SHORT REPORT

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Atypical Chemical Profiles of Wild Yarrow (*Achillea millefolium* L.) Essential Oils

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Abstract: Chemical composition of yarrow (*Achillea millefolium* L. ssp. *millefolium*) essential oils was presented in the study. Plant material was collected at four populations where two forms, white (f. *millefolium*) and pink (f. *rosea*) of *A. millefolium* L. are growing together (in Lithuania). The essential oils of different plant organs (inflorescences and leaves) were obtained by hydro-distillation and analyzed by GC and GC/MS. The application of PCA and AHC grouped the sixteen oils into seven segments, that allowed to distinguish four new chemical profiles of yarrow essential oils. For the first time, atypical chemotypes with predominant *cis*-chrysanthenol (27.3%), selin-11-en-4α-ol (24.0%), viridiflorol (13.7%) and 10-*epi*-γ-eudesmol (10.0-15.6%, three samples) have been described for yarrow leaf oils.

Keywords: *Achillea millefolium* L. ssp. *millefolium* f. *millefolium* and f. *rosea*; essential oil; *cis*-chrysanthenol; viridiflorol; selin-11-en-4α-ol; 10-*epi*-γ-eudesmol, statistical data analysis.

1. Plant Source

Achillea millefolium L. (yarrow) represents a polyploidic complex of hardly distinguishable species, subspecies, forms and hybrids. The plant is a well known remedy from ancient times due to it's anti-inflammatory, antirheumatic, antiseptic, antispasmodic, astringent, carminative, diaphoretic, digestive, expectorant, haemostatic, tonic, stomachic etc. properties. Up-to-now it is widely used in folk and modern medicine, veterinary, food and cosmetic industry [1-3]. Numerous medicinal properties of yarrow herb, extracts or volatile oils may be due to the various chemical profiles. The *European Pharmacopoeia* prescribes Millefolii herba with essential oil content not less than 2 mL/kg and amount of proazulenes at least 0.02% in it [3].

The present research evaluated four atypical chemotypes of essential oils within *A. millefolium* species native to Lithuania.

2. Previous Studies

High biodiversity within yarrow species determinates a wide range of chemical variability of secondary metabolites. Essential oils of *A. millefolium* have been the subject of

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numerous studies over the years. The investigations have shown high level of chemical polymorphism of yarrow volatile oils [4-20].

3. Present Study

The aerial parts (~35cm) of *Achillea millefolium* L. ssp. *millefolium* plants were collected from four sites of natural populations in Lithuania at full flowering stage. Voucher specimens were deposited in the Herbarium of the Institute of Botany (BILAS), Vilnius, Lithuania. Growing localities have chosen such where both forms of yarrow grow together. Code numbers of localities of the plants with white (W, f. *millefolium*) and pink (P, f. *rosea*) flowers were: Rokiskis district - 59438 (AW), 65286 (AP); Vilnius distr. - 59441 (BW), 65289 (BP); Vilnius city - 59442 (CW), 65283 (CP) and Kedainiai distr. - 65275 (DW), 65276 (DP). The essential oils were prepared by hydro-distillation (3h) of air-dried material in a Clevenger-type apparatus. The oil yields were 0.7-1.2% and 0.1-0.4% (v/w) for inflorescences and leaves, respectively. Oils were dried over anhydrous sodium sulphate, stored in a refrigerator and before analysis were diluted in pentane and diethyl ether (1:1). Essential oil concentration was about 0.002-0.004% in the samples for analyses.

GC/MS: Analyses by GC/MS were performed using a chromatograph HP 5890 interfaced to an HP 5971 mass spectrometer (ionization voltage 70 eV, scan time 0.6 s, *m/z* scan range 35-400 Da) and equipped with a capillary column CP-Sil 8 CB (50 m × 0.32 mm i.d., film thickness 0.25 μm). The oven temperature was held at 60°C for 2 min, then programmed from 60 to 160°C at a rate 5°C/min, held for 1 min, then increased from 160°C to 250°C at 10°C/min rate and ending with 2 min at 250°C. He was used as a carrier gas (1.0 mL/min, split ratio 1:30.). The temperatures of injector port and detector were 250°C. Injection volume was 2 μL. Qualitative analysis was based on comparison of retention times and indexes on both columns, co-injection of some terpenes references and C_8-C_{28} n-alkane series; and mass spectra with corresponding data in the literature [21] and computer mass spectra libraries (Wiley and NBS 54K).

