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Volatile composition of *Helichrysum arenarium* field accessions with differently coloured inflorescences

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The composition of essential oils obtained from six colour inflorescences of *Helichrysum arenarium* field accessions was analysed by GC and GC–MS. A total of 68 compounds were identified. Sesquiterpene hydrocarbons were shown to be the main group of constituents in all samplesand to account for 20.6–41.2% of total essential oil. The rather small monoterpene fraction (6.7–14.8%) consisted mainly of oxygenated monoterpenes (5.5–13.6%). On the basis of the major constituents, four chemical profiles of essential oils were distinguished. *Trans*-caryophyllene, δ-cadinene, and heneicosane were prevalent in essential oils from citric-yellow, orange and brown-orange inflorescences. The composition of essential oils from citric inflorescences differed by the third major constituent – 1,8-cineole. Oils from yellow inflorescences contained tetradecanoic acid, whereas nonadecane was the major constituent of oils from yellow-brown inflorescences. Chemical analysis of essential oils revealed a chemical variability that was more or less reflected in the morphological variability exposed by the colour of inflorescences.

Key words: *Helichrysum arenarium*, essential oil composition, GC-MS, sesquiterpene hydrocarbons

INTRODUCTION

The genus *Helichrysum* Miller comprises 16 species native to Europe [1]. Only one species, *H. arenarium* (L.) Moench, is spontaneous in Lithuania, growing on dry, poor sandy soils. It is a clone perennial, semi-rosette herb with yellow to reddish-orange or even brown inflorescences of various colour intensity [2].

The inflorescences of *H. arenarium* (*Helichrysi flos*) are widely used in folk medicine treating gall-bladder and gastric disorders, cystitis, and arthritis. The therapeutical effect of raw material is attributed mainly to the presence of flavonoids and polyphenols [3–5]. Significant antimicrobial properties have also been attributed to oils of *Helichrysum* species [6–8].

Previously, the chemical composition of essential oils had been investigated on wild populations of *H. arenarium* from Eastern Lithuania [9]. Of late, a report appeared from Hungary on the composition of the essential oil of *H. arenarium* of Caucasus origin [10]. The main constituents of essential oil were used as chemotaxonomic markers of the species *Helichrysum* [11]. Most of the reports concerning the volatile chemistry of the genus *Helichrysum* described the composition of essential oils of Mediterranean taxa. The different chemical composition of *H. italiacum* (Roth) G. Don ssp. *italicum* and *H. italiacum* ssp. *microphyllum* (Willd.) Nyman essential oils were reported from various countries. The oxygenated monoterpenes (neryl acetate, geraniol and geranyl acetate) were present mainly in essential oils of *H. italiacum* ssp. *italicum* from Greece [6] and France [12]. The main compounds of *H. italiacum* ssp. *microphyllum* growing in Greece were the sesquiterpene hydrocarbons, β-selinene and γ-curcumene [13], whereas essential oils of this subspecies growing in Italy were rich in oxygenated monoterpenes [14, 15]. The essential oil of *H. italicum* ssp. *serotinum* (Boiss.) Fourn. was found to be dominated by oxygenated compounds (geraniol, nerol) [11]. Monoterpenoids were found as the most abundant constituents in essential oils of *H. picardii* Boiss. et. Reuter (carene), *H. ambiguum* (Pers.) C. Presl. (α-pinene), *H. amorginum* Boiss. et Orph in Boiss. (geraniol, geranyl acetate and neryl acetate), *H. stoechas* L. (α-pinene), and *H. taenari* Rothm. (geraniol and camphene) [6–8]. Caryophyllene derivatives were prevalent in essential oils of *H. orientale* (L.) Gaertner, *H. heldreichii* Boiss., *H. litoreum* Guss., and *H. stoechas* (L.) Moench ssp. *barrelieri* (Ten.) Nyman [7, 13, 16]. The main metabolites of *H. doerfleri* Rech. were oxygenated sesquiterpenes (α-, β-, γ- and epi-γ-eudesmol) [14]. The corresponding reported data have revealed monoterpenes and sesquiterpenes as the principal constituents in essential oils of *Helichrysum* species.

