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Chemical composition of essential oils produced by pink flower inflorescences of wild *Achillea millefolium* L.

Asta Judþentienë, Danutë Mockutë

Institute of Chemistry, Goštauto 9, LT-2600, Vilnius, Lithuania
E-mail judzent@ktl.mii.lt

Fourteen samples of *Achillea millefolium* L. with pink inflorescences were collected in 14 habitats. Essential oils were produced by hydrodistillation and analysed using GC and GC/MS. The main constituents of the oils were sabinene, β-pinene, 1,8-cineole, β-caryophyllene, (E)-nerolidol, caryophyllene oxide and selinen-11-en-4-α-ol. The first major component of the essential oils was (E)-nerolidol (11.6–31.9%, 7 oils) or β-pinene (9.0–23.1%, 6 samples) or 1,8-cineole (14.1%, one oil). Nine samples of inflorescence oils did not contain chamazulene, 4 oils contained only ≤0.5% and one sample 5.7% of this compound. The 64 constituents found in the oils made up 72.0–97.6%. The essential oils formed by yarrow inflorescences with pink flowers differed from these with white flowers by absence of chamazulene and borneol chemotypes and by presence of larger amounts of (E)-nerolidol.

Key words: *Achillea millefolium* L., *Compositae*, chemical composition of essential oils, inflorescences, (E)-nerolidol, β-pinene, 1,8-cineole

INTRODUCTION

Achillea millefolium L. plants with pink flowers grow together with plants with white inflorescences. Yarrow with white flowers is widely spread all over Lithuania, while that with pink flowers is much rarer [1]. Yarrow plant is used for healing of different diseases [2–9], and the bioactive properties of the plant are attributed mainly to azulenes and proazulenes. Data on these compounds have been reviewed in [9–11] . Plants only from some habitats of Lithuania contain proazulenes, which produced azulenes during hydrodistillation [9–14].

Reports on the chemical composition of essential oils produced by *A. millefolium* from different countries were reviewed in [9–11, 15, 16]. The first major components in the oils were chamazulene, sabinene, β-pinene, 1,8-cineole, linalool, α-thujone, β-thujone, ocimene, camphor, ascaridole, caryophyllene oxide, β-eudesmol and α-bisabolol [11]. Chamazulene was the first main constituent only in the inflorescence oils from Canada [15], Estonia [17] and Lithuania [11]. White colour of flowers was mentioned only in several papers on the chemical composition of essential oils. Authors of a large part of investigations did not indicate the colour of inflorescences, while the decorative usage of coloured yarrow flowers was mentioned in [18].

Plants with different colours of inflorescence are offered as medical plants in markets. Yarrow essential oils as well as plants themselves are used for healing [19]. However, only essential oils biosynthesized by plants with white flowers have been earlier investigated in Lithuania.

The chemical composition of essential oils produced by pink-flowered yarrow markedly differed from that of the oils biosynthesized by white flowers.

MATERIALS AND METHODS

The aerial parts (~35 cm) of plants (0.1–0.5 kg) growing wild in 14 localities of Lithuania were collected in August 2000 and 2001.

Voucher specimens were deposited in the Herbarium of the Institute of Botany (BILAS), Vilnius, Lithuania: A – No 65288, Rûdninkai (Ðalèininkai district); B – 65285, Viðtytis (Vilkaviðkis district); C – 65291, Vilnius center; D – 65272, Antakalnis (Vilnius city); E – 65281, Rokantiðkës (Vilnius city); F – 65290, Vidugiriai (Trakai district); G – 65279 and H – 65277, Tauras hill (Vilnius city); J – 65286, Aleknos (Rokiðkis district); K – 59444, Paþeimenë (Ðvenèionys district); L – 65289, Skaliðkës (Vilnius district); M – 65276, Pilioniai (Këdainiai district); N – 65280 and P – 65283, Þirmûnai (Vilnius city).

