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Full Length Research Paper

Volatiles in the *Lysimachia clethroides* Duby by head space solid phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS)

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We studied for the first time, the volatile compounds in the *Lysimachia clethroide* Duby by using head space solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). The results showed that 14 compounds were identified from the stem of *L. clethroides*, 13 compounds were identified from the leaves, and 16 compounds from the flowers. 8 compounds were mutual components, accounting for 92.99, 94.59 and 90.32% of the identified components in stem, leaves and flowers, respectively. Limonene was the main compound in the three parts, and the contents were up to 52.59, 54.57 and 44.98%, respectively. These showed that the volatiles had not much difference in the three parts; maybe the three parts have the same medicinal effect, and could provide certain theoretical basis for further development and utilization.

Key words: *Lysimachia clethroides* Duby, solid phase microextraction, volatiles.

INTRODUCTION

Lysimachia clethroides Duby, belonging to the Primulaceae family, is found in mild region of northeast China, southwest and other east provinces. Korea and Japan also has distribution (Guo et al., 1995). Phytochemical research showed that the genus *Lysimachia* has flavonoids, saponins andorganic acid (Kitagawa et al., 1967; Yasukawa and Takido, 1986; Kim et al., 1993). Pharmacological investigations showed that the flavonoids in the genus *Lysimachia* can eliminate the superoxide radical and hydroxyl radical (Zhang et al., 1999; Beate, 2001). Triterpenoid saponins in the genus has immunoregulation, anti-tumor and anti-fungal effects (Xu et al., 2004).

In recent years, reports on *L. clethroides* are few, no research has so far been conducted concerning the chemical constituents of essential oil of *L. clethroides*. In order to identify the chemical constituents of the essential oil in the stem, leaves and flowers of *L. clethroides*, and to illuminate the difference among them, the present study was undertaken. This paper reports the components in the essential oil of *L. clethroides* using the HS-SPME technique subsequently analysed by GC-MS for the first time.

MATERIALS AND METHODS

Plant and extract preparation

Air-dried plants of *L. clethroides* were collected in Guizhou, China, in June 2010, and identified by Professor Deyuan Chen (Guiyang College of Traditional Chinese Medicine). Voucher specimen was deposited in the Institute of Chinese Materia Medica, Henan University. By manual SPME holder together with 5 ml vials and Polydimethylsiloxane-Divinylbenzene (PDMS-DVB) fibers (Supelco Inc. Bellefonte, USA) volatiles were extractacted from stem, flowers and leaves of *L. clethroides*. The stem, leaves and flowers powders about 0.7 g were placed in 5 ml vials, then the SPME fiber was exposed in the upper space of the sealed vial for 30 min at 80ºC to adsorb the analytes. After that, the fiber was withdrawn and directly inserted into the GC-MS inlet to desorb the volatiles for 1 min.

Determination conditions

The volatiles were analyzed by HS-SPME-GC-MS. Analysis was

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Table 1. Volatiles from stem, leaves, flowers of *L. clethroides.*

Number	Compound	KI	Percentage (%)		
			Stem	Leaves	Flowers
1	*n*-hexane		0.51	0.36	-
2	1*R*-*α*-Pinene	928	5.15	4.87	3.56
3	Camphene	943	1.92	1.46	0.95
4	1*S*-*β*-Pinene	971	4.02	3.91	-
5	*β*-Myrcene	988	6.26	6.32	4.95
6	Limonene	1030	52.59	54.57	44.98
7	Linalool	1101	1.67	1.36	2.98
8	ethenyl-Cyclooctane	1160	-	0.38	-
9	Borneol	1167	0.39	-	-
10	Menthone	1180	-	-	0.68
11	Citral B	1240	10.68	11.18	11.82
12	Citral A	1272	13.83	14.00	15.11
13	Acetic acid, isobornyl acrylate	1280	0.60	-	-
14	(1-Methylethyl)-cyclohexane	1321	-	0.41	-
15	Copaene	1365	0.33	-	1.09
16	Caryophyllene	1405	0.89	0.83	0.97
17	Geranylaceton	1442	-	0.34	-
18	(*Z*)-*β*-Farnesene	1447	0.52	-	-
19	Heneicosane	1832	-	-	2.32
20	Docosane	2017	-	-	0.34
21	Phytane	2030	-	-	2.12
22	Tetracosane	2187	-	-	2.06
23	Pentacosane	2322	-	-	1.12
24	Hexacosane	2443	-	-	0.94
Total (%)			99.35	99.99	99.77

carried out using an Agilent 6890 N gas chromatograph equipped with a capillary column HP-5 MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector spectrometer from the same company.

GC conditions

The temperature of front inlet was kept at 250ºC in split-less mode. The temperature program was as below: the initial column temperature was 50ºC (held at 50ºC for 1 min), and then programmed to 120ºC at a rate of 3ºC min^{-1} (held at 120ºC for 2 min); finally programmed to 210ºC at a rate of 4ºC min^{-1} (held at 210ºC for 10 min). Helium was used as a carrier gas with flow rate of 1.0 ml min^{-1}.

