VOLATILE CONSTITUENTS FROM THE FLOWERS OF TWO SPECIES OF HONEYSUCKLE (*LONICERA JAPONICA* AND *LONICERA CAPRIFOLIUM*)

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Abstract

The aim of this paper was to evaluate the chemical composition of volatile oil isolated from the flowers of *Lonicera japonica* and *Lonicera caprifolium* cultivated in Romania. One oil sample from *Lonicera caprifolium* and two oil samples from *Lonicera japonica* obtained by hidrodistillation were analysed by gas chromatography - mass spectrometry (GC-MS). The main compounds found belong to sesquiterpene hydrocarbons (germacrene D 11.26 - 33.09%) and sesquiterpene alcohols (farnesol 26.16 - 50.98%, nerolidol 1.28 - 2.63%, α-cadinol 0.68 - 2.86%). The monoterpene alcohol linalool was identified in our samples from 1.93% in *Lonicera caprifolium* oil to 11.64% in *Lonicera japonica* oil. Other quantitatively important compounds were the esters: 3-hexenyl tiglate 2.62 - 6.79%, 3-hexenyl benzoate 0.57 - 5.24%, farnesyl acetate 0.11 - 5.23%. Cis-jasmone was detected in reduced, but apreciable quantities (0.58 - 0.85%) in oil samples from *Lonicera japonica*.

Rezumat

Scopul acestei lucrări a fost evaluarea compoziţiei chimice a uleiului volatil izolat din flori de *Lonicera japonica* şi *Lonicera caprifolium* cultivate în România. O probă de ulei volatil de *Lonicera caprifolium* şi două probe de ulei de *Lonicera japonica,* obţinute prin hidrodistilare, au fost analizate prin gaz cromatografie - spectrometrie de masă (GC-SM). Principalii compuşi identificaţi aparţin hidrocarburilor sesquiterpenee (germacren D 11,26 - 33,09%) şi alcoolilor sesquiterpenei (farnesol 26,16 - 50,98%, nerolidol 1,28 - 2,63%, α-cadinol 0,68 - 2,86%). Alcoolul monoterpene, linalool, a fost identificat în probele noastre cu un procent de 1,93% în uleiul de *Lonicera caprifolium* şi 11,64% în uleiul de *Lonicera japonica*. Alţi compuşi importanţi din punct de vedere cantitativ au fost esterii: 3-hexenil tiglat 2,62 - 6,79%, 3-hexenil benzoat 0,57 - 5,24% şi acetatul de farnesil 0,11 - 5,23%. Cis-jasmona a fost detectată în cantităţi mai mici, dar apreciable (0,58 - 0,85%), în probele de ulei volatil de *Lonicera japonica*.

Keywords: volatile oil, GC-MS, *Lonicera japonica, Lonicera caprifolium*.

Introduction

The *Lonicera japonica* Thunb. and *Lonicera caprifolium* plants are two species of honeysuckle belonging to the *Caprifoliaceae* family. Various parts of *Lonicera japonica* and *Lonicera caprifolium* plants were used in herbal traditional medicine for their antiviral, antibacterial and antioxidant activities. A few recent pharmacological studies have used essential oil or solvent plant extracts to confirm these activities [2,3,7,10,11]. Despite the fact that the essential oil from *Lonicera* is incorporated into many herbal medicinal and cosmetic preparations, its chemical composition was very little investigated and the scientific literature is poor in information concerning its chemical data [6,13,14].

Several previous papers have reported that chemical composition of volatile oil of *Lonicera japonica* varies at different flowering stages [13,14], but no data concerning *Lonicera caprifolium* oil has been reported yet. Other papers have shown that volatile compounds isolated from *Lonicera japonica* flowers have a notable cytotoxic activity against different cancer cells [4,7]. The fruits of *Lonicera caerulea* were shown by romanian researchers to be rich in anthocyanosides, flavonosides and carotenoids [10].

The aim of this study was to investigate the chemical composition of essential oil isolated from flowers of *Lonicera caprifolium* and *Lonicera japonica* harvested from Romanian flora.

Materials and Methods

Chemicals

Dichloromethane Suprasolv for gas-chromatography and anhydrous Na_2SO_4 granulated for organic trace analysis were purchased from Merck, Darmstadt, Germany. The n-alkanes C_8 - C_{20} solutions in hexane used for the determination of Kovats indices were also purchased from Merck.

