Volatile compounds of the aerial parts of wild St. John's wort (*Hypericum perforatum* L.) plants

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The chemical composition of the essential oil of *Hypericum perforatum* L. growing wild in 10 habitats in Eastern Lithuania has been studied. The samples collected at full flowering were analysed by GC and GC/MS. Fourty six identified constituents made up 87.8–98.5% of the oils. The oils fell into three chemotypes – β-caryophyllene (4 samples, 10.5–19.1%), caryophyllene oxide (4 samples, 13.3–35.8%) and germacrene D (2 samples, 16.1–31.5%). The oils of the first two chemotypes contained 17.3–46.9% of constituents with a caryophyllane skeleton. The germacrene D chemotype had not been detected earlier in *H. perforatum* species. The sesquiterpene hydrocarbons and oxygenated sesquiterpenes made up 62.0–81.8% of the oils. The identified aliphatic compounds varied from 1.7 to 19.6%.

Key words: *Hypericum perforatum* L., Hypericaceae, essential oil composition, β-caryophyllene, germacrene D, caryophyllene oxide

INTRODUCTION

Six species of *Hypericum genus* grow wild in Lithuania [1]. *H. perforatum* and *H. muculatum* are widespread. The other four species (*H. hirstum, H. montanum, H. humifusum, H. acutum*) are rare or very rear.

Documented pharmacological activities, including antidepressant, antiviral and antibacterial effects, provide supporting evidence for some of the traditional uses stated for St. John's wort [2]. Many pharmacological activities appear to be attributable to hypericin, hyperforin and to the flavonoid constituents. The main constituents of the oils of the genus *Hypericum* pinenes and caryophyllene are bioactive [3–6].

The essential oils of *Hypericum* plants contained aliphatic compounds (2-methyl octane, n-nonane, n-decane, n-undecane, n-tetradecanol, etc) along with terpenoids. Terpenoids comprise the largest part of *H. perforatum* [2, 7–12] and the other species of *Hypericum* oils [9–11, 13–15]. Some oils contained n-nonane [13, 15] or 2-methyl octane [12] as the major constituents. The authors of [11] proposed that the main constituents of the oils of *H. perforatum* were sesquiterpenes, while the large part of the other species oils contained monoterpenes as major constituents [8–11, 16]. Recent studies of *H. perforatum* from India [12] and Turkey [7] showed that the main (>60%) constituent of oils was monoterpene α-pinene. Analysis of the oils of *H. perforatum* collected in other countries such as Serbia [8] and France [11] have shown that the caryophyllene was the main constituent of the essential oils.

The major constituents of the oils under study from plants collected in different localities of Eastern Lithuania were the compounds with caryophyllane skeleton or germacrene D.

MATERIALS AND METHODS

The aerial parts of *Hypericum perforatum* were collected in 2001 in different localities of Lithuania (Table 1). The plants were air-dried at room temperature (20–25 °C). The essential oils (0.1–0.4%) were prepared by hydrodistillation of 10–100 g air-dried samples. Voucher specimens have been deposited in the Herbarium of the Institute of Botany, Lithuania (BILAS) (Table 1).

The analysis of the essential oils was carried out by GC and GC-MS. A HP 5890II chromatograph equipped with FID and an HP-FFAP capillary column (30 m × 0.25 mm) was used for quantitative analysis. The GC oven temperature was set at 70 °C for 10 min and then programmed from 70 to 210 °C at a rate of 3 °C min^{-1}, using He as a carrier gas (0.7 ml min^{-1}) The injector and detector temperatures were 200 and 250 °C, respecti-

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Table 1. **Identification system of essential oils from *Hypericum perforatum* L. plants**

Symbol	Habitat	Vaucher number
A	Mištūnai (Varėna district)	59955
B	Valkininkai (Varėna district)	59961
C	Mardasavas (Alytus district)	59960
D	Skališkes (Vilnius district)	59956
E	Baltamiškis (Elektrėnai district)	59959
F	Žolynas (Vilnius city)	59954
G	Sapieginė (Vilnius city)	59958
H	Aleknos (Rokiškis district)	59957
K	Rokantiškės (Vilnius city)	59953
L	Mardasavas (Lazdijai district)	59962

vely. Analysis by GC-MS was performed, using a 5980II chromatograph interfaced to an HP 5971 mass spectrometer (ionization voltage 70 eV) and equipped with a CP-Sil 8 CB capillary column (50 × × 0.32 mm). The oven temperature was kept at 60 °C for 1 min, then programmed from 60 to 70 °C at the rate of 3 °C min^{-1} and then to 170 °C at the rate of 15 °C min^{-1}, kept for 8 min, then programmed at the rate of 5 °C min^{-1} to 250 °C, using He as a carrier gas (2.0 ml min^{-1}). The injector and detector temperatures was 250 °C.

