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Comparative Analysis of Volatile Oils from *Lonicera japonica* Thunb. var. *chinensis wakey* by HS-SPME and GC-MS

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The volatile oils of the flowering stages, *i.e.*, the flower bud stage, silver flowering stage, gold flowering stage as well as the leaf from *Lonicera japonica* Thunb. var. *chinensis wakey* were extracted and analyzed by headspace solid-phase microextraction and GC-MS. In this study, *Lonicera japonica* Thunb. was used as contrastive species. The fiber coating of 65 μm PDMS/DVB was chosen to extract the volatile oils. A total of 46, 50, 51 and 20 volatile compounds were identified, constituting of 98.16, 97.31, 95.99 and 97.84 % of oil composition of the flower bud stage, silver flowering stage, gold flowering stage and leaf from *L. japonica* var. *chinensis wakey*, respectively. The dominant constituents of the above parts were hydrocarbons (37.79 %), oxygenated monoterpenes (28.98 %), alcohols (25.44 %) and esters (53.29 %). It is concluded that the esters in flowering period contributed to the attractive aroma of *L. japonica* var. *chinensis wakey*.

Key Words: *Lonicera japonica* Thunb. var. *chinensis wakey*, Volatile oils, Analysis, Comparison, HS-SPME-GC-MS.

INTRODUCTION

Lonicera japonica Thunb. (Jin Yin Hua or Ren Dong in Chinese, Caprifoliaceae plants) is one of the most common traditional Chinese medicines and used for treating various diseases, including arthritis, diabetes mellitus, fever, infections, sores and swelling[1]. Pharmacological studies indicated that their extracts have a broad spectrum of biological activities, such as antibacterial, antiinflammatory, antioxidant, antipyretic, antiviral and hepato-protective effects[2-6]. It is sprawling and twining lianas; young stems pubescent; leaves ovate, elliptic, oblong or broadly lanceolate, branch of the year and blade common green. The flowering period can be divided into three stages, *i.e.*, the flower bud stage, the silver flowering stage and the gold flowering stage[7]. Now as an ornamental groundcover, *L. japonica* is commonly planted in many areas for sprawling habit, numerous sweetly fragrant white flowers and attractive evergreen foliage, becoming naturalized in Argentina, Brazil, Mexico, Australia, New Zealand and United States.

L. japonica var. *chinensis wakey*, growing quickly and adaptable, with purple, essentially glabrous leaves, red flowers and attractive aroma, is a mutation of *L. japonica*. The aroma of *L. japonica* var. *chinensis wakey* is more fragrant and attractive than *L. japonica*. It is a good ornamental and medicinal plant.

Headspace solid-phase microextraction (HS-SPME) is a solvent free sampling technique first introduced by Pawliszyn and co-workers[8]. At present, HS-SPME has been widely used in environmental, biological and food analysis for its simplicity, speed and sensitivity[9,10]. HS-SPME combined with GC/MS analysis has being a suitable technique for the analysis of volatile oils in many fields[11-13].

To our best of knowledge, there is no report about the chemical analysis of the volatile oils from *L. japonica* var. *chinensis wakey* in literature. The aim of this paper is to systematically compare the volatile oils of *L. japonica* var. *chinensis wakey* with those of *L. japonica* in the flowering stages, *i.e.*, the flower bud stage, silver flowering stage, gold flowering stage as well as the leaf, for the clear understanding of this plant.

EXPERIMENTAL

The fresh materials of *L. japonica* var. *chinensis wakey* "Yatehong" and *L. japonica* "Yate" were collected in May 2011 from Shandong Analysis and test center. The species were authenticated by Pro. Fa-zeng LI (College of Life Science, Shandong Normal University, Jinan, China).

GC-MS was performed with an Agilent 6890N-5973N; Capillary column HP-5MS (30 m × 0.25 mm, 0.25 μm) quartz capillarity columniation. The following SPME fibers (Supelco, Bellefonte, PA, USA) were used for the extraction procedure: 65 μm PDMS/DVB, 70 μm CW/DVB and 100 μm PDMS.