MS conditions

The MS detector was used in the EI mode with an ionization voltage of 70 eV. The ion source temperature was at 230ºC. The transfer line was at 280ºC. The spectra were collected at 3 scans/s over the mass rang (*m*/*z*) 30 to 440.

Retention indices were calculated by using the retention times of *n*-alkanes (C_8 to C_{26} alkanes) that were injected at the same chromatographic conditions. The compounds were identified by comparing their relative retention indices and computer matching with the WILEY275.L library. The volatiles (Table 1 and Figures 1 to 3) were identified by comparison of their linear retention indices that were relative to C_8 to C_{26} alkanes on the DB-5MS column and mass spectra with authentic standards, as well as standards from RTLPEST 03.L and NIST 05.L. The percentage composition of the volatile was computed from the GC peak areas normalization without any corrections (Kang et al., 2011).

RESULTS

The volatiles in the stem, flowers and leaves of *L. clethroides* are presented in Table 1 and Figures 1 to 3. Fourteen compounds in the stem were identified, which comprised 99.35% of the volatile fraction. The main volatile components in the stem were limonene (52.59%), citral A (13.38%), citral B (10.68%), *β*-myrcene (6.16%). Thirteen compounds in the leaves were identified, which comprised 99.99% of the volatile fraction. The main compounds were limonene (54.57%), citral A (14.00%), citral B (11.18%), *β*-myrcene (6.32%). Sixteen compounds were identified from the flowers, and the main compounds were the same with both the stem and leaves. And the contents were 44.98, 15.11, 11.82 and 4.95%, respectively. Some main compounds, such as

