia et al., 1998). Solid phase micro-extraction ‘(SPME) technology’ is rapid and simple to extract the volatiles without organic solvent (Wang and Kang, 2009; Kang et al., 2009), and is easier to operation and lower cost than that of solvent extraction, steam distillation, pressing, chromatography, grease separation and carbon dioxide extraction methods.

The volatiles for *Lysimachia* genus, *L. Paridiformi*, *L. Pentapetala*, *L. punctata* and *L. circaeoides* are reported (Shi et al., 2010; Kang

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et al., 2011; Dtterl and Schffler, 2007; Wei et al., 2011). To the best of our knowledge, the volatile compositions of *L. paridiformis* var. *stenophylla*, *L. fortumei* and *L. chikungensis* have not been reported. So, this paper reported the volatile compounds in the *L. paridiformis* var. *stenophylla*, *L. fortumei* and *L. chikungensis* using head space solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) for the first time.

Materials and methods

Plant materials

The leaves of *L. paridiformis* var. *stenophylla* were collected in Guiyang, China, in August 2009, the leaves of *L. fortumei* were collected in Guiyang, China, in June 2010, and the leaves of *L. chikungensis* were collected in Sandu country, Guizhou, China, in July 2010. They were identified by Professor Zhiyou Guo (Qiannan Normal College for Nationalities), Professor Deyuan Chen (Guiyang College of Traditional Chinese Medicine), and Professor Zhiyou Guo, respectively. Voucher specimens were deposited in the Institute of Chinese Materia Medica, Henan University.

Volatiles preparation

Volatiles were extracted by the manual solid phase micro-extraction (SPME), instrument together with 5 mL vials and 65 μm Polydimethyl siloxane-divinyl benzene (PDMS-DVB), fiber head that purchased from Supelco Inc. Bellefonte, USA. The leaves of *L. paridiformis* var. *Stenophylla*, *L. fortumei*, and *L. chikungensis* were rapid dehydrated with allochroic silicagel. Three pulverized samples about 0.7 g were placed in vials (5 mL), then the SPME fiber heads were exposed in the upper space of the sealed vial for 30 min at 80℃ to adsorb the analytes. And then, pull the fiber head out immediately and directly insert into to desorb for 1 min.

Determination conditions

By an Agilent 6890 N gas chromatograph equipped with HP-5 MS quartz elastic capillary column (0.25 μm × 30.0 m × 250 μm) and coupled with a 5975B mass selective detector spectrometer to analyze the volatile constituents. Determination conditions set as follows: The front inlet was kept at 250℃ in split-less mode. The temperature program was as below: the initial column temperature was 50℃, held for 1 min, and then programmed to 120℃ at a rate of 4℃. min-1 and held for 2 min; finally programmed to 230℃ at a rate of 6℃. min^{-1}, held at 230℃ for 5 min. As a carrier gas helium at 1.0 mL·min-1 was used. MS conditions: The MS detector was used in the EI mode with an ionization voltage of 70 eV. The ion source temperature was at 230℃. The transfer line was at 280℃.The spectra were collected at 3 scans/s over the mass rang (m/z) 30-440.

Retention indices were calculated by using the retention times of n-alkanes that were injected at the same chromatographic conditions. The volatile constituents (Table 1), were identified by comparison of their linear retention indices (relative to C_6-C_{26} alkanes on the HP-5MS column), and mass spectral database search (Nist 08.L library). The percentage composition of the volatile was computed from the GC peak areas normalization without any corrections.

Results

The volatiles in the leaves of *L. paridiformis var stenophylla*, *L. fortumei* and *L. chikungensisi* are presented in Table 1 and Figure 1, Figure 2 and Figure 3. Twenty-nine compounds in the leaves of *L. paridiformis* var. *stenophylla* were identified (in Table 1), which comprised 89.17% of the total volatile fraction. The main constituents were ethanol (13.58%), linalool (8.4%), and *β*-ionone (8.05%). Monoterpenoids and sesquiterpenes, such as linalool and *β*-ionone were the main aroma constituents in *L. paridiformis* var. *stenophylla*.

