NPC Natural Product Communications

2012
Vol. 7
No. 5
641 - 644

Chemical Composition of the Essential Oils from the Flower, Leaf and Stem of *Lonicera japonica*

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Received: January 5th, 2012; Accepted: March 23rd, 2012

The essential oils from different aerial parts of *Lonicera japonica* have been extracted by hydro-distillation and analyzed by gas chromatography and gas chromatography coupled with mass spectrometry. Quantitative and qualitative differences were found between the analyzed plant parts. A total of eighty-nine compounds were identified. The main constituents were (*Z*,*Z*)-farnesole (16.2%) and linalool (11.0%) for the flowers fraction, hexadecanoic acid (16.0%) and linalool (8.7%) for the leaves fraction, and hexadecanoic acid (31.4%) for the stems. Monoterpene hydrocarbons were absent from all the oils, and oxygenated sesquiterpenes were not identified in the essential oil of the stem.

Keywords: *Lonicera japonica*, essential oils, GC, GC/MS, *Caprifoliaceae*.

Lonicera japonica Thunb., family *Caprifoliaceae*, known as Japanese Honeysuckle, is native to eastern Asia, including Japan, Korea, and northern and eastern China [1,2], but is an invasive species in other parts of the world [3].

L. japonica has traditionally been used as a medicinal plant [4]. Pharmacological studies show that it possesses many biological functions, including hepatoprotective, cytoprotective, antimicrobial, antioxidative, antiviral and anti-inflammatory [2,5]. This plant contains iridoid glycosides [6], polyphenolic compounds [4,7], triterpene saponins, fatty acid esters and long chain hydrocarbons [8]. The main polyphenolic components in *L. japonica* are chlorogenic acid, caffeic acid, hyperoside and luteolin [4,6]. Different parts of the plant have different medicinal properties; the flower buds anticancer and anti-inflammatory [9], the leaves antioxidant and tyrosinase inhibition [10], and the stem xanthine oxidase inhibition, tyrosinase inhibition, and nitrite-scavenging activities [10,11]. The volatile oil from flowers of *L. japonica* is often used in foods, cigarettes and cosmetics, and also shows biological activities [2,12].

To the best of our knowledge, investigation of the volatiles from the leaves and stems of *L. japonica* has not yet been undertaken. In addition, there are no data about volatiles from the flowers of this plant grown in Europe. This prompted us to carry out a detailed GC and GC-MS analysis of *L. japonica* flower, leaf and stem oils from the region of central Serbia.

Chemical compositions of the essential oils obtained from the fresh parts of *L. japonica* (flowers, leaves and stems) are presented in Table 1, while the number of volatiles divided into different chemical classes is summarized in Table 2. Altogether, eighty-nine compounds were identified by GC and GC-MS.

Flower oil: Analysis of the oil derived from the flowers using GC and GC-MS identified fifty-six components (98.6% of total). The oil was characterized by a high proportion of esters (12 components, 32.7 %), oxygenated sesquiterpenes (10 components, 24.6 %) and oxygenated monoterpenes (4 components, 19.6%). The major constituents were (*Z*,*Z*)-farnesole (16.2%), linalool (11.0%), methyl linoleate (9.0%), hexadecanoic acid (7.1%), *Z*-hexenyl tiglate (6.9%), geraniol (4.8%) and germacrene D (4.8%). Besides the terpenic compounds, two hydrocarbons were present (2.8%). Aromatic compounds formed 0.1% of the total and ketones 1.4%.

Leaf oil: Sixty compounds were identified in the leaf oil, representing 99.2% of the total. The oil was characterized by a high proportion of sesquiterpenes (14 components, 23.2%), acids (5 components, 23.2%) and oxygenated monoterpenes (7 components, 16.3%). The main constituents were hexadecanoic acid (16.0%), linalool (8.7%), (*E*,*E*)-α-farnesene (7.1%) and (*Z*,*Z*)-farnesole (5.5%). The diterpene, phytol (5.6%), and the ester, bis(2-ethylhexyl)phthalate (4.6%), were also dominant components. Other compounds were present in amounts less than 2.9%.

Stem oil: A total of twenty-nine components were identified in the stem oil, accounting for 98.9% of the total oil. Acids constituted the major fraction (37.1%), hexadecanoic acid being (31.4%) the main representative. The second most abundant fraction was represented by sesquiterpene hydrocarbons (6 components, 16.2%), with germacrene D (3.9%), (*Z*,*E*)-α-farnesene (3.6%) and (*E*,*E*)-α-farnesene (3.4%) as the major compounds. Also, bis(2-ethylhexyl)phthalate (5.8%), (*E*)-β-damascenone (5.5%) and linalool (4.2%) were observed in high amounts.