The percentage composition of the essential oils was computed from GC peak areas without correction factors. Qualitative analysis was based on a comparison of retention times and retention indices on both columns and the mass spectra with corresponding data in the literature [17–20] and the computer mass spectra libraries (Wiley and NBS 54K).

Mass spectra (GC–MS) of the unknown compounds, m/z (relative intensity):

Unknown 1: (RI ~ 1457) 204 (4.03), 161 (5.09), 148 (12.95), 111 (28.59), 97 (59.3), 83 (82.14), 36 (90), 55 (96.43).

Unknown 2: (RI ~ 1910) 223 (6.04), 205 (3.05), 121 (2.01), 104 (2.08%), 76 (2.01), 57 (2.08), 41 (3.03).

RESULTS AND DISCUSSION

The main constituent of 8 oils from the 10 samples under study was β-caryophyllene or caryophyllene oxide (Table 2). The amounts of compounds with a caryophyllane skeleton in the above oils varied from 17.3 to 46.9%. Four oils from the plants collected in A–D localities were of caryophyllene oxide (13.4–35.8%) chemotype and the other 4 samples of oils from E–H habitats — of β-caryophyllene (10.5–19.1%) chemotype. Three oils (samples A, C, D) of caryophyllene oxide chemotype contained β-caryophyllene (11.1–12.0%) and the one oil (sample B) – germacrene D as the second major constituent. The third major constituent in A and B oils was spathulenol (7.5–8.0%) and in C and D samples – germacrene D (7.8–10.1%).

All the oils of β-caryophyllene chemotype (E–H) included caryophyllene oxide (7.0–15.7%) as the second major constituent. Spathulenol (6.9–8.5%) in F and H oils and germacrene D in E and G samples was the third major constituent.

Germacrene D (16.1–31.5%) was the main constituent in two oils (K, L; germacrene D chemotype). Caryophyllene oxide (13.1%) and β-farnezene

Table 2. **Chemical composition of essential oils of *Hypericum perforatum* L. growing wild in Eastern Lithuania***

Compound	RI	A	B	C	D	E	F	G	H	K	L
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Dimethylheptane	850	**6.6**	2.1	5.5	3.1	1.9	2.3	2.9	2.2	2.5	0.6.
2-Methyl octane	858	–	1.2	0.8	t*	t	0.2	t	t	0.1	0.7
n-Nonane	900	t	0.8	1.2	0.9	t	0.1	0.9	t	0.2	0.1
α-Pinene	939	6.5	1.1	1.6	**6.9**	2.5	4.9	3.9	1.2	3.4	2.6
β-Pinene	980	t	2.7	0.2	1.8	–	t	0.6	0.2	0.7	0.5
n-Decane	1000	–	t	t	t	t	0.6	t	0.2	0.5	0.2
n-Undecane	1100	t	–	0.6	t	t	0.6	0.7	t	0.6	0.1
Isoborneol	1156	t	–	–	t	–	0.2	1.0	–	t	t
α-Dihydroterpineol	1161	t	–	–	–	t	t	t	t	t	–
α-Copaene	1376	t	–	–	t	t	0.1	t	0.7	–	1.3
β-Bourbonene	1384	t	2.2	–	t	t	–	1.1	2.9	–	–
β-Caryophyllene	1418	**11.1**	**5.2**	**11.4**	**12.0**	**18.3**	**19.1**	**13.2**	**10.5**	5.2	5.1
β-Gurjunene	1432	–	–	–	–	t	t	–	0.9	0.8	t
β-Farnezene	1443	t	1.5	**8.0**	2.4	3.2	0.9	0.6	**6.1**	**8.2**	3.2
α-Humulene	1454	t	0.9	0.9	t	1.3	1.1	0.7	0.8	0.7	1.1
Unknown 1	1457	t	6.7	t	–	1.3	1.1	0.7	0.8	0.7	1.1