Twenty-one compounds were identified from the leaves of *L. fortumei* (Table 1), which comprised 94.72% of the total volatile fraction. The main constituents were alkanes, such as tricosane (14.72%), docosane (11.02%), tetracosane (10.77%) and pentacosane (9.81%).

Thirty-two compounds were identified from the leaves of *L. chikungensis* (in Table 1), which comprised 88.58% of the total volatile fraction. Typical compounds detected in *L. chikungensis* were *cis*-3-hexenyl pentanoate (13.33%), followed by ethanol (12.13%), hexadecanoic

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acid, ethyl ester (7.78%) and heneicosane (5.38%).

Table 1: Volatiles from the leaves of *L. paridiformis var stenophylla*, *L. fortumei* and *L. chikungensisi*

No.	Compound	Percentage (%)			KI
		L. paridiformis var. *Stenophylla*	*L. fortumei*	*L. chikungensis*	
1	3-Hydroxy-*N*-methylphenethylamine	-	1.23	-	-
2	Ethanol	13.58	9.11	12.13	-
3	Trichloromethane	1.81	-	2.23	-
4	Isopentaldehyde	1.74	-	-	648
5	Hexanoic acid, ethyl ester	1.14		-	999
6	Leaf alcohol	-		1.59	850
7	(*Z*)-3-Hexenoic acid ethyl ester	0.77	-	-	1004
8	Benzyl alcohol	-	-	1.13	1035
9	Linalool	8.4	5.88	1.63	1099
10	Phenylethyl alcohol	2.36	1.54	3.38	1110
11	Estragole	1.72	-	-	1193
12	*β*-Cyclocitral	1.16	-	0.77	1212
13	*cis*-3-Hexenyl pentanoate	-	-	13.33	1234
14	Eugenol	-	-	1.46	1346
15	Tetradecane	0.52	-	-	1398
16	2-methyl-5-(1,1,5-trimethyl-5-hexenyl)-Furan	-	2.59	-	1432
17	Geranylacetone	1.35	1.53	1.55	1440
18	*β*-Ionone	8.05	2.72	4.18	1468
19	trans- *β*-Ionone epoxide	-	-	1.29	1472
20	Pentadecane	3.85	3.49	2.29	1498
21	Dihydroactinidiolide	1.6	-	2.4	1511
22	*cis*-3-Hexenyl benzoate	-	-	1.88	1564
23	Ethyllaurate	2.46	1.37	1.5	1593
24	Hexadecane	4.57	2.75	2.48	1598
25	2,6,10-trimethyl-Pentadecane	-	-	1.2	1646
26	6,9-Heptadecadiene	-	-	0.74	1664
27	Heptadecane	5.15	0.99	2.57	1697
28	2,6-dimethyl-Heptadecane	4.46	-	1.42	1699
29	2,6,10,14-tetramethyl-Pentadecane	2.27	0.14	1.61	1700
30	Ethyl myristate	1.54	1.34	0.96	1790
31	Octadecane	2.14		1.11	1796
32	2,6,10,14-tetramethyl-Hexadecane	2.86		1.89	1802
33	9-Octadecyne	-	1.15	-	1855
34	6,10,14-trimethyl-2-Pentadecanone	1.3	-	-	1836
35	Diisobutyl phthalate	1.21	-	-	1849
36	Nonadecane	0.74	-	-	1895
37	Butyl isobutyl phthalate	0.76	-	-	1943
38	Ethyl palmitate	5.8	4.94	7.78	1988

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39	Heneicosane	-	4.49	5.38	2012
40	Phytol	-	-	0.7	2099
41	Linoleic acid ethyl ester	2.21	-	1.2	2151
42	(*Z,Z,Z*)-9,12,15-Octadecatrienoic acid ethyl ester	-	3.14	3.08	2157
43	Ethyl Oleate	3.65	-	-	2159
44	Docosane	-	11.02	-	2200
45	Tricosane	-	14.72	0.82	2294
46	Squalene	--	-	2.9	2349
47	Tetracosane	-	10.77		2400
48	Pentacosane	-	9.81		2498
Total (%)		89.17	94.72	88.58	