Our results showed qualitative and quantitative differences between the oils isolated from different aerial parts of *L. japonica*. As Table 1 shows, the percentage of oxygenated monoterpenes decreased in the order flower oil > leaf oil > stem oil, which was followed by a decrease in the concentration of linalool from 11.0% to 4.2%. However, oxygenated sesquiterpenes were not identified in the stem oil, and (*Z*,*Z*)-farnesole was present in higher amount in the flower oil than in the leaf oil. On the other hand, the level of hexadecanoic acid increased in the order flower oil < leaf oil < stem oil (from 7.1% to 31.4%).

Table 1: Qualitative and quantitative composition of essential oil from flowers, leaves and stems of *Lonicera japonica.*

Compound[a]	RI[b]	flowers %	leaves %	stems %
Aromatic compounds				
Toluene [c, d, e]	773	tr[f]	0.2	0.4
p-Xylene [c, d, e]	885	0.1	0.3	1.6
Ethylbenzene [c, d, e]	868	/	/	/
m-Xylene [c, d, e]	869	/	/	3.6
o-Xylene [c, d, e]	894	/	0.6	/
2,6-Diisopropylnaphthalene [c, d]	1725	/	0.8	3.0
subtotal		0.1	1.9	8.5
Hydrocarbons				
1-Undecene [c, d]	1092	/	0.5	/
1.19-Eicosadiene [c, d]	1836	/	1.2	/
Docosane [c, d, e]	2200	/	2.3	/
Tricosane [c, d, e]	2300	/	1.1	/
Tetracosane [c, d, e]	2400	/	0.6	/
Pentacosane [c, d, e]	2500	/	0.3	1.2
Heptacosane [c, d, e]	2700	2.2	2.2	2.0
Octacoasane [c, d, e]	2800	/	1.4	0.4
Nonacoasane [c, d, e]	2900	0.6	3.5	/
subtotal		2.8	13.1	3.5
Alcohols				
3-Hexanol [c, d]	803	tr[f]	0.2	1.9
2-Hexanol [c, d]	806	tr[f]	0.3	0.3
(*Z*)-3-Hexenol [c, d]	857	tr[f]	1.0	/
(*Z,Z,Z*)-9,12,15-Octadecatrien-1-ol[c,d]	2055	/	2.1	/
subtotal		tr[f]	3.6	2.2
Aldehydes				
Hexanal [c, d]	800	tr[f]	0.4	2.9
2-Vinyl-crotonaldehyde [c, d]	839	/	0.3	1.1
(*E*)-2-Hexenal [c, d]	854	/	0.2	/
Benzaldehyde [c, d, e]	961	/	0.1	/
Lilac aldehyde (isomer I) [c, d]	1149	/	0.2	/
Lilac aldehyde (isomer II) [c, d]	1156	/	0.3	/
subtotal		tr[f]	1.5	4.0
Ketones				
3-Hexanone [c, d, e]	784	tr[f]	0.3	2.4
2-Hexanone [c, d, e]	792	tr[f]	/	/
cis-Jasmone [c, d]	1406	0.3	/	/
2-Pentadecanone [c, d]	1672	0.5	/	/
(*E.E*)-Farnesyl acetone [c, d]	1921	0.6	0.3	/
subtotal		1.4	0.6	2.4
Acids				
Dodecanoic acid [c, d, e]	1580	0.4	1.4	/
Tetradecanoic acid [c, d, e]	1780	1.7	1.8	2.6
Pentadecanoic acid [c, d, e]	1878	0.4	/	2.2
Hexadecanoic acid [c, d, e]	1991	7.1	16.0	31.4
Linoleic acid [c, d, e]	2134	/	1.1	1.0
Octadecanoic acid [c, d, e]	2160	0.1	2.9	/
subtotal		9.9	23.2	37.1
Esters				
Butyl acetate [c, d, e]	812	tr[f]	0.1	2.0
Benzyl acetate [c, d, e]	1165	tr[f]	/	/
Z-Hexenyl tiglate [c, d]	1275	6.9	/	/