GC/FID: An HP 5890 II gas chromatograph, equipped with a capillary column HP-FFAP (30 m × 0.25 mm; 0.25μm) was used for quantitative analysis. The GC oven temperature was set at 60°C for 2 min, then programmed to 160°C at a rate of 5°C/min, held for 1 min, then from 160°C to 230°C at 10°C/min and finally isothermal at 230°C for 12 min, using He as a carrier gas (0.7 mL/min). The injector port and detector temperatures were 230°C and 250°C, respectively. Injection volume was 2 μL.

Chemical variability: Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) were performed using XLSTAT (Addinsoft, 2009) on all the oils data of individual compounds expressed as a percentage (over 5%). AHC was done using various methods and aggregation criterions, and for definition of oil chemotypes was chosen Euclidean distance, unweighted pair-group average as a criterion.

Existence of intraspecific chemical polymorphism of *A. millefolium* was visible demonstrated in this study. Data of main chemical composition of inflorescence (F, even numbers) and leaf (L, odd numbers) essential oils of *A. millefolium* two morphotypes (f. *millefolium* and f. *rosea)* were presented in Table 1 (total sixteen samples) and submitted to statistical analysis. On a basis of major constituents (≥5%) the oils were attributed to main seven subclusters by AHC (Figure 1).

Oil sample 13, representing the first segment in AHC, contained as major component *cis*-chrysanthenol (27.3%) (Figure 1). Other representative compounds were found to be caryophyllene oxide (14.5%) and 1,8-cineole (9.3%) in the leaf oil (DWL, Table 1). Only

insignificant amounts of *cis*-chrysanthenol were determined in some other previous studies [9, 11, 12, 14, 20].

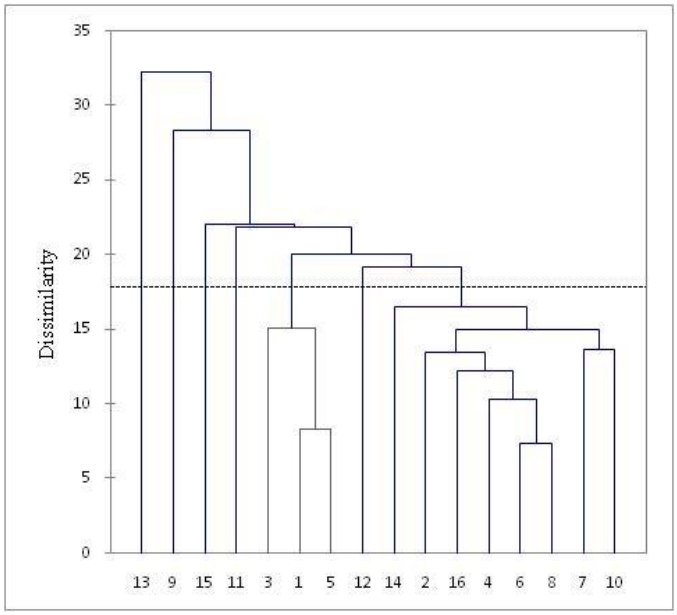

Figure 1. Dendrogram of sixteen leaf and inflorescence oils of *A. millefolium* (ssp. *millefolium*, f. *millefolium* and f. *rosea*) obtained by Agglomerative Hierarchical Clustering (AHC), using Euclidean distance, aggregation criterion - unweighted pair-group average. The oils were clustered into seven groups: I (sample 13), II (sample 9), III (sample 15), IV (sample 11), V (samples 1, 3 and 5), VI (sample 12) and VII (samples 2, 4, 6, 7, 8, 10, 14 and 16).