Plant material

The flowers of *Lonicera caprifolium* at their full maturity were collected from Bucharest flora (June 2008). The flowers of *Lonicera japonica* at the same stage of flowering were collected from Targoviste region of Romania (June 2009, 2010). The species were identified on the basis of their morphological characteristics. The voucher specimens were deposited in the herbarium of the Botanical Department, Faculty of Pharmacy, University of Medicine and Pharmacy “Carol Davila” Bucharest.

Volatile oil extraction

500 g fresh flowers were hydro-distilled in a Neo Clevenger apparatus for 4 h. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4˚C until GC-MS analysis. The oil sample was diluted in dichloromethane (1/200) and 2 µL were injected for GC-MS analysis.

Gas-chromatography – mass spectrometry

GC-MS analysis of *Lonicera* volatile oil was carried out using a Fisons Instruments GC 8000 with an electron impact quadrupole, MD 800 mass spectrometer detector. The electron ionisation energy was 70 eV. A fused silica column 5% phenylpoly(dimethylsiloxane) (SLB - 5ms, 30 m x 0.32 mm i.d., film thickness = 0.25 µm) was employed. The operating conditions were the following: a split-splitless injector (split ratio, 1/30) at 280˚C, ion-source temperature 200˚C and the interface temperature 280˚C; initial column temperature, 40˚C for 3 min, raised at 4˚/min to 280˚C and finally held isothermally for 20 min; the carrier gas (helium) flow rate was 2 mL/min; sample volume injected, 2 µL. Data acquisition was performed with MassLab Software for the mass range 30-600 u with a scan speed of 1 scan/s. The identity of volatile oil components was established from their GC Kovats retention indices and from mass spectra by computer matching with mass spectra library (NIST, WILEY and a personal library of 600 spectra). The linear retention indices were determined in relation to a homologous series of n-alkanes (C_8 - C_{20}). The experimental value of Kovats indices were compared with those reported in literature [1,9,15]. Component relative concentrations were calculated from GC peak areas without using correction factors.

Results and Discussion

The essential oil yields, calculated on dry weight, varied between 0.16 - 0.23% for *Lonicera caprifolium* sample and 0.31 - 0.58% for *Lonicera japonica* samples, range that includes the value (0.43%) reported by Rahman [12]. The GC separation chromatogram of volatile oil isolated from *Lonicera* flowers is shown in Figure 1.

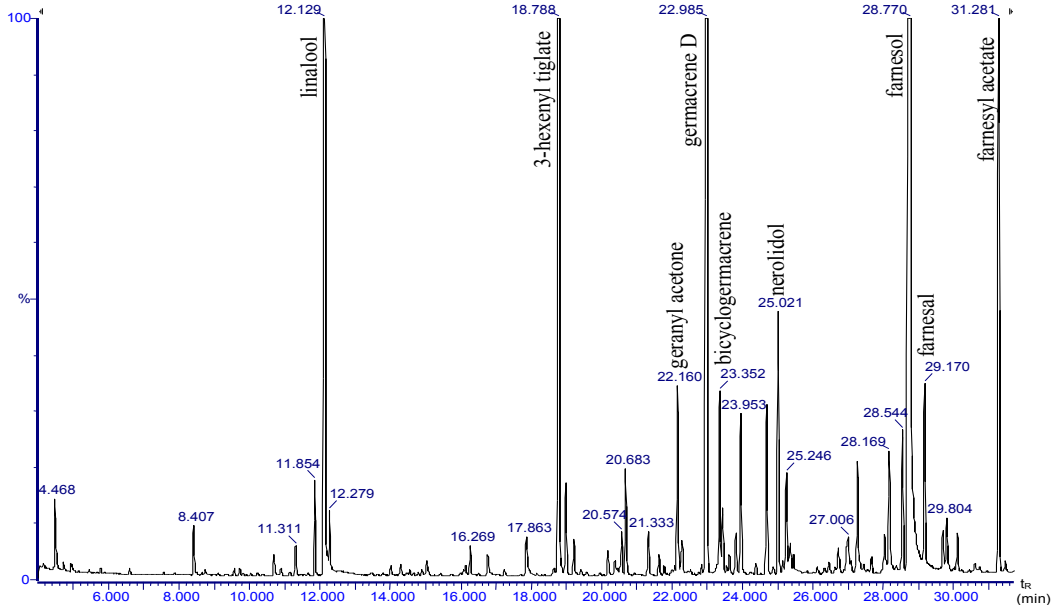

Figure 1
The chromatogram of *Lonicera japonica* (2009) volatile oil.