(*Z*)-3-Hexenyl benzoate [c, d]	1570	1.0	/	/
Benzyl benzoate [c, d]	1762	3.4	/	/
Farnesyl acetate [c, d]	1811	4.1	0.4	/
Methyl hexadecanoate [c, d, e]	1926	3.6	0.6	/
Methyl linoleate [c, d, e]	2093	9.0	/	/
Ethyl linoleate [c, d, e]	2159	1.2	/	/
Butyl hexadecanoate [c, d]	2188	0.6	/	/
Bis(2-ethylhexyl)phthalate [c, d]	2526	/	4.6	5.8
Methyl docosanoate [c, d, e]	2538	1.5	/	/
Methyl tetracosanoate [c, d, e]	2740	1.5	/	/
subtotal		32.7	5.7	7.8
Phenylpropanoids				
Eugenol [c, d, e]	1356	/	2.8	3.1
subtotal		/	2.8	3.1
Oxygenated monoterpenes				
cis-Linalool oxide [c, d, e]	1074	/	0.2	/
Linalool [c, d, e]	1098	11.0	8.7	4.2
Hotrienol [c, d]	1106	/	1.3	/
p-Menth-1-en-9-al [c, d]	1187	/	0.8	/
α-Terpineol [c, d, e]	1189	0.5	1.2	/
Nerol [c, d, e]	1230	3.2	2.0	/
Geraniol [c, d, e]	1255	4.8	2.1	2.6
subtotal		19.6	16.3	6.7
Norisoprenoides				
(*E*)-β-Damascenone [c, d, e]	1381	tr[f]	1.0	5.5
Geranyl acetone [c, d]	1461	0.8	0.3	/
(*E*)-β-Ionone [c, d, e]	1488	/	0.4	/
subtotal		0.8	1.7	5.5
Sesquiterpene hydrocarbons				
α-Copaene [c, d, e]	1376	0.1	/	/
β-Bourbonene [c, d]	1384	tr	/	/
β-Cubebene [c, d]	1388	tr	/	/
β-Elemene [c, d]	1391	tr	/	/
β-Caryophyllene [c, d, e]	1418	0.1	1.9	0.6
Thujopsen [c, d]	1430	/	0.2	2.1
Aromadendrene [c, d, e]	1438	/	0.6	2.7
α-Humulene [c, d, e]	1454	tr[f]		
Germacrene D [c, d, e]	1480	4.8	0.8	3.9
(*Z,E*)-α-farnesene [c, d]	1492	/	0.3	3.6
(*E,E*)-α-Farnesene [c, d]	1507	/	7.1	3.4
Germacrene A [c, d]	1508	0.4	/	/
β-Bisabolene [c, d]	1510	/	0.9	/
δ-Cadinene [c, d, e]	1523	0.5	/	/
Germacrene B [c, d]	1556	0.5	1.0	/
subtotal		6.4	12.6	16.2
Oxygenated sesquiterpenes				
β-Elemol [c, d]	1550	0.2	2.9	/
(*E*)-Nerolidol [c, d, e]	1568	1.5	/	/
Ledol [c, d]	1582	0.5	/	/
Vulgarol B [c, d]	1605	/	1.5	/
10-*epi*-α-Muurolol [c, d]	1639	0.9	/	/
τ-Muurolol [c, d]	1641	0.7	/	/
α-Cadinol [c, d, e]	1650	0.5	/	/
(*Z*)-α-Bisabolene epoxide [c, d]	1675	/	0.2	/
(*Z,Z*)-Farnesole [c, d, e]	1713	16.2	5.5	/
(*E,Z*)-Farnesole [c, d]	1742	3.9	0.1	/
β-Costol [c, d]	1785	0.3	0.4	/
subtotal		24.6	10.6	/
Diterpenes				
Geranyllinalool [c, d, e]	2004	0.3	/	/
(*E*)-Phytol [c, d, e]	2109	/	5.6	/
Squalene [c, d, e]	2818	/	/	1.9
subtotal		0.3	5.6	1.9
total		98.6	99.2	98.9

[a]Essential oil compounds sorted by chemical families and percentage calculated by GC/FID on non-polar HP-5MS capillary column; [b]Retention indices calculated on non-polar HP-5MS capillary column; [c]Compound identified by mass spectral comparison with Wiley7NIST library; [d]Compounds identified by RI and mass spectra; [e]co-injection with authentic compound; tr[f] – below 0.1%.