Table 2 (continued)

1	2	3	4	5	6	7	8	9	10	11	12
cis-Muurola-4(14),5-diene	1460	–	–	1.9	1.5	–	0.8	1.1	–	2.8	3.0
β-Acoradiene	1466	–	–	t	–	t	t	t	2.4	–	–
γ-Muurolene	1477	t	2.4	2.2	4.5	2.1	2.9	2.7	5.5	t	2.3
Germacrene D	1480	5.9	**9.8**	**10.1**	**7.8**	**10.1**	**6.8**	**5.9**	4.5	**16.1**	**31.5**
β-cis-Guaiene	1490	t	–	t	1.8	–	t	t	1.1	–	t
Viridoflorene	1493	t	–	t	0.2	t	–	–	–	t	t
α-Muuralene	1499	t	–	–	t	2.5	1.1	1.2	1.9	–	–
β-Bisabolene	1509	–	1.8	2.1	t	–	1.7	0.2	–	3.3	1.1
β-Curcumene	1512	–	–	–	t	–	t	–	t	1.1	–
γ-Cadinene	1513	t	1.7	1.6	2.0	1.3	1.6	1.8	2.7	4.5	4.6
δ-Cadinene	1524	t	2.6	2.5	3.4	2.6	1.7	2.5	6.7	3.5	t
Cadina-1,4-diene	1532	–	–	–	–	–	t	t	t	t	–
α-Cadinene	1538	–	–	–	t	–	t	t	0.8	t	–
α-Calacorene	1542	t	–	t	t	t	t	t	0.7	t	t
E-Nerolidol	1564	4.7	2.8	3.8	3.3	3.8	2.7	2.5	3.2	3.6	2.0
3-(Z)-Hexenyl benzoate	1570	–	–	t	t	t	t	–	1.1	t	t
Spathulenol	1576	**8.0**	**7.5**	7.1	5.4	**7.1**	**8.5**	**5.6**	**6.9**	**5.6**	3.9
Caryophyllene oxide	1581	**35.8**	**17.5**	**14.2**	**13.4**	**15.7**	**12.5**	**11.8**	**7.0**	**13.1**	6.1
Ledol* *	1583	t	1.8	2.4	1.2	t	3.1	1.9	1.1	2.1	t
β-Capaneol-4	1584	–	–	–	–	t	1.9	2.2	–	t	2.7
1-Hexadecene	1593	4/1	2.6	2.2	2.5	–	2.9	3.2	3.1	1.4	–
Humulene oxide II	16-6	-	4.0	–	–	2.5	–	–	–	–	–
Tetradecanal	1611	–	–	1.1	–	1.8	–	–	–	–	–
α-Muurolol	1645	–	–	1.0	2.1	–	t	1.9	3.1	–	–
α-Cadinol	1653	4.5	**5.3**	3.9	2.2	2.5	2.9	2.7	2.5	3.4	**6.2**
n-Tetradecanol	1676	7.3	4.9	4.2	3.9	2.2	1.6	1.1	2.9	2.1	–
α-Bisabolol	1683	2.7	–	–	–	1.6	t	t	2.8	1.2	–
α-Muurolene-14-hydroxy	1775	t	3.3	1.6	3.4	–	1.9	2.4	2.7	1.6	**9.1**
n-Pentadecanol	1778	–	–	–	1.7	–	0.9	1.2	1.7	1.6	–
Unknown 2	1910	–	2.3	1.5	5.2	t	0.7	1.3	5.0	1.8	t
Phytol	1949	–	t	2.1	t	3.3	1.9	5.6	t	3.5	t
n-Eicosane	2000	–	t	2.0	–	t	0.6	4.0	1.0	t	t
Total identified		97.2	85.3	96.2	88,4	90.1	87.4	86.4	87.9	95.9	86.7
Monoterpene hydrocarbons		6.5	3.8	1.8	9.7	2.5	4.9	4.8	1.4	4.1	3.1
Oxygenated monoterpenes		t	–	–	t	t	0.2	1.0	t	t	t
Sesquiterpene hydrocarbons		17.0	27.7	42.7	35.6	41.4	37.7	31.0	47.5	48.8	51.9
Oxygenatd sesquiterpene		55.7	42.2	36.1	31.0	33.2	35.1	31.0	31.0	30.5	30.0
Aliphatic hydrocarbon		10.7	6.7	10.3.	6.5	1.9	6.3	11.7	3.4	8.8	1.7
Oxygenated aliphatics		7.3	4.9	5.3	5.6	7.3	3.4	7.9	4.6	3.7	t
Caryophyllane skeleton		46.9	22.7	25.6	25.6	34.0	31.6	25.0	17.3	18.3	11.2