Results of qualitative and quantitative analyses of essential oils isolated from different colour inflorescences of *H. arenarium* grown in a field collection are presented in the work, and a relationship between the colour and essential oil composition is discussed.

MATERIALS AND METHODS

Plant material. The plant material was inflorescences of eleven *H. arenarium* accessions presently grown in the field collection.

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Accessions were originated from wild populations and propagated by clone sprouts. Inflorescences were sampled at the beginning of flowering and sorted into six colour groups: citric, citric-yellow, yellow, yellow-brown, orange, and orange-brown. The colour of inflorescences was identified according to the standard chart of the Royal Horticultural Society (1995). Voucher specimens of accessions were deposited in the Herbarium of the Institute of Botany (BILAS, Vilnius, Lithuania).

Essential oil analysis. Essential oils were isolated from ten grams of air-dried inflorescences of each accession by hydrodistillation in a Clevenger-type apparatus for 3 h using a mixture of hexane and diethyl ether (1 : 1) as an organic solvent. Analysis of essential oils was carried out by GC and GC / MS. A HP 5890(II) chromatograph equipped with a flame ionization detector (FID) using a capillary column HP-FFAP (30 m × 0.25 mm, film thickness 0.3 μm) was applied for quantitative analysis. Oil solution (1–2 μl) was injected in a split mode. The oven temperature programme was set at 70 °C and kept isothermal for 10 min, then increased from 70 to 210 °C at the rate of 3 °C min^{-1} and then increased up to 250 °C at the rate of 5 °C min^{-1}. The injector and detector temperatures were 200 and 250 °C, respectively. The carrier gas was He with a flow rate of 0.7 ml min^{-1}. Analysis by GC-MS was performed using a HP 5890(II) 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 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 with the increase rate of 5 °C min^{-1}, held for 1 min, then increased up to 250 °C at the rate of 10 °C min^{-1} and kept isothermal for 5 min at 250 °C, using He as a carrier gas (1.0 ml/min^{-1}). The injector and detector temperatures were 250 °C.

The identity of the components was assigned by a comparison of their retention indices (RI) and mass spectra with the corresponding data in the literature and the computer mass spectra libraries (Wiley and NBS 54K). RI meanings match the Kovats indices presented by Adams [17] on a J & W DB-5 column whose polarity is equivalent to that of the column CP-Sil8CB applied in our analysis.

Essential oil of each sample was analysed in triplicate, and then the mean percentage composition in dry weight was presented.

RESULTS AND DISCUSSION

With regard to the morphological differences exposed by the colour variability of inflorescences, a chemical study of essential oils was performed. Sixty-eight compounds were identified in oil samples on the basis of their mass spectra and retention indices. The components identified accounted for up to 67.1–84.7% of total oil. Their mean percentages are given in Table 1 where the components are listed in the order of their RI.

Sesquiterpene hydrocarbons were shown to be the main group of constituents in all essential oils which accounted for 20.6–41.2% of total oil, whereas monoterpene hydrocarbons were present in very low amounts (0.5–3.0%). *Trans*-caryophyllene and δ-cadinene were important constituents in all essential oils (4.4–8.8% and 3.7–8.2%, respectively). Aliphatic hydrocarbons were prevalent in essential oil E (20.5%), nonadecane being the major one (12.2%). The total oxygenated compounds, which account for 18.7–25.2% of total essential oil, were represented by oxygenated monoterpenes (5.5–13.6%), oxygenated sesquiterpenes (10.3–12.2%) and oxygenated aliphatics (5.3–11.4%). The most important differences in the amount of compounds were attributed to 1.8-cineole (1.6–7.0%), caryophyllene oxide (1.0–5.7%), and tetradecanoic acid (0–7.8%). The sesquiterpene fraction, ranging from 30.9 to 52.7% of total essential oil, was dominant in all the oils analysed, while monoterpenes occurred in considerably lower amounts (5.6–14.8%). Sesquiterpenoids were determinated as the dominant compounds in volatile oils of *H. arenarium* wild populations, too [9].

Although all the study oils contained a similar array of constituents, their relative contribution influenced differences in chemical profiles. Based on the major constituents, four chemical profiles of oils were recognized.