The composition of the flower oil also showed qualitative and quantitative differences when compared with those reported for *L. japonica* from China [12]. In fact, (*Z,Z*)-farnesole, *Z*-hexenyl tiglate and germacrene D, occurring as major volatiles in the Serbian (European) populations of *L. japonica*, were absent in the flower oil from China. However, the oil isolated from fresh leaves from the Himalayan region of India was characterized by high contents of heneicosane (37.5%) and octadecane (34.5%) [8], whereas our sample contained only 13.1% of hydrocarbons.

With (*E*)-nerolidol and caryophyllene oxide as major compounds, the oil derived from the air dried floral parts of *L. japonica* from Korea [2] showed significant differences compared with our sample. In regard to the previously reported contents of the flower essential oil of *L. japonica*, qualitative and quantitative differences between the present work and earlier studies indicate that environmental factors, as well as the condition of the samples (fresh or dried), have a strong influence on its chemical composition.

Table 2: Total percentage of grouped components and number of identified constituents for each class of component, and oils yield.

	Number of identified compounds		
	flowers	leaves	stems
Aromatic compounds	2	4	4
Hydrocarbons	2	9	3
Alcohols	3	4	2
Aldehydes	1	6	2
Ketones	5	2	1
Acids	5	5	4
Esters	12	4	2
Phenylpropanoids	/	1	1
Oxygenated monoterpenes	4	7	2
Norisoprenoids	2	3	1
Sesquiterpene hydrocarbons	10	8	6
Oxygenated sesquiterpenes	9	6	/
Diterpenes	1	1	1
Total	56	60	29
	98.6 %	99.2 %	98.9 %
Yield	0.45 %	0.26 %	0.16 %

To our knowledge, this is the first report on the chemical composition of the leaf and stem essential oils of *L. japonica*, as well as that from flowering parts of this plant growing in Europe.

Experimental

Plant material: The aerial parts (flowers, leaves and stems) of *Lonicera japonica* were collected in June 2011 from natural populations in the region of Serbia, Kragujevac, Divostin (position: 43° 57' 16" N, 20° 53' 31" E, altitude: 750 m). A voucher specimen is deposited in the Herbarium of the Department of Botany, Faculty of Biology, University of Belgrade (BEOU 16506).

Isolation of the oils: The fresh plant materials (700 g, each) were subjected to hydro-distillation for 5 h using a Clevenger type apparatus. The oils were dried over anhydrous sodium sulfate overnight and stored in sealed vials at low temperature (4ºC) before analysis. The oil yields were calculated on a dry weight basis as 0.45% (flowers), 0.26% (leaves) and 0.16% (stems).

GC-MS and GC analysis: Analyses were carried out in an Agilent 6890N gas chromatograph fitted with a HP-5MS fused silica column (5% phenyl methyl polysiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm), interfaced with an Agilent mass selective detector 5975B (Agilent Technologies, USA) operated by HP Enhanced ChemStation software. Analytical conditions were injector and transfer line temperatures 250 and 280ºC, respectively, oven temperature programmed at 60ºC (isothermal for 5 min), with an increase of 4ºC/min to 130ºC (isothermal for 10 min), then 4ºC/min to 240ºC; carrier gas, helium at 1 mL/min; injection of 2 μL (10% *n*-hexane solution); split ratio 1:50, whereas split flow was 50 mL/min; standard electronic impact (EI) MS source temperature: 230ºC; MS quadruple temperature: 150ºC; mass scan range: 35-500 amu at 70 eV; scan velocity: 3.12 scans/s; resulting EM voltage: 1200 V.

GC analyses were performed on an Agilent 6890N gas chromatograph with FID detector using a HP-5MS column. The chromatographic conditions were the same as for GC/MS analyses.

Calculation of Kovats Retention Indices (RI): The oils were spiked with a standard mixture of homologous *n*-alkanes (C_6-C_{30}). Retention indexes were directly obtained by application of Kovats procedure [13,14].

Qualitative and quantitative analyses: The constituents of the volatile oils were identified by comparing their retention indices relative to C_6-C_{30} *n*-alkanes and their mass spectral fragmentation patterns with those reported in the literature [15] and stored in the MS library (Wiley7Nist). In many cases, the essential oils were subjected to co chromatography with authentic compounds (Sigma, Aldrich, Fluka). The quantification of the components was performed on the basis of their GC peak areas on a HP-5MS column.

Acknowledgments - This research was financially supported by Serbian Ministry of Science (Grand number III41010), VEGA (1/0501/12) and KEGA (013SPU-4/2012) scientific grants.

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