Preparation of samples and extraction of volatile oils: The sample preparation for solid-phase microextraction was as follows: 3 g of sample was cut into pieces and instantly introduced into a 15 mL headspace vial. The solid-phase microextraction device was inserted in the sealed vial containing the sample prepared as described above and the fiber was exposed to the sample headspace for 0.5 h in a water bath (30 ºC). The following solid-phase microextraction fibers (Supelco, Bellefonte, PA, USA) were used for the extraction procedure: 65 µm PDMS/DVB, 70 µm CW/DVB and 100 µm PDMS.

Conditions of the GC-MS analysis: The capillary GC analysis was conducted using an Agilent 6890N gas chromatograph system (Agilent Technologies). The temperature program used for analysis was as follows: (1) isothermal temperature at 50 ºC for 2 min; (2) heating from 50-120 ºC at a rate of 2 ºC/min; (3) isothermal processing at 120 ºC for 5 min; (4) heating from 120-160 ºC at a rate of 5 ºC/min; (5) isothermal processing at 160 ºC for 10 min; (6) heating from 160-240 ºC at a rate of 10 ºC/min; (7) isothermal processing at 240 ºC for 5 min. Helium (99.999 %) was used as the carrier gas at a flow-rate of l mL/min. The injector temperature was set at 240 ºC and desorption was performed at the injector for 3 min. The injection was performed in the splitless model. For identification, the electron impact ionization conditions were as follows: ion energy 70 eV and the mass range scanned was 30-500 amu in the full scan acquisition mode.

Identification of constituents: Retention indices of all the components were determined by Kovats method using *n*-alkanes (C_6-C_{30}) as standards (Supelco, Bellefonte, PA, USA). The constituents of the volatiles were identified by the Wiley 138, NIST 02 and HPCH 1607 (Allured Corp., Carol Stream, IL) libraries and with data published in the literatures[14-16].

RESULTS AND DISCUSSION

SPME fiber selection: Three fiber coatings, *i.e.*, 65 µm PDMS/DVB, 70 µm CW/DVB and 100 µm PDMS were used to test the volatile oils present in the flower bud stage of *L. japonica* var. *chinensis wakey*. Fig. 1 showed the chromatograms obtained using these three fibers. The 65 µm PDMS/DVB fiber coating was chosen for the further study considering its better performance in extracting more variety of compounds and larger amount.

Analysis of samples: The GC-MS analysis of the volatile oils from the flower bud, silver flower, gold flower and leaf of *L. japonica* var. *chinensis wakey* and *L. japonica* are presented in Table-1. Altogether, 57 volatile compounds were identified by GC-MS with HP-5MS column.

The flower bud volatile oils were revealed the presence of 46 and 45 components, representing 98.16 and 97.87 % of the total oils in *L. japonica* var. *chinensis wakey* and *L. japonica*. The major compounds in the flower bud volatile oils of *L. japonica* var. *chinensis wakey* were 1-allyl-2-methylcyclopentane (37.79 %), *cis*-3-hexenyl-2- methylbutyrate (7.85 %) and linalool (7.01 %), while in *L. japonica* they were linalool (40.36 %), 1-allyl-2-methylcyclopentane (19.72 %) and δ-cadinene (3.80 %). A total of 50 and 47 components were identified at the silver flowering stage with linalool (28.88 %),

