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Comparison of chemical composition of the essential oil from *Marrubium vulgare* L. and *M. incanum* Desr. during the second year of cultivation

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Abstract

Horehound herb (*Marrubium vulgare* L.) is harvested from plantations in Poland. In our country, there are also favorable conditions for *M. incanum* Desr. growing. The aim of the study was to compare the chemical composition of essential oils from *M. vulgare* L. and *M. incanum* Desr. in the second year of cultivation. The study revealed the presence of 31 compounds in the essential oil from *M. vulgare* L. and 24 compounds in the essential oil from *M. incanum* Desr. Chromatographic analysis revealed that the main compounds in the essential oil of *M. vulgare* L. from a two-year plantation were as follows: E-caryophyllene (34.51–36.78%), germacrene D (22.45–27.18%), bicyclogermacrene (9.54–11.12%), δ-amorphene (6.15–8.18%), and carvacrol (4.71–6.64%), whereas the following compounds prevailed in the oil from *M. incanum* Desr.: germacrene D (28.75–32.14%), E-caryophyllene (23.18–29.57%), α-cadinol (13.59–20.84%), and carvacrol (2.08–7.47%).

Keywords: *Marrubium vulgare* L.; *Marrubium incanum* Desr.; E-caryophyllene; germacrene D; δ-amorphene

Introduction

The best-known species of the genus *Marrubium* sp. are *M. vulgare* L. and *M. incanum* Desr. [1,2]. The herb of the horehound (Marrubi herba) [3] is harvested from plantations in Poland [4]. In our country, there are also favorable conditions for growing *M. incanum* Desr. [5].

Marrubium vulgare L. herb contains about 0.1% of essential oil [4]. The oil has relaxant, expectorant [2], and antioxidant [6] properties. Antimicrobial properties were found in the essential oil both from *M. vulgare* L. [7] and *M. incanum* Desr. [8]. *M. incanum* Desr. is recommended for soothing stomach and heart diseases [9]. *Marrubium vulgare* L. and *M. incanum* Desr. raw material is recommended to drink as a panacea, especially as an agent accelerating the digestion processes [10]. *Marrubium vulgare* L. herb is sometimes replaced with *M. incanum* Desr. herb [11]; therefore, the aim of the study was to compare the chemical composition of essential oils from *M. vulgare* L. and *M. incanum* Desr. in the second year of cultivation.

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Material and methods

Plant material

The study was carried out in 2010–2011 in the experimental section of the Department of Vegetable and Medicinal Plants, University of Life Sciences in Lublin (51°14′ N 22°34′ E). The plantation was established from seedlings produced in a greenhouse. Seeds of *M. vulgare* L. and *M. incanum* Desr. came from the Botanical Garden of the Maria Curie-Skłodowska University in Lublin. Plants were grown at 30 × 40 cm spacing. The experiment was conducted using two-year-old horehound plants. The herb of *M. vulgare* L. and *M. incanum* Desr. was harvested at the flowering stage (July). According to Wolski et al. [2], essential oil is extensively excreted just during this developmental stage. Plants were cut at a height of 8 cm, since the lower part of the stem was woody. Herbage was then dried in a drying chamber at 30°C for four days.

Oil isolation

Essential oils from *M. vulgare* L. and *M. incanum* Desr. were obtained from dry herb in a glass Clevenger-type distillation apparatus. Woody stems were discarded before the distillation process. Essential oils were distilled during the steam process according to the "Polish Pharmacopoeia" VIII [12]. The distillation time was three hours. 40 g of herb and 400 ml of distilled water were used for distillation. The distillation process was carried out in three replicates.

GC-MS

The GC-MS instrument ITMS Varian 4000 GC-MS/MS (Varian, USA) was used, equipped with a CP-8410 auto-injector and a 30 m × 0.25 mm i.d. VF-5ms column (Varian, USA), film thickness 0.25 µm; carrier gas, helium at a rate of 0.5 ml/min; injector and detector temperature, 220°C and 200°C, respectively; split ratio, 1:20; injection volume, 1 µl. A temperature gradient was applied (60°C for 0.5 min, then incremented by 3°C/min to 246°C and held at this temperature for 10 min); ionization energy, 70 eV; mass range, 40–1000 Da; scan time, 0.80 s.

GC-FID

A Varian 3800 Series (Varian, USA) instrument with a DB-5 column (J&W, USA) was used, operated under the same conditions as GC-MS; FID, 256°C; split ratio 1:50.

The qualitative analysis was carried out on the basis of MS spectra, which were compared with the spectra of the NIST library [13] and with data available in the literature [14]. The identity of the compounds was confirmed by their retention indices, taken from the literature [14].

Results

The two-year study showed that the essential oil content in the dry herb of *M. vulgare* L. was on average 0.06% and in the dry herb of *M. incanum* Desr. – 0.04% (Tab. 1).

Tab. 1 Percentage of essential oil in the herb of *Marrubium vulgare* L. and *M. incanum* Desr. during the second year of cultivation.