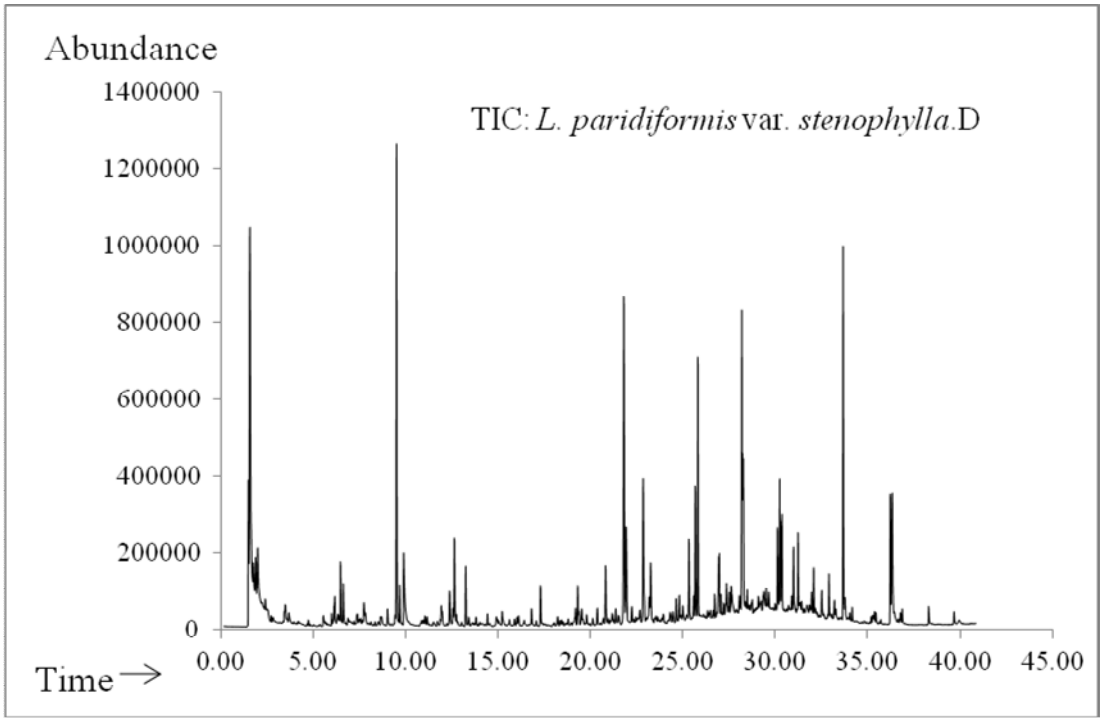

Figure 1: Ion Chromatogram of leaves of *L. paridiformis* var. *Stenophylla*

Discussion

Chemical compositions of the essential oils reported in *Lysimachia* genus were organic acid and its esters, terpenoids and sesquiterpenes derivatives, aromatic compounds and their oxygenated derivatives, such as alcohols, aldehydes, ketones, phenols, ethers, lactones, and some of its nitrogen-containing and sulfur-containing compounds (Shi et al., 2010; Kang et al., 2011; Dtterl and Schffler, 2007; Wei et al., 2011). Components and contents of compounds of volatile oil are different among different plants. Volatiles in leaves of *L. paridiformis* var. *stenophylla* had alkanes (28.37%), alcohols (21.98%), esters (21.14%), ketones (10.7%), aldehydes (2.9%) and terpenoids (1.72%). Volatiles in leaves of *L. fortumei* had alkanes (59.63%), alcohols (14.99%), esters (10.79%), ketones (4.25%), aldehydes (4.13%) and alkynes (1.15%), and in leaves of *L. chikungensis* had alcohols (32.13%), alkanes (22.55%), esters (20.59%), ketones (7.02%), alkenes (3.64%), phenols (1.46%), alkynes (1.15%) and aldehydes (0.77%).