All samples were collected at full flowering stage. The plants were dried at room temperature (20–25 °C). Flowers were separated from stems and leaves before drying. Essential oils were prepared by hydrodistillation for 3 h of 15–50 g of

air-dried plants. The inflorescence oil yield was of 0.7–1.2%.

The analysis of the essential oils was carried out by GC and GC-MS. The HP 5890 II chromatograph equipped with FID and capillary column HP-FFAP (30 m × 0.25 mm) was used for quantitative analysis. The GC oven temperature was set at 60 °C for 2 min, then programmed at a rate of 5 °C min^{-1} to 160 °C, kept for 1 min, then programmed from 160 to 230 °C at a rate of 10 °C min^{-1} and finally kept isothermal at 230 °C for 12 min. The injector and detector temperatures were 250 °C.

Analysis by GC-MS was performed using an HP 5890 chromatograph interfaced to an HP 5971 mass spectrometer (ionization voltage 70 eV) and equipped with a CP-Sil 8 CB capillary column (50 m × × 0.32 mm). The oven temperature was kept at 60 °C for 2 min, then programmed from 60 to 160 °C at a rate of 5 °C min^{-1}, kept for 1 min, then programmed from 160 to 250 °C at a rate of 10 °C min^{-1} and kept isothermal at 250 °C for 2 min, using He as the carrier gas (2.0 ml min^{-1}). The temperatures of the injector and detector were 250 °C and 280 °C, respectively.

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 indexes on both columns and mass spectra with corresponding data in the literature [20, 21] and computer mass spectra libraries (Wiley and NBS 54K).

The following mass spectral data (m/z – relative intensity) of unknown components were recorded:

Unknown 1: 220 (16), 202 (14), 177 (13), 159 (79), 131 (32), 119 (64), 109 (75), 93 (79), 91 (100), 79 (65), 67 (50), 55 (58), 41 (78),

Unknown 2: 207 (3), 177 (3), 159 (7), 145 (3), 126 (29), 108 (75), 93 (58), 79 (36), 67 (32), 55 (36), 43 (100).

RESULTS AND DISCUSSION

White inflorescences of yarrow from 21 habitats produced essential oils containing as the main constituents β-pinene/1,8-cineole (8 samples), 1,8-cineole/β-pinene (2 oils), chamazulene/β-pinene (2), β-pinene/chamazulene (2), (E)-nerolidol/β-pinene (3), borneol/β-pinene (2), β-pinene/borneol (1) and β-pinene/sabinene (1 oil) as has been shown in the previous study (Table 1) [11].

The chemical composition of essential oils of yarrow with pink flowers differed from that of plants with white flowers in principle. The inflorescence oils of pink flowers collected in 14 habitats did not contain chamazulene among the main constituents (Table 2). Nine oils were azulene-free, four samples (B, E, G, K) contained ≤0.5% of chamazulene, and only one oil (P) contained 5.7% of it. Inflorescences with pink flowers biosynthesized borneol in lower amounts (Table 2, tr-5.6%) than plants with white flowers (11.5–13.2%) of the borneol group [11]. Bor-

Table 1. **The main constituents of inflorescence essential oils of *Achillea millefolium* L. with white flowers [11]**

Sample No	First component	Second component	Third component
3	Borneol	1,8-Cineole	β-Pinene
4	–"–	β-Pinene	1,8-Cineole
5	β-Pinene	Chamazulene	–"–
6	–"–	–"–	–"–
7	Chamazulene	β-Pinene	**(E)-Nerolidol**
8	–"–	–"–	β-Caryophyllene
13	**(E)-Nerolidol**	–"–	Sabinene
15	–"–	–"–	–"–
14	–"–	–"–	1,8-Cineole
23	1,8-Cineole	–"–	**(E)-Nerolidol**
25	–"–	–"–	α-Pinene
16	β-Pinene	Borneol	**(E)-Nerolidol**
22	–"–	Sabinene	1,8-Cineole
20	–"–	1,8-Cineole	α-Pinene
24	–"–	–"–	Sabinene
31	–"–	–"–	–"–
38	–"–	–"–	–"–
9	–"–	–"–	β-Caryophyllene
34	–"–	–"–	–"–
35	–"–	–"–	–"–
39	–"–	–"–	**(E)-Nerolidol**