*Capital letters indicate the plant growing localities, t – traces.
**Unidentified isomer.

(8.2%) in the K oil and α-muurolene-14-ol (9.1%) and α-cadinol (6.2%) in the L oil were the second and the third major constituents. The L oil contained the lowest amount of compounds with a caryophyllane skeleton (11.2%).

Oils of β-caryophyllene and caryophyllene oxide chemotypes included 8.0–19.6% of aliphatic compounds, while L oil of germacrene D chemotype contained only 1.7% of aliphatic hydrocarbons.

The largest number (130) of constituents was found in sample B. A large number of the peaks was under 0.01%. Fourty six compounds were identified (Table 2). The constituents listed in Table 2 made up 87.8–98.5% of the oils. The amount of the main constituent groups, sesquiterpene hydrocarbons and oxygenated sesquiterpenes, comprised 62.0–81.8% of oils in all the samples studied. The largest variation of the number of constituents was

detected for sesquiterpene hydrocarbons (Table 2, 17.0–51.9%). Oxygenated sesquiterpenes made up 30.0–55.7% of the oils. Aliphatic compounds varied from 1.7 to 19.6%. Monoterpenoids (1.4–9.7%) and the concentration of "other" compounds (tr. – 9.0%) were never more than 10% of the oils. β-Caryophyllene (5.1–19.1%), spathulenol (3.9–8.5%), α-cadinol (2.2–6.2%) and E-nerolidol (2.0–4.7%) were found in a significant quantity (>2%) in all the oils studied. Some samples of the oils contained dimethylheptane (A, C), α-pinene (A, D), β-farnezene (C, K), γ-muuralene (H), n-tetradecanol (A), α-muurolene-14-hydroxy (L) and phytol (G) in considerable amounts (>5%). The content of a lot of constituents in the oils varied in a wide range (Table 2).

H. perforatum producing β-caryophyllene chemotype oil is widespread all over Europe. They were found in such countries as France [10], Serbia [8], and Lithuania (Table 2). The composition of the oils of caryophyllene oxide chemotype was similar to that of the oils of the above chemotype. The oils of germacrene D chemotype have not been detected in *H. perforatum* species.

CONCLUSIONS

Fourty six identified constituents made up 85.3–97.2% of essential oils of *Hypericum perforatum* L. growing wild in Eastern Lithuania. The content of sesquiterpenoides reached 62.0–81.8% of the oils. The major constituents were β-caryophyllene, germacrene D, caryophyllene oxide and spathulenol. Three chemotipes (β-caryophyllene, caryophyllene oxide, germacrene D) of essential oils were identified according to the first major constituent. The germacrene D chemotype has not been noted early in *H. perforatum* species before.

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LAUKINIŲ JONAŽOLIŲ *HIPERICUM PERFORATUM* L. LAKIEJI JUNGINIAI

S a n t r a u k a

Žydinčios jonažolės buvo surinktos 10 augimviečių. Eteriniai aliejai pagal pirmąjį komponentą priskirti 3 chemotipams: β-kariofileno, kariofileno oksido ir germakreno D. Pirmieji du chemotipai buvo rasti ir kitose šalyse, o trečiasis, germakreno D, mūsų nustatytas tirtose 2 augimviečių jonažolėse. Keturiasdešimt šeši identifikuoti junginiai sudaro 85,3–97,2% tirtų eterinių aliejų. Didžioji dalis (62,0–81,8%) lakiųjų junginių yra seskviterpenoidai.