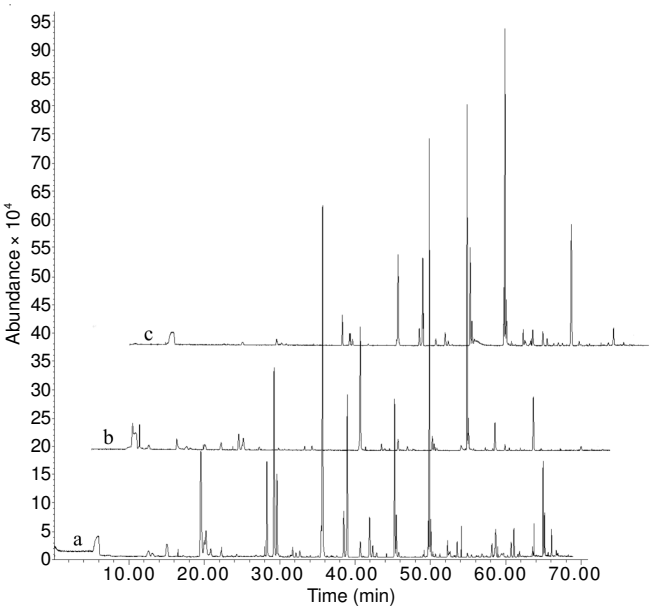

Fig. 1. GC-MS chromatograms of the comparison three fiber coatings used for SPME (a) 65 µm PDMS/DVB; (b) 70 µm CW/DVB; (c) 100 µm PDMS)

1-allyl-2-methylcyclopentane (19.49 %) and (Z)-3-hexen-1-ol (6.77 %) as the major compounds in the volatile oils of *L. japonica* var. *chinensis wakey* and linalool (36.57 %), 1-allyl-2-methylcyclopentane (10.43 %) and (E)-germacrene D (7.65 %) as major compounds in *L. japonica*. A total of 51 and 47 components were identified, respectively in *L. japonica* var. *chinensis wakey* and *L. japonica* at the gold flowering stage. The main compounds at this stage in *L. japonica* var. *chinensis wakey* were linalool (23.58 %), (Z)-3-hexen-1-ol (16.19 %) and 1-allyl-2-methylcyclopentane (13.61 %). Comparatively, they are linalool (33.95 %), 1-allyl-2-methylcyclopentane (11.06 %) and (E)-germacrene D (8.01 %) in *L. japonica*. Twenty components accounting for 97.84 and 98.44 % of constituents of the leaf volatile oils from *L. japonica* var. *chinensis wakey* and *L. japonica* were identified. The major compounds were (Z)-3-hexen-1-ol, acetate (27.28 %), (Z)-3-hexen-1-ol (23.39 %) and (E)-2-hexen-1-ol, acetate (13.07 %) in *L. japonica* var. *chinensis wakey* and (Z)-3-hexen-1-ol (64.98 %), 1-hexanol (17.76 %) and acetic acid, hexyl ester (3.55 %) in *L. japonica*.

The volatile compounds were separated into eight chemical classes and the chemical distribution of the volatile oils was summarized in Table-2. The dominant constituents of the flower bud volatile oils were hydrocarbons (37.79 %) and esters (30.04 %) in *L. japonica* var. *chinensis wakey*, while oxygenated monoterpenes (40.47 %) and hydrocarbons (19.72 %) featured in *L. japonica*. The oxygenated monoterpenes (28.9 % in *L. japonica* var. *chinensis wakey* and 21.20 % in *L. japonica*) and sesquiterpene hydrocarbons (36.61 % in *L. japonica* var. *chinensis wakey* and 32.48 % in *L. japonica*) were the major constituents of the silver flowering stage. Eight esters and five alcohols were identified in the leaf volatile oils from *L. japonica* var. *chinensis wakey* which contributed to 53.29 and 37.67 % of total oils. Nine esters and four alcohols indentified in leaf volatile oils from *L. japonica* accounted for 13.33 and 83.59 % of total oils. Apparently, terpenoids in leaf were fewer compared with flowers.

TABLE-1
VOLATILE OILS CONSTITUENTS OF *L. japonica* var. *chinensis wakey* (A) AND *L. japonica* (B)[a]