Table 2. **Chemical composition of inflorescence essential oils of *Achillea millefolium* L. with pink flowers**

Compound	$R.I._{CP\text{-}Sil8\ CB}$	A	B	C	D	E	F	G	H	J	K	L	M	N	P
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
Tricyclene	926		tr.						tr.			tr.			
α-Thujene	931		tr.					tr.	tr.	0.1		tr.	0.5	0.1	0.1
α-Pinene	939	1.8	3.0	6.3	2.0	1.9	1.3	3.6	0.1	4.9	0.9	6.3	**8.9**	2.5	3.5
Camphene	953	tr.	2.0		1.9	1.1	0.4	tr.	0.1	2.4	tr.	2.1	tr.	0.5	2.5
Sabinene	976	6.5	4.4	5.0	6.0	5.0	3.5	4	6.6	**7.7**	4.0	**6.9**	3.9	**7.4**	6.2

Table 2 (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
β-Pinene	980	**20.0**	**11.1**	**12.6**	**12.1**	**5.0**	4.1	4.6	**18.0**	**9.0**	**15.0**	**17.9**	**16.0**	**23.1**	**9.0**
Myrcene	991	tr.	0.3	1.0	0.8	0.1	0.4		0.1	0.1	0.8	1.3	2.0	1.9	0.2
α-Terpinene	1018	tr.	0.3	0.5	tr.	0.4	0.5	tr.	tr.	0.6	0.7	tr.	0.2	tr.	0.1
p-Cymene	1026	tr.	0.7	0.8	tr.	0.4	0.7		0.1	0.5	0.6	0.3	0.7	tr.	tr.
1,8-Cineole	1033	**8.3**	**9.9**	2.7	5.5	4.2	1.4	3.5	5.4	5.7	**14.6**	**10.3**	**8.3**	tr.	**14.1**
γ-Terpinene	1062	tr.	1.4	1.2	2.7	1.2	0.4	0.8	2.5	2.3	1.6	1.8	2.3	3.4	tr.
Terpinolene	1088	tr.	0.1	0.5	tr.	0.2	0.5	2.3	tr.	0.4	0.3	tr.		tr.	tr.
Chrysanthenone	1123			**7.2**											
trans-Pinocarveol	1139			tr.			0.2								
Camphor	1143	tr.	3.8		2.3	2.8	1.5	1.0	1.7	4.1	1.9	4.1	tr.	4.5	2.3
cis-Chrysanthenol	1162		0.2	0.5	tr.	0.3	0.3		tr.	0.1	4.4	tr.		tr.	0.1
Borneol	1165	tr.	0.9	1.0	4.2	4.1	0.9	1.5	5.6	4.9	3.6	3.1	tr.	3.2	4.2
Terpinen-4-ol	1177	1.1	1.2	1.4	3.1	2.0	0.5	1.3	2.6	2.8	3.9	2.3		6.1	2.5
α-Terpineol	1189	1.3	1.4	0.4	0.9	0.8	0.4	0.5	tr.	1.4		1.9	0.6	tr.	2.6
Carvotanacetone	1246			2.1											
cis-Chrysanthenyl acetate	1262		0.3				1.7		tr.	0.3	0.7				
Myrtenol	1270									0.4				1.2	
Bornyl acetate	1285	3.0	3.3	0.3	1.8	1.1	0.9	3.6	3.9	0.4	0.8	0.6		0.5	0.8
Levandulyl acetate	1289	0.6	0.4		0.7	0.5				0.2					
α-Cubebene	1351		0.2	0.2		0.1	tr.	0.4					0.7		tr.