Despite the differences in colour of inflorescences essential oils from citric-yellow, orange and brown-orange inflorescences (A, D and F, respectively) showed the same chemical profiles according to the major constituents: *trans*-caryophyllene > δ-cadinene > heneicosane. The chemical profile from citric inflorescence oils (B): *trans*-caryophyllene > δ-cadinene >1,8-cineole, differed from the above-mentioned ones in a third major constituent – 1,8-cineole. Essential oils from yellow inflorescences (C) contained tetradecanoic acid as major compound and exposed following profile: tetradecanoic acid > *trans*-caryophyllene > caryophyllene oxide. Tetradecanoic acid was absent in oils A and B and seems to have appeared in oils from darker colour inflorescences. Yellow-brown inflorescence essential oils (E) were characterized by a greater importance of nonadecane as major constituent. The chemical profile of E oils was recognized as follows: nonadecane > tetradecanoic acid > *trans*-caryophyllene. *Trans*-caryophyllene, δ-cadinene and heneicosane were found to be the principal constituents in inflorescence oils of wild *H. arenarium* [9]. *Trans*-caryophyllene was common to all chemical profiles and seems to be the most consistent component in essential oils within the other *Helichrysum* species [11].

Essential oils revealed the chemical variability that was more or less reflected in the morphological variability exposed by the colour of inflorescences. On the other hand, essential oil from orange inflorescences whose colour is characteristic of the morphological form *H. arenarium* f. *aurantiacum* (Pres.) Bleck. did not relate to a particular composition. The colour of inflorescences seems to be more related to the other active compounds of *H. arenarium*, such as flavonoids.

As published analyses of essential oils on *H. arenarium* are scarce, a comparison of our results with published data is difficult. In view of previous reports, the main difference between the percentages of volatile constituents of *H. arenarium* from Lithuania and other *Helichrysum* species from the Mediterranean region, seems to be a lower content of monoterpenes and a higher level of sesquiterpene hydrocarbons. *H. arenarium* from Lithuania could be characterized as low aromatic taxa of the genus *Helichrysum*. Remarkable differences were seen in the composition oils obtained for our plant material and those recorded for *H. arenarium* from the Caucasus region [10]. The composition of essential oil referred in corresponding literature contained aliphatic acids and their esters in higher quantities, whereas the concentration of caryophyllene was much lower than in oils of Lithuanian accessions.

Table. **Mean percentage composition of essential oils from differently coloured inflorescences of Helichrysum arenarium field accessions (n = 11)**