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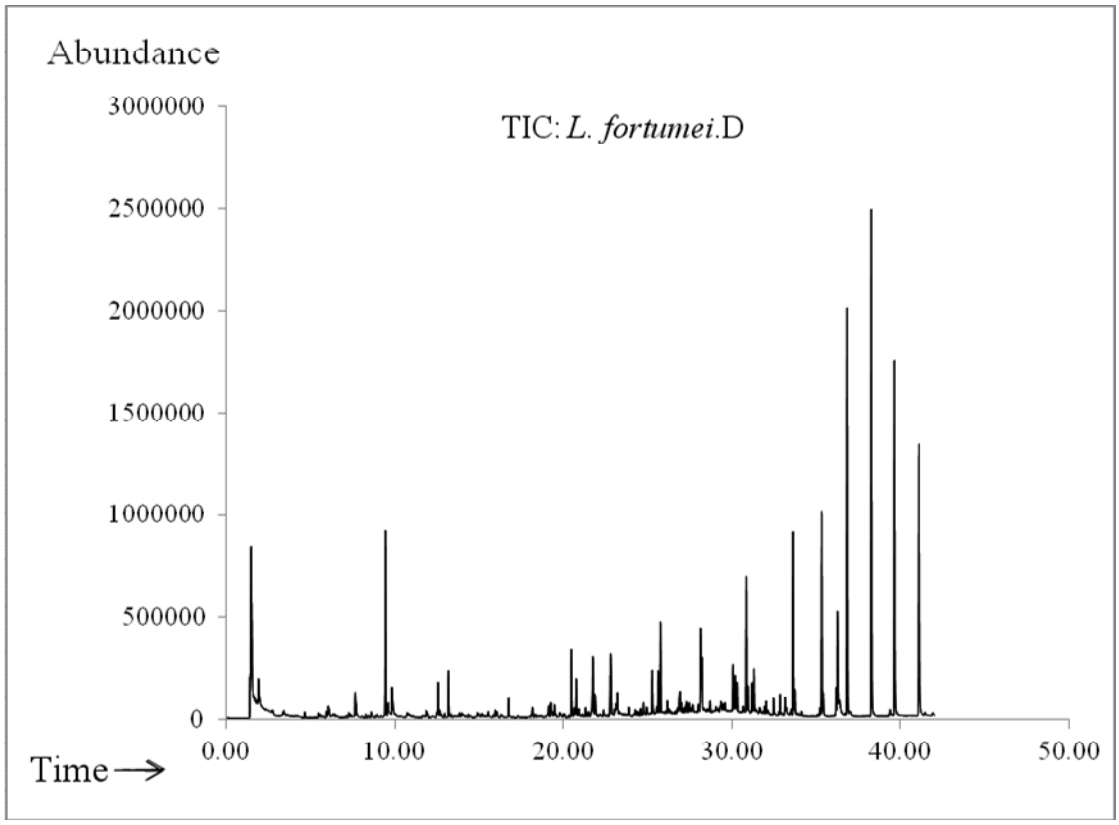