β-Bourbonene	1384		0.3	tr.	tr.	0.1	0.1	0.1	0.1	0.2			tr.	tr.	tr.
β-Elemene	1404		0.8						1.2	0.4		0.4			
β-Caryophyllene	1418	2.2	7.7	4.2	5.5	2.4	1.7	1.5	**6.7**	3.9	3.3	3.1	6.5	5.7	6.6
α-Humulene	1454	tr.	1.2	1.1	0.9	0.6	0.3	0.3	1.2	1.3	0.5	0.6	1.1	0.9	1.0
β-Farnesene	1460		0.5					0.6		0.2			0.6		1.4
allo-Aromadendrene	1469	0.7	0.3					tr.	tr.	0.3		0.3		2.5	1.2
α-Acoradiene	1475								tr.	0.3					
Germacrene D	1480	1.8	4.7	3.8	3.0	1.7	2.2	**7.4**		2.0	2.1	2.0	1.0	0.7	1.9
γ-Curcumene	1485		1.2					0.4		1.1		0.5			
Bicyclogermacrene	1494		tr.	0.6		0.8	0.3	1.2		0.6	0.4	0.4	0.5		
α-Muurolene	1499					0.7	0.5								
β-Himachalene	1504		0.5				0.5	0.7		0.2					
β-Bisabolene	1509	1.1	0.3	0.5	0.7	0.4		1.7	1.2		1.0	0.4			
Sesquicineole	1514			0.2			2.3								
δ-Cadinene	1524		1.8	0.6	1	2.0	0.8	**7.6**		2.0	2.7	0.9	0.5	0.7	tr.
trans-Nerolidol	1564	**31.9**	**11.6**	**15.8**	**14.8**	**12.0**	**16.0**	**21.8**	**14.0**	**6.2**	**7.6**	6.8	3.4	2.4	3.6
Spathulenol	1576	tr.		0.8	tr.	2.0	3.2	tr.	tr.	0.8	0.7		0.4		tr.
Sesquisabinene hydrate	1579		1.2						1.6	1.2					
Caryophyllene oxide	1581	2.8	7.5	3.5	3.5	2.1	**4.8**	2.3	3.7	3.0	2.4	2.4	5.1	**8.2**	3.9
Globulol	1585	0.8	0.5		2.3					0.7		1.5	0.5	2.3	tr.
Viridiflorol	1590			1.0	1.8	2.0	2.8		2.0	0.5	0.9	0.3	0.4	0.5	
Humulene epoxide	1606		0.6							0.9					
Eudesmol	1622		1.3	3.0	**8.4**	2.2	4.3	2.4	3.4	5.3		2.2	0.8	0.8	
Caryophylla-4(14), 8(15)-dien-5-ol	1635	0.6		1.2	1.1	1.5	1.8			0.7	0.8	0.6	1.0	2.6	
epi-α-Cadinol	1640									1.5					
Himachalol ?	1647							1.7		1.1		0.9	1.3	1.5	1.2
Selin-11-en-4-α-ol	1660		3.2		2.5	**8.0**	**5.1**	0.2	4.0		4.0	4.3	5.9	6.7	
14-Hydroxy-9-epi-β-caryophyllene	1669														
α-Bisabolene oxide	1682			0.3		0.5			0.8	2.8	1.1		1.8		**7.1**
Unknown 1	1685			1.9		1.3	2.3				1.6				
Unknown 2	1682			0.7		1.8	1.4				2.3				
(2Z, 6E)-Farnesol	1701								3.5	0.5		0.5	1.4		

Table 2 (continued)