Compounds	RI	A (2 oils)	B (2 oils)	C (2 oils)	D (3 oils)	E (1 oil)	F (1 oil)	Mean	SE
α-Pinene	939	1.1	tr	0.4	0.2	0.6	0.3	0.4	0.15
β-Pinene	976	1.8	1.2	0.3	0.3	0.5	0.3	0.7	0.26
1.8-Cineole	1033	3.3	7.0	1.6	3.6	2.3	4.4	3.7	0.77
Terpinolene	1089	0.1	tr	0.1	tr	0.1	0.2	0.1	0.02
Undecane N	1100	0.1	tr	0.2	0.1	tr	0.1	0.1	0.02
Nonanal N	1101	1.0	1.9	0.7	1.2	0.7	1.0	1.1	0.18
endo-Fenchol	1117	0.1	tr	tr	0.1	0.3	tr	0.1	0.04
Nonen-1-al	1162	0.4	1.3	0.3	0.1	tr	0.1	0.4	0.19
β-*trans*-Terpineol ?	1163	1.7		0.6	0.7	1.1	1.5	0.9	0.26
Borneol	1169	tr	1.5	–	–	–	–	0.3	0.25
α-Terpineol	1189	0.8	2.2	0.3	0.5	0.3	0.8	0.8	0.29
Safranal	1197	0.2	0.1	tr	tr	0.9	0.2	0.3	0.13
Dodecane N	1200	0.1	tr	0.1	0.1	tr	0.1	0.1	0.01
Decanal N	1202	1.6	2.2	1.4	1.8	0.3	1.4	1.5	0.26
Isobornyl acetate	1286	0.1	tr	–	tr	tr	tr	0.1	0.01
Lavandulyl acetate	1290	0.7	0.4	0.8	0.6	–	tr	0.4	0.14
trans-Sabinyl acetate	1291	0.2	tr	–	tr	–	tr	0.1	0.03
Decadienal	1317	0.2	–	0.3	tr	tr	tr	0.1	0.05
Eugenol	1359	0.3	–	0.2	tr	0.1	–	0.1	0.05
α-Ylangene	1375	0.5	0.6	0.3	0.4	0.3	0.4	0.4	0.05
α-Copaene	1377	3.0	3.6	2.6	2.2	2.3	2.4	2.7	0.22
Tetradecane N	1400	0.4	0.9	0.5	0.6	0.4	0.5	0.6	0.08
Methyl eugenol	1404	0.1	tr	0.2	0.5	tr	0.1	0.2	0.07
Dodecanal	1409	0.2	tr	0.2	tr	0.4	0.5	0.2	0.07
α-Gurjunene	1410	tr	–	tr	–	tr	tr	0.0	0.01
trans-Caryophyllene	1419	5.3	8.8	6.4	6.5	4.4	7.8	6.5	0.65
β-Cedrene	1421	tr	0.1	0.1	0.3	0.2	tr	0.1	0.04
Aromadendrene	1441	0.2	0.4	0.2	tr	0.2	tr	0.2	0.05
α-Humulene	1455	1.7	2.9	2.1	2.3	1.5	2.3	2.1	0.20
β-Farnesene	1457	2.0	2.9	2.1	2.5	2.3	2.9	2.5	0.16
allo-Aromadendrene	1460	0.3	tr	0.3	0.1	tr	–	0.1	0.05
trans-Cadina-1(6).4-diene	1477	0.4	0.8	0.4	0.4	tr	0.3	0.4	0.10
γ-Muurolene	1480	1.6	2.7	1.7	1.9	1.4	1.8	1.9	0.18
α-Amorphene	1485	0.4	0.8	0.4	0.4	0.5	0.4	0.5	0.07
β-Ionone	1489	0.9	1.9	0.7	1.0	0.5	0.9	1.0	0.20
trans-Muurola-4(14). 5-diene	1494	0.8	1.4	0.3	0.6	–	0.9	0.7	0.20
γ-Amorphene	1496	0.5	1.0	0.4	0.4	0.3	0.6	0.5	0.10
α-Muurolene	1500	1.0	1.5	1.4	1.5	1.1	1.1	1.3	0.09
Lavandulyl isovalerate	1510	0.3	0.5	0.3	–	tr	0.4	0.3	0.08
γ-Cadinene	1514	1.9	3.2	1.9	2.4	1.5	2.2	2.2	0.24
σ-Cadinene	1523	5.1	8.2	4.6	5.8	3.7	5.7	5.5	0.62
α-Cadinene	1539	0.7	0.9	0.6	0.7	0.5	0.6	0.7	0.06
α-Calacorene	1546	0.8	0.9	0.5	0.7	0.4	0.6	0.7	0.08
trans-Nerolidol	1563	0.5	0.5	0.4	0.3	0.3	0.3	0.4	0.04