	Essential oil content in dry herb (%)		
Species	**2010**	**2011**	**Mean**
Marrubium vulgare L.	0.07	0.06	0.06
Marrubium incanum Desr.	0.05	0.03	0.04

The study revealed the presence of 31 compounds in the essential oil from *M. vulgare* L. and 24 compounds in the essential oil from *M. incanum* Desr. during the second year of growing. Sesquiterpene hydrocarbons dominated in the essential oil from *M. vulgare* L. In addition to the dominant sesquiterpene hydrocarbons in the essential oil from *M. incanum* Desr., also oxygenated sesquiterpenes accounted for a significant portion of oil (Tab. 2, Tab. 3).

Chromatographic analysis revealed that the main compounds in the essential oil of *M. vulgare* L. from a two-year plantation were as follows: E-caryophyllene (34.51–36.78%), germacrene D (22.45–27.18%), bicyclogermacrene (9.54–11.12%), δ-amorphene (6.15–8.18%), and carvacrol (4.71–6.64%; Tab. 2), whereas the following compounds prevailed in the oil from *M. incanum* Desr.: germacrene D (28.75–32.14%), E-caryophyllene (23.18–29.57%), α-cadinol (13.59–20.84%), and carvacrol (2.08–7.47%; Tab. 3).

The study revealed that bicyclogermacrene was also a dominant constituent in the essential oil of *M. vulgare* L., whereas in the oil of *M. incanum* Desr. the content of this compound was low. The content of δ-amorphene in the essential oil from *M. vulgare* L. was much the same, which was many times greater than that from *M. incanum* Desr. Carvacrol was present at a level of 4.71–6.64% in the essential oil from *M. vulgare* L. and 2.08–7.47% from *M. incanum* Desr. (Tab. 2, Tab. 3).

Discussion

The content of essential oil from *M. vulgare* L. herb collected in the second year of cultivation was 0.06–0.07%. A similar level of oil content was reported by Zawiślak [15] for annual herbal plants containing 0.06% of essential oil. The content of essential oils in the herb of *M. incanum* Desr. in the second year of cultivation ranged between 0.03% and 0.05%, which was the same as in the herb harvested during the first year of cultivation, as determined by Zawiślak [5].

Studies on essential oil in other plants of the genus *Marrubium* sp. have also revealed its low content. The herb of *M. peregrinum* L. contained 0.07% of essential oil, that of *M. parviflorum* Fisch & C.A. Mey – 0.08% [16], while in *M. cuneatum* Russell – up to 0.15% [17].

According to Zawiślak [5], the number of identified compounds in the essential oils from *M. vulgare* L. and *M. incanum* Desr. in the first year of cultivation remained at a similar level and it was 34 and 20 compounds, respectively. Asadipour et al. [18] indicated the presence of 43 compounds, among which 20 were identified. However, Abadi and Hassani [19] identified 50 different compounds in the essential oil from *M. vulgare* L.

Sesquiterpene hydrocarbons were dominant in the essential oil from *M. vulgare* L., which is consistent with the research findings of many authors [7,20]. Sesquiterpene hydrocarbons were also dominant in *M. thessalum* [21]. In the report of Zawiślak [5], the essential oil from annual *M. incanum* Desr. was also abundant in these compounds.

E-caryophyllene and germacrene D were also the major components of the essential oil from *M. vulgare* L. as determined by Khanavi et al. [16], Zawiślak [22] as well as El-Leith et al. [23]. Petrović et al. [8] also pointed out to E-caryophyllene (27.0%) and germacrene D (26.2%) as the main compounds in the *M. incanum* Desr. essential oil. Germacrene D was the prevailing component in essential oils from other species of the genus *Marrubium* sp.: *M. cuneatum* Russell [17], *M. parviflorum* Fisch & C.A. Mey – 0.08% [16], and *M. thessalum* [21].

The content of δ-amorphene in the essential oil from *M. vulgare* L. was 6.15–8.18% and from *M. incanum* Desr. 0.05–1.32% in the second year of cultivation. Zawiślak [5] reported more than twice higher content of δ-amorphene in the essential oil of *M. vulgare* L. than from *M. incanum* Desr. harvested during the first year of cultivation.

A study performed by Salama et al. [20] revealed that carvacrol was also the dominant component in the essential oil of *M. vulgare* L. with its content of 4.35%. This study showed a higher content of carvacrol in the oil of *M. vulgare* L.

Tab. 2 Percentage composition of essential oil of *Marrubium vulgare* L. during the second year of cultivation.