The identified compounds and their relative area are listed in Table I.

Fifty seven compounds were identified in the *Lonicera japonica* oil samples representing 95.20 and 90.85% of the total oil area. Thirty-nine compounds were reported by Rahman [12] and twenty-seven by Schlotzhauer [13] for the volatile oil of *Lonicera japonica*. With very few exceptions the compounds reported by Rahman [12] were also detected in the samples analyzed in this study. The data concerning the chemical composition of essential oil of *Lonicera caprifolium* has not been reported earlier. The chemical composition of the two samples of *Lonicera japonica* harvested in different years (2009 and 2010) showed no essential differences. In the volatile oil of *Lonicera caprifolium* only thirty eight compounds were identified.

Table I
Chemical composition of *Lonicera* volatile oils

No.	Compound	KI* exp	KI lit	Area %		
				***Lonicera caprifolium* Bucharest**	***Lonicera japonica* Targoviste**	
				2008	**2009**	**2010**
1	3-hexen-1-ol	872	875	-	0.01	0.51
2	benzaldehyde	964	964	-	0.01	0.11
3	6-methyl-5-hepten-2-one	988	987	-	0.23	0.13
4	p-cymene	1025	1026	-	0.05	0.11
5	limonene	1030	1031	-	0.05	0.11
6	1,8-cineole	1033	1033	-	0.05	0.11
7	trans-linalool oxide	1073	1074	-	0.01	0.16
8	cis-linalool oxide	1088	1087	-	0.01	0.14
9	methyl benzoate	1093	1094	-	0.47	0.20
10	linalool	1101	1100	1.93	11.64	9.61
11	nonanal	1106	1108	-	0.45	0.11
12	benzyl acetate	1164	1164	-	0.01	0.11
13	3-hexenyl butanoate	1172	1178	-	0.07	0.12
14	methyl salicylate	1191	1190	-	0.05	0.12
15	α-terpineol	1195	1197	-	0.10	0.20
16	dodecane	1200	1200	0.03	0.01	0.11
17	decanal	1208	1209	-	0.05	0.11
18	3-hexenyl-3-methylbutanoate	1238	1239	-	0.14	0.23
19	geraniol	1254	1255	-	0.15	0.24
20	n-propyl benzoate	1271	1284	-	0.05	0.11
21	indole	1291	1292	-	0.32	0.20
22	3-hexenyl tiglate	1324	1322	5.66	6.79	2.62
23	hexyl tiglate	1331	1331	0.01	0.50	0.47
24	methyl anthranilate	1340	1337	0.01	0.22	0.23
25	α-cubebene	1346	1345	0.18	0.05	0.12
26	α-copaene	1375	1376	0.22	0.14	0.18
27	3-hexenyl hexoate	1381	1381	0.01	0.01	0.20
28	β-bourbonene	1382	1384	0.10	0.05	0.11
29	β-elemene	1388	1389	0.80	0.25	0.36
30	Z-jasmone	1392	1394	0.01	0.58	0.85
31	tetradecane	1400	1400	0.12	0.01	0.11