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
(2Z, 6Z)-Farnesol	1718	1.1	0.6	1.3	4.5	1.0	1.5	0.2	0.5	0.6	0.6	1.6	0.5	4.5	1.6
(2E, 6E)-Farnesol	1725	1.9							2.4						
Chamazulene	1732		0.5			0.3		0.5			0.1				5.7
(2Z, 6E)-Farnesyl acetate	1822	1.9	2.0		2.1		0.5	0.7		2.3		1.7	0.2	3.2	0.5
Total		89.4	93.2	84.2	96.1	74.6	72.0	78.4	93.0	88.9	85.9	90.3	77.0	97.6	83.9
Monoterpene hydrocarbons		28.3	23.2	27.4	25.5	15.1	11.3	13.0	27.5	27.6	23.6	36.6	34.5	38.9	21.6
Oxygenated monoterpenes		14.3	21.5	16.1	18.5	16.0	8.3	13.7	19.2	20.7	30.2	22.3	8.9	15.5	26.6
Sesquiterpene hydrocarbons*		5.8	20.0	11.0	11.1	9.1	5.4	22.4	10.4	12.5	10.1	8.6	10.9	10.5	17.8
Oxygenated sesquiterpenes		41.0	28.5	29.7	41.0	34.4	46.0	29.3	35.9	28.1	22.0	22.8	22.7	32.7	17.9

* Including chamazulene.

neol was between six major constituents only in 2 oils (Table 2, H – the fifth, J – the sixth one).

The essential oils under study (except sample P) were divided into two groups: (E)-nerolidol (7 samples) and β-pinene (6 oils).

(E)-Nerolidol was among the four predominant constituents in 11 oils out of 14 under study (Tables 2 and 3). Seven oils (A–G) were of (E)-nerolidol chemotype (11.6–31.9%), one sample (H) contained this compound as the second (14.0%), two samples (J, K) as the third (6.2–7.6%) and one oil (L) as the fourth main constituent (6.8%). (E)-Nerolidol content in white inflorescence essential oils (9.3–13.5%) in the (E)-nerolidol group [11] was markedly lower than in the corresponding oils of plants with pink flowers (Table 2, 11.6–31.9%) of the same group. Four oils (A–D) of (E)-nerolidol chemotype included β-pinene, two (E, F) samples contained selin-11-en-4-α-ol and one oil (G) δ-cadinene as the second dominant component. The third main compound was sabinene (2 samples) or 1,8-cineole (1 oil) in the white inflorescence oils (Table 1). 1,8-Cineole was the third major constituent in two out of the 7 oils of (E)-nerolidol chemotype studied (Tables 2 and 3). The above position was occupied by chrysanthanone, eudesmol, β-pinene, caryophyllene oxide and germacrene D in other five oils. β-Pinene was the second or the third dominant compound in 5 from 7 oils of (E)-nerolidol chemotype (Table 3). This compound was the fourth in the oil G and the fifth in the sample F (Table 2).

Six essential oils (H-N) out of 14 under study contained β-pinene (9.0–23.1%) as the first dominant constituent (Tables 2 and 3). 1,8-Cineole was the second main component in two essential oils produced by inflorescences with pink flowers (Tables 2 and 3, K, L), while this compound occupied the same position in 8 out of 10 oils of β-pinene chemotype biosynthesized by white inflorescences (Table 1). The second major constituents were (E)-nerolidol, sabinene, α-pinene and caryophyllene oxide in the other oils of β-pinene chemotype under study (Table 3). The third position in the row of main constituents was occupied by (E)-nerolidol, β-caryophyllene, 1,8-cineole and sabinene in the oils produced by yarrow inflorescences with both pink and white flowers (Tables 1–3).

1,8-Cineole, β-pinene and α-bisabolene oxide were the first three main components in the oil H (Table 2 and 3). α-Bisabolene oxide was found in 6 oils in low quantities (Table 2, 0.3–2.8%), while 7 samples did not contain this compound. The essential oils with the same first and second predominant constituents produced by yarrow with white flowers [11] differed in other main components from the oil H (Table 2).