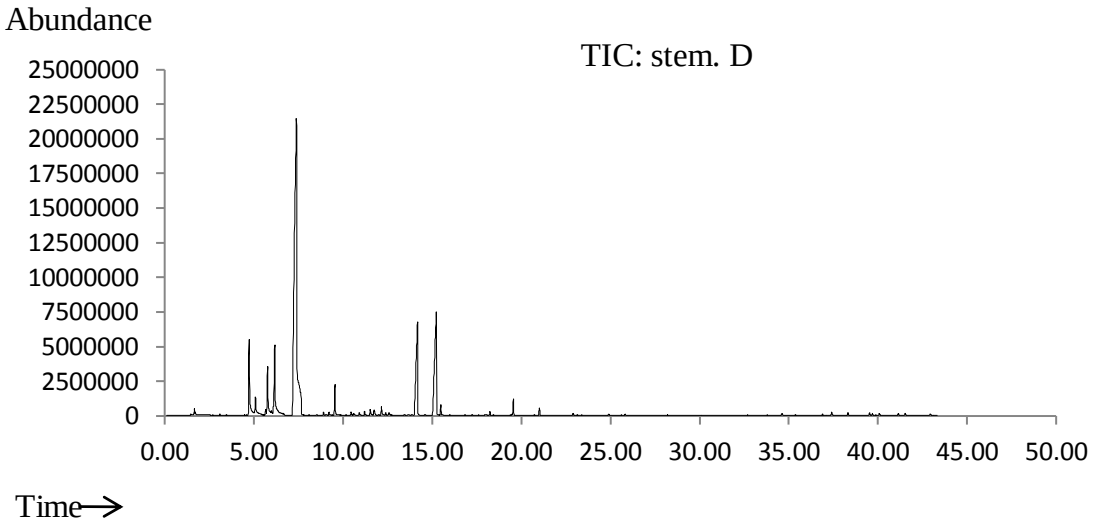

Figure 1. Total ion chromatogram of stem of *L. clethroides.*

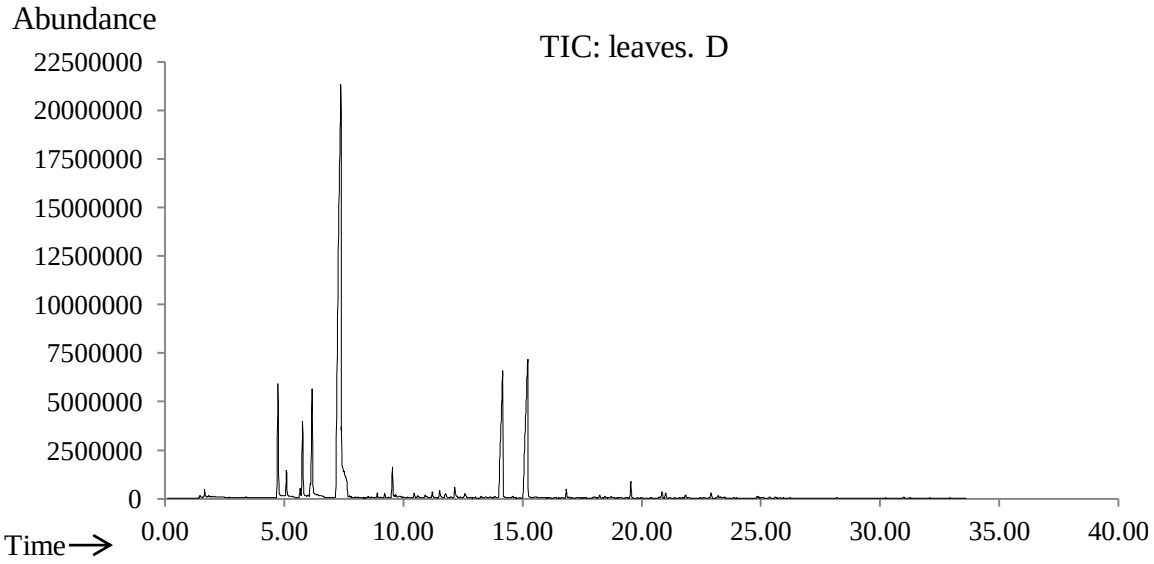

Figure 2. Total ion chromatogram of leaves of *L. clethroides.*

limonene, citral A, citral B, *β*-myrcene, 1*R*-*α*-pinene,camphene, linalooland caryophyllene were mutual in leaves, stem and flowers, accounting for 92.99, 94.59 and 90.32% of the identified components, respectively. Limonene was the highest content compound in the three samples.

DISCUSSION

In Table 1, 14 compounds were identified and accounted for 99.35% of total oils in stem, 13 compounds in leaves accounted for 99.99% of total oils in leaves and 16 compounds in flower accounted for 99.77% of total oils in flower. 1*R*-*α*-pinene, camphene, *β*-myrcene, limonene, linalool, citral B, citral A and caryophyllene were mutual compounds in three parts of *L. clethroides* and the ratio of mutual compounds in three parts were as high as 90%. The content of limonene was about 50%, which showed that the volatiles are not much different in the stem, flowers and leaves; maybe the three parts have the same medicinal effect.

β-Myrcene, which is colorless or yellowish liquid, often has light fragrance. It can be used as flavor composition.

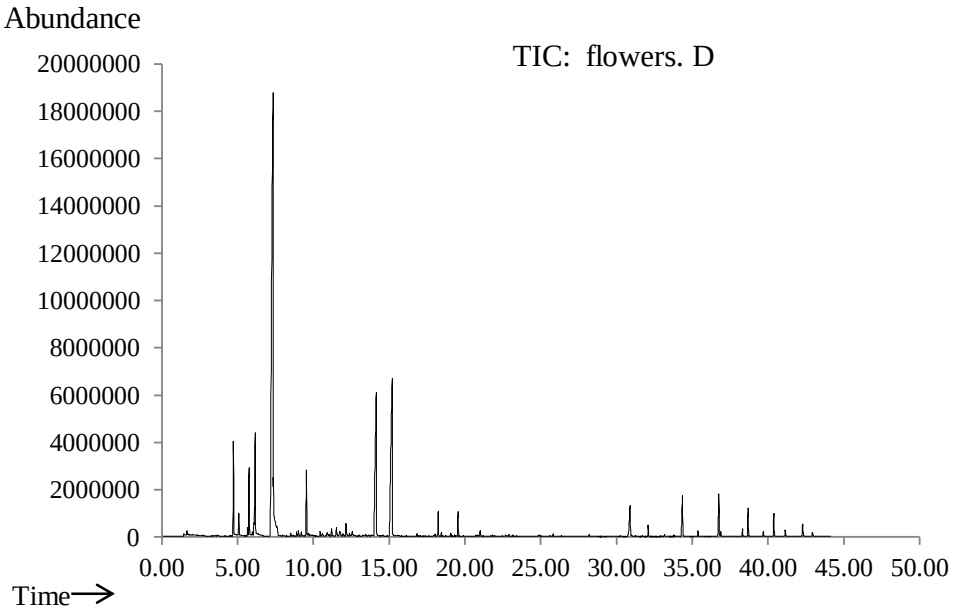

Figure 3. Total ion chromatogram of flowers of *L. clethroides*.

Limonene, colorless liquid, which has lemon fragrance, can be wildly used in food spice. So, it is considered that both of the two ingredients are the main fragrance of the flowers, and can be the spice sources. Limonene, a natural and functional monoterpene, is used as a flavor and fragrance additive in food widely. Studies from Xue-mei Wang have shown that limonene had better antimicrobial ability to *Saccharomyces* sp. and *Aspergillus niger* than that of potassium sorbate and sodium benzoate (Wang et al., 2010). Research from Wang (2005) revealed that limonene has a lot of pharmacological effects, such as cough expectorant, anti-inflammatory, pain reliever, especially strong anticancer activity. It is wildly used in medicine industry. Therefore, whether limonene is the effective component in suppressing cough and calming panting is required for further study.

ACKNOWLEDGEMENT

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