Table. **Continued**

Compounds	RI	A (2 oils)	B (2 oils)	C (2 oils)	D (3 oils)	E (1 oil)	F (1 oil)	Mean	SE
Caryophyllenyl alcohol	1572	0.5	0.5	0.4	0.3	0.3	0.6	0.4	0.05
Caryophyllene oxide	1582	1.0	1.4	5.7	2.5	3.7	3.4	3.0	0.70
Gleenol	1587	1.1	0.8		0.5	0.3	tr	0.5	0.18
Hexadecane N	1600	0.7	0.9	0.6	0.7	0.5	0.6	0.7	0.06
1.10-di-*epi*-Cubenol	1619	0.8	0.9	0.7	0.5	0.7	0.7	0.7	0.05
1-*epi*-Cubenol	1629	1.4	1.8	0.9	1.3	0.8	1.2	1.2	0.15
epi-a-Cadinol	1640	2.8	4.0	2.1	2.7	2.0	2.6	2.7	0.29
α-Muurolol	1646	0.8	0.8	0.5	0.5	0.6	0.5	0.6	0.06
α-Cadinol	1654	2.3	0.8	1.5	1.9	1.6	1.8	1.7	0.20
Tetradecanol-N	1673	1.2		0.4	0.5	0.7	0.4	0.5	0.16
Chamazulene	1725		0.5	0.2	–	–	–	0.1	0.08
Methyl tetradecanoate	1728	0.3	tr	0.3	tr	0.2	1.6	0.4	0.24
Tetradecanoic acid	1750	–	–	7.8	4.6	7.0	1.7	3.5	1.41
Octadecane N	1800	0.5	0.4	0.3	0.3	0.5	0.3	0.4	0.04
Hexadecanol	1876	0.4	0.6	tr	0.5	0.4	0.8	0.5	0.10
Nonadecane N	1900	0.5	3.4	0.3	0.7	12.2	3.8	3.5	1.85
m/z-149 (phthalide)	1905	4.3	0.6	5.6	3.9	2.4	3.7	3.4	0.70
Eicosane	2000	0.4		1.3	1.8	1.6	1.6	1.1	0.30
Heneicosane	2100	5.1	1.5	4.4	5.1	4.9	5.0	4.3	0.58
Tricosane N	2300	0.6	1.5	0.4	0.4	0.4	0.3	0.6	0.18
Total identified (%)		67.1	82.7	69.3	69.5	70.2	73.7	72.1	2.30
Grouped components									
Monoterpene hydrocarbons		3.0	1.2	0.8	0.5	1.2	0.8	1.3	0.37
Oxygenated monoterpenes		8.7	13.6	4.7	7.0	5.5	8.3	8.0	1.29
Sesquiterpene hydrocarbons*	26.2	41.2	26.5	29.1	20.6	30.0		28.9	
Oxygenated sesquiterpenes		11.2	11.5	12.2	10.5	10.3	11.1	11.1	0.28
Aliphatic hydrocarbons		8.4	8.6	8.1	9.8	20.5	12.3	11.3	1.95
Oxygenated aliphatics		5.3	6.0	11.4	8.7	9.7	7.5	8.1	0.94

tr – traces (≤0.05%), * including chamazulene, SE – standard error,

RI – retention indices whose values match the Kovats indices presented by Adams [17] on J & W DB-5 column.

Essential oils from inflorescences of different colour: A – citric-yellow, B – citric, C – yellow, D – orange, E – yellow-brown, F – brown.

The differences in chemical composition probably depend on the place of growth and climate. On the other hand, the array of the same principal components was detected while comparing the volatile composition of wild populations and field accessions. Investigations indicated the infraspecific variation and chemical polymorphism that might arise on the genetic background, since the influence of environmental factors appears to be not substantial.

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LAKIŲJŲ JUNGINIŲ SUDĖTIS *HELICHRYSUM ARENARIUM* KOLEKCINIUOSE SKIRTINGOS SPALVOS ŽIEDYNŲ PAVYZDŽIUOSE

Santrauka

Tirta smėlyninio šlamučio, augančio lauko kolekcijoje, skirtingos spalvos žiedynų eterinių aliejų kiekybinė bei kokybinė sudėtis. Eteriniai aliejai išskirti distiliuojant vandens garais, o jų analizė atlikta dujų chromatografijos ir masių spektrofotometrijos metodais. Buvo identifikuoti 68 lakūs junginiai. Žiedynų eteriniuose aliejuose vyravo seskviterpenai hidrokarbonai, kurie sudarė 20,6–41,2% viso jų tūrio. Monoterpenų frakciją daugiausia sudarė oksiduoti monoterpenai (5,5–13,6%). Pagal dominuojančius pagrindinius junginius eteriniai aliejai buvo suskirstyti į keturis aliejų tipus. Citriniškai geltonos, oranžinės, rudai oranžinės spalvos žiedynuose vyravo *trans*-kariofilenas, δ-kadinenas ir heneikosanas. Citrininės spalvos žiedynų eterinis aliejus skyrėsi didesniu 1,8-cineolo kiekiu, geltonos spalvos žiedynuose buvo gausu tetradekanoinės rūgšties, o geltonai ruduose – nonadekano. Tyrimu nustatytą smėlyninio šlamučio žiedynų eterinio aliejaus sudėties įvairavimą sunku tiesiogiai susieti su skirtinga žiedynų spalva, jis, matyt, nulemtas genotipo.