Figure 2: Ion Chromatogram of leaves of *L. fortumei*

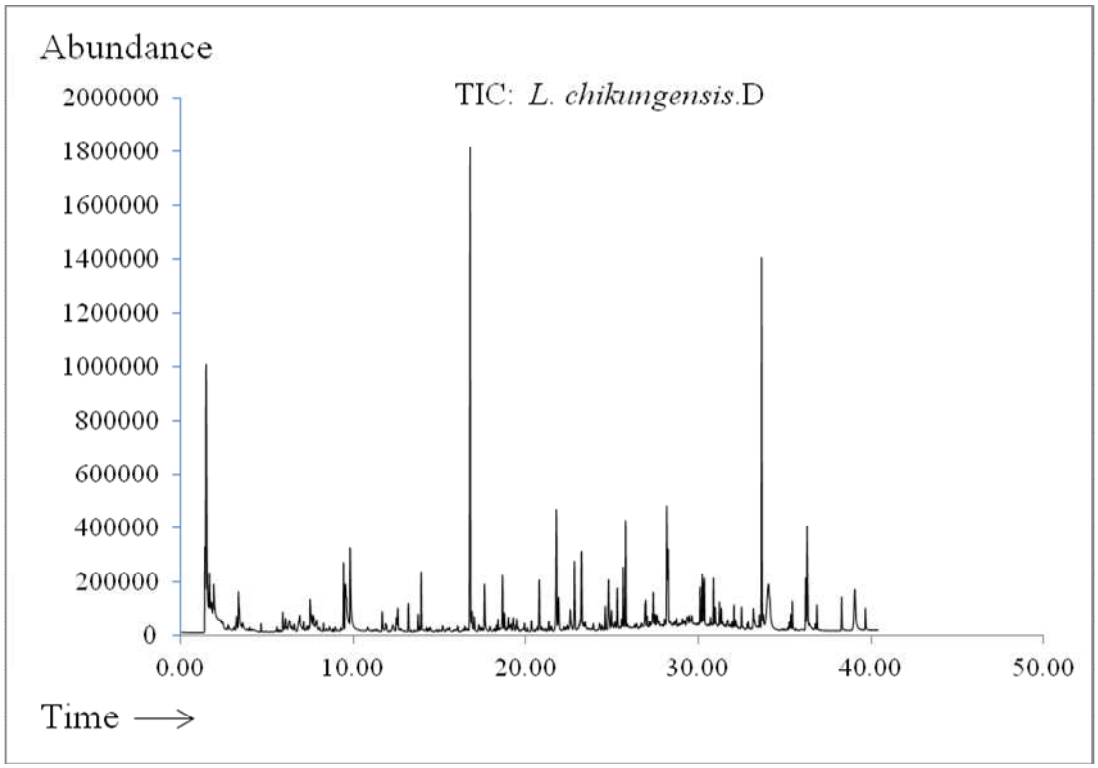

Figure 3: Ion Chromatogram of leaves of *L. chikungensis*

The common components of three plants were alkanes, alcohols, esters, ketones and aldehydes, and the contents of alkanes, alcohols and esters were the highest. Aliphatic saturated hydrocarbon was main ingredient of plant wax, it played a important role in protection of the plant, and once formed, it was no longer participate in metabolism and product of the end of metabolism (Zhou and Duan, 2005).

Alcohols accounted for 21.95%, 14.99% and 20.59% in the leaves of *L. paridiformis var stenophylla*, *L. fortumei* and *L. chikungensisi* respectively, but ethanol was up to 13.58%, 9.11% and 12.13% respectively, accounting for sixty percent of the alcohols. It could be seen that

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volatiles of the three plants were rich in ethanol, but ethanol could not be detected in the volatiles of *L. paridiformis* (Wei et al., 2011). This result may be conducted with the difference of drying methods. As known to all, water was a respiratory reaction medium; plant water content had great influence on respiration. *L. paridiformis* var. *stenophylla*, *L. fortumei* and *L. chikungensis* were rapidly dehydrated by allochroic silica gel, and made plant cell to a point of dehydration in a short time, normal aerobic respiration is hindered, glycolytic pathway turned into anaerobic respiration, NADH was generated in the dehydrogenation stage for the reduction of pyruvic acid, pyruvic acid was turned into acetaldehyde in pyruvate decarboxylase catalyst by oxidation decarboxylation pathway, and then, acetaldehyde was reduced to ethanol by alcohol dehydrogenase catalyst, while NADH was oxidized to NAD^+. The leaves of *L. paridiformis* was dried naturally at room temperature, slow dehydration had little effect on aerobic respiration. Intracellular respiratory substrates were completely oxidized to CO_2 and H_2O. Therefore, ethanol was not detected in the volatiles.

Linalool and *β*-ionone were the main aroma constituents in *L. paridiformis* var. *stenophylla*. Linalool and *β*-ionone also existed in *L. fortumei* and *L. chikungensis*. Linalool and its oxides, with fragrance and sweet taste, had effects of antibacterial, antifungal and antiviral (State Drug Administration of Information Center of Chinese Traditional and Herbal Drugs, 1986), and they could inhibit the growth of *aspergillus flavus*, thereby and the generation of aflatoxin. So, they could be used as food additive. In addition, the three plants contained hexadecanoic acid, ethyl ester, which could be used to synthesis reaction of flavors and perfume.

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