RI	Compounds	Area (%)							
		Flower bud		Silver flower		Gold flower		Leaf	
		A	B	A	B	A	B	A	B
825	2-Methyl-1-butanol	0.70	0.49	0.61	0.17	0.44	0.25	–	–
850	2-Methyl-butanoic acid, methyl ester	0.29	–	0.28	–	0.21	–	–	–
902	(E)-2-Butenoic acid, ethyl ester	0.09	0.93	0.09	–	–	–	–	–
905	2-Methyl butanoic acid, ethyl ester	0.26	0.51	0.35	0.02	0.15	–	–	–
910	(Z)-3-hexen-1-ol	3.72	3.10	6.77	3.64	16.19	4.82	23.39	64.98
915	Anti-2-methyl-butyl aldoxime	0.88	0.82	0.59	0.45	0.40	0.60	–	–
917	1-Hexanol	–	–	–	–	–	–	11.97	17.76
918	2-Methylene-butanoic acid, methyl ester	1.34	0.53	1.64	0.38	2.75	0.61	–	–
940	2-Heptanol	0.98	0.62	0.37	0.22	0.61	0.46	0.77	0.45
955	1,4-Hexadiene	–	–	–	–	–	–	0.43	0.14
969	Ethyl tiglate	0.39	0.67	1.27	0.07	0.71	0.10	–	–
998	1-Octen-3-ol	–	–	–	–	–	–	0.66	0.40
1007	dl-6-Methyl-5-hepten-2-ol	2.20	0.97	1.06	0.52	3.32	0.74	–	–
1010	3-Octanol	0.14	–	0.16	–	0.32	0.12	0.88	–
1012	Hexanoic acid, ethyl ester	–		–				–	0.52
1018	(Z)-3-Hexen-1-ol, acetate	1.79	2.58	1.46	2.93	4.89	3.55	27.28	4.20
1022	Acetic acid, hexyl ester	1.52	1.41	0.31	0.74	0.99	1.00	10.96	3.55
1024	(E)-2-hexen-1-ol, acetate	–	–	–	–	–	–	13.07	0.45
1038	Limonene	–	0.18	0.11	0.11	0.06	0.07	–	–
1045	2-Ethyl-hexanoic acid, methyl ester	0.52	–	0.11	–	0.23	–	–	–
1050	(Z)-3,7-Dimethyl-1,3,6-Octatriene	0.71	0.82	0.42	0.26	0.29	0.23	4.79	0.13
1056	3-Octen-1-ol	–	–	–	0.22	4.26	0.59	–	–
1071	(Z)-Linalool oxide (furanoid)	0.41	0.11	0.10	0.03	0.19	0.11	–	–
1088	(Z)-3-Hexen-1-ol, propanoate	0.22	–	0.56	–	1.62	–	0.39	0.83
1090	Linalool	7.01	40.36	28.88	36.57	23.58	33.95	–	–
1112	Terpinolene	–	0.06	0.04	0.05	0.23	0.04	–	–
1123	*cis*-3-hexenyl isobutyrate	1.30	0.81	1.33	0.48	1.02	0.49	0.29	0.69
1150	Butanoic acid, (Z)-3-hexenyl ester	0.41	0.51	1.34	0.37	0.88	0.41	–	–
1155	Benzoic acid, ethyl ester	–	0.85	0.33	1.15	0.19	0.96	–	–
1166	Butanoic acid, (E)-3-hexenyl ester	3.29	0.30	0.67	0.27	0.38	0.20	0.79	1.41
1171	Isobutyric acid, hexyl ester	1.24	0.13	0.12	0.03	0.07	0.05	–	–
1176	(Z)-3-Octen-1-ol, acetate	–	0.12	0.33	0.19	0.71	0.33	–	–
1209	Cis-3-hexenyl-2-methylbutyrate	7.85	1.64	1.94	0.55	1.61	0.61	0.40	1.48
1212	2-Butenoic acid, (E,Z)-3-hexenyl ester	2.77	1.85	1.76	0.62	1.32	0.59	–	–
1217	2-Butenoic acid, hexyl ester	0.35	0.29	0.33	0.10	0.22	0.15	–	–
1229	2,7-Dimethyl-2,6-octadien-1-ol	–	–	–	0.02	0.31	0.06	–	–
1279	1-Allyl-2-methylcyclopentane	37.79	19.72	19.49	10.43	13.61	11.06	0.86	0.80
1282	Tiglic acid, isobutyl ester	6.16	1.70	1.49	0.49	0.82	0.45	–	–
1286	(-)-Isocaryophyllene	0.45	0.41	0.48	0.66	0.34	0.61	–	–
1300	α-Cubebene	0.25	0.31	0.47	0.64	0.27	0.59	–	–
1316	(Z)-3-Hexen-1-yl hexanoate	0.24	–	0.20	0.11	0.18	0.07	0.10	0.20
1322	Copaene	0.60	0.50	0.65	0.94	0.47	0.97	–	–
1338	*cis*-Jasmone	0.65	0.33	1.58	1.42	1.10	1.17	–	–
1359	(+)-Calarene	0.41	0.44	0.93	1.80	0.52	1.75	–	–
1369	β-Cubebene	0.29	0.33	0.60	1.09	0.33	1.07	–	–
1380	(-)-Alloaromadendren	0.13	0.14	0.43	0.36	0.28	0.37	–	–
1393	(-)-Cloven	0.24	0.15	0.28	0.64	0.14	0.65	–	–
1405	(+)-Epi-bicyclosesquiphellandrene	0.13	0.14	0.28	0.45	0.15	0.41	–	–
1428	α-Amorphene	1.36	2.15	2.46	3.63	1.31	3.27	0.10	0.08
1444	(E)-Germacrene D	2.73	2.1	3.68	7.65	2.15	8.01	–	–
1469	(+)-Calarene	0.77	1.05	1.40	2.16	0.73	1.98	–	–
1480	α-Muurolene	0.78	0.69	1.22	1.27	0.69	1.15	0.29	0.08
1497	β-Cadinene	0.13	0.26	0.40	0.64	0.19	0.56	–	–
1517	γ-Muurolene	1.37	2.11	2.41	3.31	1.40	2.91	0.16	0.08
1531	δ-Cadinene	2.70	3.80	4.36	5.66	2.53	5.03	0.27	0.23
1556	1,4-Cadinadiene	0.15	0.23	0.34	0.43	0.17	0.39	–	–
1565	Naphthalene, 1,2,4a,5,6,8a-	0.43	0.64	0.81	1.13	0.45	1.01	–	–