Sample indicated by number 9, was attributed to the second segment (Figure 1), is the leaf oil (CWL) with first principal constituent selin-11-en-4α-ol (24.0%), while content of the compound ranged from 0 to 8.5 % in other samples (Table 1). The oil contained also appreciable quantities of caryophyllene oxide (10.0%) and *trans*-nerolidol (9.6%). Selin-11-en-4-α-ol was determined in minor percentages or in appreciable amounts in some previous investigations [7, 9, 11, 14, 20], but never as the first predominant compound.

A third unit of cluster (sample 15, Figure 1) is a leaf oil of pink flowering yarrow (DPL) containing such main constituents: viridiflorol (13.7%), caryophyllene oxide (11.7%) and selin-11-en-4α-ol (8.5%) (Table 1). Viridiflorol was mainly found only in insignificant amounts (0-2.8%) in the rest oils (Table 1). However, this sesquiterpene alcohol has not even been detected or it amounts were found only in minor percentages in some earlier investigated yarrow oils [9, 15, 18]. Appreciable amounts of viridiflorol (≤6.5%) have been detected in yarrow during previous investigations performed in our laboratory [11, 14] and in the oils of cultivated yarrow in the Botanical garden of Nikita, in Crimea [8]. The oils from cultivated plants were characterized by large amounts of fragranol (≤51.6%), while viridiflorol (≤7.2%) was the second principal component in the above study.

Three oils (samples 1, 3 and 5, Figure 1) were attributed to the same subcluster. The leaf oils were characterized by appreciable amounts of 10-*epi*-γ-eudesmol. Other representative components were caryophyllene oxide, borneol, β-pinene, 1,8-cineole and selin-11-en-4α-ol in the above oils (Table 1). 10-*epi*-γ-Eudesmol was found among main constituents in our recently published research [11], but in contrast, the earlier analyzed oils were characterized by

predominance of *trans*-nerolidol. In fact, other isomers such as α-, β- or γ-eudesmol were found in less or more significant amounts in some previous studies [5, 7, 10, 11, 14, 15, 19].

Leaf and inflorescence oils of the same plant (CPL and CPF, Table 1) were attributed to the separate segments (sample 11 and 12, Figure 1). Sample 11 contained appreciable amount of caryophyllene oxide (19.0%), while β-pinene (23.0%) was the major constituent in the sample 12.

The rest eight oils (seven of flowers and one leaf oil: 14, 2, 16, 4, 6, 8, 7 and 10) were grouped together in one unit, where the flower oil 14 (DWF, Table 1) containing main components *cis*-chrysanthenol (10.9%), 1,8-cineole (10.2%) and β-pinene (9.2%) mostly different from this group.

Table 1. Main composition (over 5%) of inflorescence (F) and leaf (L) essential oils of white (W, f. *millefolium*) and pink (P, f. *rosea*) flowering *Achillea millefolium* L. (growing wild in Lithuania)