Table 3. **The main constituents of inflorescence oils of *Achillea millefolium* L. with pink flowers**

Habitat	First component	Second component	Third component
A	**(E)-Nerolidol**	β-Pinene	1,8-Cineole
B	–"–	–"–	–"–
C	–"–	–"–	Chrysanthanone
D	–"–	–"–	Eudesmol
E	–"–	Selin-11-en-4-α-ol	β-Pinene
F	–"–	–"–	Caryophyllene oxide
G	–"–	δ-Cadinene	Germacrene D
H	â-Pinene	**(E)-Nerolidol**	β-Caryophyllene
J	–"–	Sabinene	**(E)-Nerolidol**
K	–"–	1,8-Cineole	–"–
L	–"–	–"–	Sabinene
M	–"–	α-Pinene	1,8-Cineole
N	–"–	Caryophyllene oxide	Sabinene
P	1,8-Cineole	β-Pinene	α-Bisabolene oxide

Different quantities of terpenoid groups were determined in the (E)-nerolidol and β-pinene chemotypes of essential oils. The amount of monoterpene hydrocarbons was lower (Table 2, 11.3–28.3%) in (E)-nerolidol chemotype oils than in β-pinene type (23.6–38.9%). Almost the same correlation was observed for oxygenated monoterpenes. No marked difference was noted in the amounts of sesquiterpene hydrocarbons. The quantity of oxygenated sesquiterpenes in (E)-nerolidol chemotype oils exceeded that in β-pinene type oils. Three oils (A, D, F) of (E)-nerolidol chemotype contained >41.0% of oxygenated sesquiterpenes, while 3 oils (K, L, M) of β-pinene type included <23.0% of these compounds.

Sixty-four compounds listed in Table 2 made up 72.0–97.6% of the essential oils, the amount of identified constituents reaching 68.3–97.6%.

CONCLUSIONS

Thirteen essential oils out of 14 produced by *Achillea millefolium* L. inflorescences with pink flowers were distributed into two chemoypes: (E)-nerolidol (7 oils) and β-pinene (6 samples). Chamazulene and borneol chemotypes of inflorescence oils produced by yarrow with white flowers were not determined in the inflorescences under study. Only 3 out of 21 inflorescence oils found in plants with white flowers in the previous study [11] were of (E)-nerolidol type, while half of the oils under study were attributed to this chemotype. Eleven from 14 inflorescence oils of plants with pink flowers contained (E)-nerolidol between the four major components. Pink inflorescences had a better biosynthesizing system for (E)-nerolidol than did inflorescences with white flowers.

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A. Judþentienë, D. Mockutë

RAUSVAI ÞYDINÈIØ LAUKINIØ KRAUJAÞOLIØ (*ACHILLEA MILLEFOLIUM* L.) ÞIEDYNØ ETERINIØ ALIEJØ CHEMINË SUDËTIS

S a n t r a u k a

Dujø chromatografijos–masiø spektrometrijos metodu buvo tiriami rausvai þydinèiø laukiniø kraujaþoliø, surinktø 14 augavieèiø, þiedynø eteriniai aliejai. Pagrindiniai komponentai ðie: sabinenas, β-pinenas, 1,8-cineolis, β-kariofilenas, (E)-nerolidolis, kariofileno oksidas ir selin-11-en-4-α-olis. Pirmas vyraujantis junginys buvo (E)-nerolidolis (11,6–31,9%) septyniuose mëginiuose, β-pinenas (9,0–23,1%) – šešiuose aliejuose ir 1,8-cineolis (14,1%) – viename aliejuje. Devyniuose aliejuose nerasta chamazuleno, keturiuose – ≤ 0,5% ir tik viename – 5,7% ðio bioaktyvaus junginio. Pagal sudëtá eteriniai aliejai suskirstyti á 2 – (E)-nerolidolio ir β-pineno – chemotipus.

Ðie aliejai savo sudëtimi labai skyrësi nuo anksèiau tirtø baltai þydinèios kraujaþolës þiedynø eteriniø aliejø, kuriuose, be minëtø (E)-nerolidolio ir β-pineno, nustatyti chamazuleno ir borneolio chemotipai, o (E)-nerolidolio kiekiai maþesni ir rasti tik 30% augavieèiø.