No.	Compound	KI* exp	KI lit	Area %		
				Lonicera caprifolium Bucharest	*Lonicera japonica* Targoviste	
				2008	2009	2010
32	α-gurjunene	1414	1419	0.91	0.29	0.28
33	aromadendrene	1445	1444	0.14	0.01	0.11
34	α-humulene	1447	1454	0.09	0.01	0.11
35	geranyl acetone	1449	1455	0.39	0.99	0.29
36	germacrene D	1482	1482	33.09	19.72	11.26
37	bicyclogermacrene	1493	1494	1.74	1.03	0.70
38	α-muurolene	1494	1495	0.28	0.01	0.11
39	benzyl tiglate	1496	1496	0.01	0.39	0.40
40	pentadecane	1500	1500	0.11	0.05	0.11
41	α-farnesene	1504	1508	0.10	0.08	0.15
42	γ-cadinene	1509	1512	0.07	0.01	0.36
43	δ-cadinene	1517	1519	2.22	0.01	0.66
44	α-cadinene	1535	1534	0.01	0.01	0.12
45	elemol	1547	1547	0.84	0.93	0.45
46	nerolidol	1561	1562	2.63	1.43	1.28
47	3-hexenyl benzoate	1570	1570	5.24	0.57	1.13
48	hexyl benzoate	1578	1576	0.01	0.01	0.58
49	α-cadinol	1655	1654	2.86	0.73	0.68
50	farnesol	1724	1722	26.16	41.50	50.98
51	farnesal	1737	1738	0.01	1.19	0.11
52	1-octen-3-ol benzoate	1772	-	1.39	0.01	0.11
53	methyl farnesoate	1778	-	0.01	0.19	0.11
54	benzyl benzoate	1768	1771	0.87	0.01	2.33
55	octadecane	1800	1800	0.28	0.01	0.11
56	farnesyl acetate	1835	1837	5.23	3.41	0.11
57	hexahydrofarnesyl acetone	1841	1845	0.05	0.07	0.11
TOTAL				**93.82**	**95.20**	**90.85**
Total monoterpene hydrocarbons				-	0.10	0.22
Total esters				18.45	12.90	9.18
Total carbonyl compounds				0.46	3.57	1.82
Total sesquiterpene hydrocarbons				39.95	21.67	14.63
Total sesquiterpene alcohols				32.49	44.59	53.39
Total monoterpene alcohols				1.93	11.89	10.05

*calculated from *Lonicera japonica* (2009) chromatogram.

The sesquiterpene fraction was the predominant terpenoid group of compounds in all three samples representing 66.26 - 72.44%. The content of sesquiterpene hydrocarbons was 39.95% in the *Lonicera caprifolium* volatile oil whereas in the *Lonicera japonica* oil samples was 21.67 and 14.63%. The sesquiterpene hydrocarbon germacrene D (33.09% from total area), was the main constituent in the oil sample from *Lonicera caprifolium*. The sesquiterpene alcohols: elemol, nerolidol, α-cadinol and farnesol were detected in all analysed volatile oils samples. Their amounts varied from 26.16% in the volatile oil of *Lonicera caprifolium* to 44.59 and 53.39% in volatile oil of *Lonicera japonica*. The sesquiterpene alcohol farnesol reported by Rahman [12] as minor constituent of the essential oil of *Lonicera japonica* grown in Korea was the main component of our *Lonicera japonica* oil samples representing 41.50 and 50.98%. Farnesol was also the major compound in the volatile oil of *Lonicera caprifolium*, 26.16% from total area.

The monoterpene alcohol linalool previously reported [13] as a major compound of *Lonicera japonica* flower volatile oil was present in our samples 9.61 and 11.64% in oil from *Lonicera japonica* and only 1.93% in oil of *Lonicera caprifolium*. The characteristic flavour of *Lonicera japonica* flowers is attributed to the compounds: linalool, linalool oxide, geraniol and α-terpineol [14]. These compounds except linalool were detected in low amounts in all three volatile oil samples.

Other quantitatively important compounds were esters 3-hexenyl tiglate and 3-hexenyl benzoate, constituting 2.62 - 6.79% and 0.57 - 5.24% of the total oil, respectively. Farnesyl acetate was also found in noticeable amounts 5.23% in *Lonicera caprifolium* oil sample and 3.41% in one oil sample of *Lonicera japonica*. The added values of carbonilic compounds present in lesser quantities were 0.46% in *Lonicera caprifolium* oil, 3.57 and 1.82% in *Lonicera japonica* oil samples. Cis-jasmone, a compound widely used in perfume formulation, was found in *Lonicera japonica* oil samples representing 0.58 and 0.85%.

Except for limonene, found in *Lonicera japonica* oil, other hydrocarbon monoterpenes were not present in the oil samples analyzed.

Conclusions

Fifty-seven compounds were identified in both samples of *Lonicera japonica* oil and only thirty-eight in the *Lonicera caprifolium* oil sample. Our research concerning the chemical composition justifies the use of *Lonicera* in traditional medicine. The high content of sesquiterpene suggests that the flowers of *Lonicera japonica* and *Lonicera caprifolium* possess a

potent antimicrobian activity. Furthermore, linalool, a main constituent of volatile oil of *Lonicera japonica,* could recommend this oil as a posible chemotherapeutic agent against a range of neoplastic diseases.

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