R.I.	Compounds	AWL_1	AWF_2	APL_3	APF_4	BWL_5	BWF_6	BPL_7	BPF_8	CWL_9	CWF_{10}	CPL_{11}	CPF_{12}	DWL_{13}	APF_{14}	DPL_{15}	DPF_{16}
939	α-Pinene	2.4	4.4	0.6	4.7	1.8	4.8	1.0	6.3	1.1	1.0	1.5	2.4	3.5	3.5	2.1	9.9
976	Sabinene	3.9	6.8	1.0	6.8	3.0	7.0	2.0	6.9	0.4	5.0	4.0	7.1	1.0	1.5	1.7	3.9
980	β-Pinene	8.0	10.0	2.3	9.0	6.7	12.4	4.5	17.9	1.0	9.2	7.2	23.0	4.5	9.2	8.0	15.0
1033	1,8-Cineole	5.1	7.3	0.9	5.5	8.6	10.0	3.7	10.3	0.1	5.3	0.9	t	9.3	10.2	7.9	10.5
1143	Camphor	2.2	2.2	t	4.1	4.4	4.4	1.9	4.1	5.8	4.2	5.4	2.7	t	t	1.4	t
1162	*cis*-Chrysanthenol	t	0.1	1.4	0.7	t	0.1		t	t	t	t	t	**27.3**	**10.9**	2.0	t
1165	Borneol	3.1	2.6	11.1	4.6	5.7	3.2	4.0	3.1	3.6	3.4	6.2	3.0	0.2	2.1	3.1	t
1177	Terpinen-4-ol	2.3	3.1	2.2	2.6	1.5	2.8	0.8	2.3	4.3	5.3	2.5	6.1	0.1	t	t	t
1285	Bornyl acetate	1.2	0.8		0.4	1.6	2.5	0.6	0.6	5.8	3.5	1.9	0.7	3.8	1.3	t	t
1418	β-Caryophyllene	2.7	3.3	3.2	3.1	1.7	3.6	2.1	3.1	2.3	1.8	2.1	5.2	0.5	4.4	2.5	6.5
1480	Germacrene D	1.2	1.4		2.3	t	2.7	1.8	2.0	t	6.8	t	1.0	t	4.1		1.0
1514	Cubebol			0.2	t	t	t			t	t					6.6	
1534	*cis*-Nerolidol	3.3	6.1	3.3	t		t		t	t		t	t		t		
1564	*trans*-Nerolidol	0.9	0.4	1.0	6.0	1.6	9.0	3.5	6.8	9.6	5.8	11.1	2.6	4.6	8.0	0.8	3.4
1576	Spathulenol	5.8	t	7.4	1.0	5.3	1.0	3.4	t	t	0.4	2.8	t	3.0	t	2.1	0.4
1581	Caryophyllene oxide	8.3	2.8	10.0	2.8	7.4	2.8	6.8	2.4	10.0	1.4	19.0	8.0	14.5	5.5	11.7	5.1
1590	Viridiflorol	1.5	t		0.7	1.6	t	2.8	0.3	0.4	1.2	0.5	0.5	t	0.4	**13.7**	0.4
1624	10-*epi*-γ-Eudesmol	**15.6**	5.1	10.0	3.1	**11.7**	4.8	1.1	2.2	0.4	3.1	t	0.8	2.7	4.2		0.8
1647	Himachalol				1.1	t	0.4	1.1	0.9	5.7	7.4	3.2	1.5	2.4	4.9	t	1.3
1660	Selin-11-en-4α-ol	5.3	5.0		0.2	7.5	6.0	5.7	4.3	**24.0**	5.2	4.5	6.5	3.8	2.7	8.5	5.9
1718	(2Z,6Z)-Farnesol	1.0	1.0	3.8	0.5	0.6	1.5	2.5	1.6	5.0	1.0	2.9	2.5	0.3	1.3		0.5
1735	Bisabolone isomer	3.2	5.7			0.6	t			t	t			t	t		

RI-retention indices on nonpolar column CP-Sil8 CB

t - traces (< 0.05%)

The first letters A-D indicated growing localities (See Supplementary Information).

Oxygenated sesquiterpenes were found to be the main fraction. All the investigated oils have been found free of chamazulene. Totally, 67 constituents were identified (comprising 76.8-94.1%), while the main composition (over 5%) of yarrow essential oils was presented (Table 1).

Results of this research differentiated from several previous investigations done in our laboratory by notably expressed atypical chemotypes of yarrow essential oils. *cis*-Chrysanthenol, viridiflorol, 10-*epi*-γ-eudesmol and selin-11-en-4α-ol have never before been reported as the most abundant constituents in yarrow (both wild and cultivated) essential oils. The new described chemical profiles were mostly characteristic for leaf volatile oils of *A. millefolium* f. *millefolium* gathered in "mixed" populations (where both forms are growing together), while these chemotypes have not been found earlier in localities of predominant white flowering plants [14]. Evaluation of new chemical profiles of volatile oils has expanded our chemotaxonomic knowledge of *A. millefolium*.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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