Compound	RI	2010	2011	Mean
α-Thujene	857	t	t	t
β-Pinene	862	t	t	t
Limonene	942	1.12	0.92	1.02
γ-terpinene	1060	1.65	0.75	1.20
Geijerene	1142	0.05	0.65	0.35
trans-Pinocamphone	1160	t	t	t
cis-Pinocamphone	1174	t	t	t
Thymol	1197	0.59	0.42	0.50
Carvacrol	1204	6.64	4.71	5.67
α-Copaene	1287	1.41	2.34	1.87
β-Bourbonene	1298	1.93	2.18	2.05
β-Elemene	1319	1.25	1.42	1.33
E-Caryophyllene	1330	34.51	36.78	35.64
β-Copaene	1341	3.66	2.24	2.95
β-Gurjunene	1342	t	t	-
(Z)-β-Farnesene	1367	t	t	-
α-Humulene	1376	0.55	3.18	1.86
Germacrene D	1385	27.18	22.45	24.81
Viridoflorene	1392	t	t	t
Bicyclogermacrene	1400	9.54	11.12	10.33
γ-Cadinene	1418	t	t	t
δ-Amorphene	1434	8.18	6.15	7.16
α-Cadinene	1452	t	t	t
β-Sesquiphellandrene	1512	t	t	t
α-Cadinene	1538	t	t	t
E-Nerolidol	1575	t	t	t
Spathulenol	1586	t	t	t
Caryophyllene oxide	1590	t	0.05	0.02
Humulene epoxide II	1618	t	t	t
epi-α-Muurolol	1632	t	t	t
α-Cadinol	1648	t	0.85	0.42
Total		98.26	96.21	97.18
Monoterpene hydrocarbons		2.82	2.23	2.57
Oxygenated monoterpenes		7.23	5.13	6.18
Sesquiterpene hydrocarbons		88.21	87.86	88.03
Oxygenated sesquiterpenes		-	0.9	0.45

t – trace (<0.05%).

Tab. 3 Percentage composition of essential oil of *Marrubium incanum* Desr. during the second year of cultivation.

Compound	RI	2010	2011	Mean
β-Pinene	945	t	t	t
Limonene	948	0.48	0.55	0.51
γ-terpinene	1065	t	t	t
Geijerene	1144	0.53	0.05	0.29
Thymol	1175	t	t	t
Carvacrol	1275	7.47	2.08	4.77
α-Copaene	1377	1.02	1.11	1.06
β-Bourbonene	1382	0.52	0.05	0.28
β-Elemene	1393	0.05	0.06	0.05
E-Caryophyllene	1410	29.57	23.18	26.37
β-Copaene	1423	0.55	3.02	1.78
α-Humulene	1445	11.08	10.49	10.78
γ-Muurolene	1455	0.05	1.01	0.53
Germacrene D	1484	28.75	32.14	30.44
Bicyclogermacrene	1495	0.15	1.98	1.06
Germacrene A	1500	3.47	2.02	2.74
γ-Cadinene	1512	0.05	1.02	0.53
δ-Amorphene	1524	1.32	0.05	0.68
α-Cadinene	1548	t	t	t
Spathulenol	1567	t	t	t
Caryophyllene oxide	1580	1.15	0.32	0.73
Humulene epoxide II	1620	t	t	t
epi-α-Muurolol	1644	t	t	t
α-Cadinol	1650	13.59	20.84	17.21
Total		99.80	99.97	99.81
Monoterpene hydrocarbons		1.01	0.60	0.80
Oxygenated monoterpenes		7.47	2.08	4.77
Sesquiterpene hydrocarbons		76.58	76.13	76.35
Oxygenated sesquiterpenes		14.74	21.16	17.94

t – trace (<0.05%).

Conclusions

The essential oils from *M. vulgare* L. and *M. incanum* Desr. were characterized by a high content of sesquiterpenes, which are responsible for the biological activity of horehound herb. E-caryophyllene and germacrene D are the dominant components of the essential oil of *M. vulgare* L. and they were also determined as the main compounds of the essential oil from *M. incanum* Desr. during the second year of cultivation.

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Competing interests

No competing interests have been declared.

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Porównanie składu chemicznego olejku eterycznego z *Marrubium vulgare* L. i *M. incanum* Desr. w drugim roku uprawy

Streszczenie

Ziele szanty zwyczajnej (*Marrubium vulgare* L.) pozyskiwane jest w Polsce z plantacji. Istnieją również odpowiednie warunki do uprawy *M. incanum* Desr. Celem przeprowadzonych badania było porównanie składu chemicznego olejków eterycznych z *M. vulgare* L. i *M. incanum* Desr. w drugim roku uprawy. Badania wykazały obecność 31 związków w olejku z *M. vulgare* L. i 24 związków w olejku z *M. incanum* Desr. Na podstawie analizy chromatograficznej stwierdzono, iż głównymi związkami w olejku z *M. vulgare* L. w drugim roku uprawy były: E-kariofilen (34.51–36.78%), germakren D (22.45–27.18%), bicyclogermakren (9.54–11.12%), δ-amorfen (6.15–8.18%) i karwakrol (4.71–6.64%), podczas gdy w olejku z *M. incanum* Desr. dominowały: germakren D (28.75–32.14%), E-kariofilen (23.18–29.57%), α-kadinol (13.59–20.84%) i karwakrol (2.08–7.47%).