[a]RI calculated from retention times relative to that of *n*-alkanes (C_6-C_{30}) on the non-polar HP-5MS column.

TABLE-2
CHEMICAL DISTRIBUTION IN THE VOLATILE OILS OF *L. japonica* var. *chinensis wakey* (A) AND *L. japonica* (B)

Constituents	Area (%)							
	Flower bud		Silver flower		Gold flower		Leaf	
	A	B	A	B	A	B	A	B
Sesquiterpene hydrocarbons	12.92 (17)	15.45 (17)	21.20 (17)	32.48 (17)	12.13 (17)	30.72 (17)	0.81 (4)	0.47 (4)
Oxygenated monoterpenes	7.42 (2)	40.47 (2)	28.98 (2)	36.61 (2)	23.77 (2)	34.07 (2)	–	–
Monoterpenes	0.71 (1)	1.06 (3)	0.58 (3)	0.42 (3)	0.57 (3)	0.33 (3)	4.79 (1)	0.13 (1)
Esters	30.04 (18)	14.83 (16)	15.91 (20)	8.51 (16)	18.96 (19)	9.59 (15)	53.29 (8)	13.33 (9)
Alcohols	7.75 (5)	5.18 (4)	8.97 (5)	4.81 (6)	25.44 (7)	7.04 (7)	37.67 (5)	83.59 (4)
Hydrocarbons	37.79 (1)	19.72 (1)	19.49 (1)	10.43 (1)	13.61 (1)	11.06 (1)	1.29 (2)	0.94 (2)
Ketones	0.65 (1)	0.33 (1)	1.58 (1)	1.42 (1)	1.10 (1)	1.17 (1)	–	–
Others	0.88 (1)	0.82 (1)	0.59 (1)	0.45 (1)	0.40 (1)	0.60 (1)	–	–

Figures in brackets are the number of compounds.

The esters in each flowering period of *L. japonica* var. *chinensis wakey* were more represented in both numbers (18, 20, 19 and 16, 16, 15 components) and the percentage (30.04, 15.91, 18.96 and 14.83, 8.51, 9.59 %) than in the volatile oils of *L. japonica* (Table-2). It could be an explanation that the aroma in *L. japonica* var. *chinensis wakey* is much more fragrant than in *L. japonica.*

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