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Composition of essential oils from horehound grown in south-eastern Poland

Skład olejku eterycznego z szanty zwyczajnej uprawianej w południowo – wschodniej Polsce

INTRODUCTION

Horehound (*Marrubium vulgare* L.) belongs to *Lamiaceae* family [10,18,21]. It is native to Mediterranean Sea shore, but can be also found as a road-side weed [13,21]. In Poland, it grows in natural habitats of wastelands, near roads, and buildings [18]; however, it is cultivated as well [10]. Horehound leaves are sometimes used in Europe as bitter spice to beer and liqueurs. Leaves of the plant are used to make a beverage in Great Britain [13].

Horehound is a perennial, the herb of which (*Marrubi herba*) is collected for medicinal purposes [20,21]. A multi-directional pharmacological properties are confirmed by many authors [1,3,6,10,11,17,20]. Compounds present in horehound herb are responsible for medicinal features: marubin (0.6-1.0%), other di-terpene compounds, ursolic acid and essential oil (about 0.1%) [10]. Marubin and marubinic acid are said to be responsible for bile-forming properties. Marubin is also considered as an expectorant, because of increasing the excretory action of bronchial mucus membranes. The essential oil contributes to diastolic, expectorating, and blood-vessel broadening actions. The horehound can serve to prepare herbal tea (4.5 g of dried herb daily) or it is a component of expectorates and digestion-improving agents [21].

The essential oils contents do not depend on the age of harvested horehound plant; instead, the harvest date influences on the essential oil content [22].

The presented study aims at evaluating the qualitative and quantitative composition of essential oil from horehound herb depending on plantation age and raw material harvest date.

MATERIAL AND METHODS

The study was performed in 2006. The plant material originated from annual and biennial plantation of horehound (*Marrubium vulgare* L.) grown at the Department of Vegetable and Medicinal Plants, University of Life Sciences in Lublin (51^{O}14'N 22^{O}34'E). The experiment was set

up on lessive soil developed from loess formations on chalk marls containing 1.6% of organic matter. The horehound plantation was established from the seedlings produced in a greenhouse. Plants were set into the field at the end of May at 30 x 40 cm spacing. The growth dynamics of annual and biennial plants was evaluated during the vegetation season. In the first year of growing, the herb was harvested only once in the second half of July (plant flowering stage), while three times (9th June, 14th July and 18th August) in the second year. Plants were cut at 10 cm level over the ground, dried under natural conditions in dry and dark place.

The essential oil was achieved from air-dried herb material by means of steam distillation in Deryng device according to Polish Pharmacopoeia VII [16]. Portion of 40 g herb and 400 ml water was used to distillation; distillation duration – 3 hours.

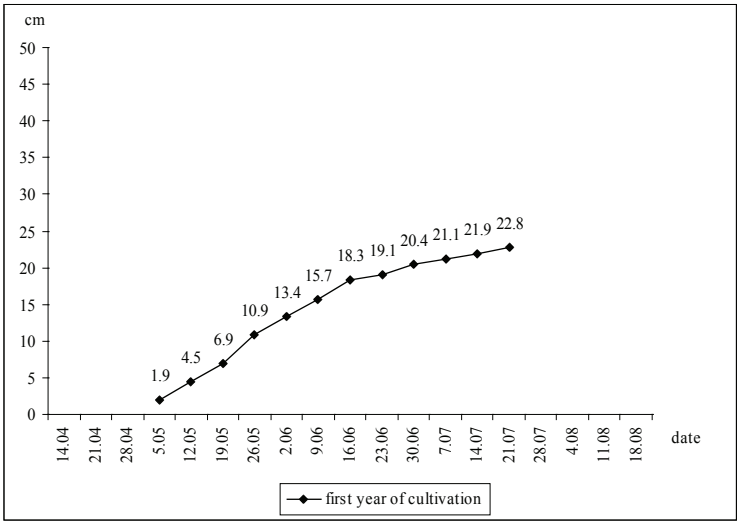
The analysis of oil chemical composition was conducted using gas chromatography method combined with mass detector, with the use of Varian 4000 GC/MS/MS chromatograph. We applied the VF-5 ms column, 30 m long, with the diameter of 0.25 mm and stationary phase thickness of 0.25 mm. The batcher temperature reached 2200C. We applied 500C temperature gradient for 1 min, and then increased to 2500C with a speed of 40C/min and 2500C for 10 min. The carrier gas was helium. Steady flow of 0.5ml/min was applied. One µl of the solution (1 µl sample in 1000 µl of hexane), batcher 2500C, split 1:100 was added. A Varian 4000 MS/MS detector with the registered range 40–1000 m/z, and the scan speed 0.8 sec./scan was used. Kovats' retention indices were determined on the basis of alkane C6-C40.

RESULTS AND DISCUSSION

Meteorological conditions during the vegetation season are presented in Table 1. Mean air temperatures since April till August were almost as those for many-year average. The rainfall sums in June and July were lower than many-year average. Drought in the mid of June had no impact on inhibition of horehound's growth neither on annual nor biennial plantation (Fig. 1). Drought at the beginning of July and low level of rainfalls in the mid of July slightly inhibited the annual and biennial plants' growth.

Table 1. Air temperature and total precipitation in 2006 year against a background of many-year averages

Month	Temperature (°C)					Precipitation (mm)				
	Decade			Mean	1951-2005	Decade			Σ	1951-2005
	I	II	III			I	II	III		
IV	6.2	7.7	12.3	8.7	7.4	19.4	10.5	0.4	30.3	40.2
V	13.5	14.6	12.8	13.6	13.0	9.0	18.4	32.1	59.5	57.7
VI	11.6	17.9	21.1	16.8	16.2	28.4	0.0	9.5	37.9	65.7
VII	21.2	20.8	23.5	21.8	17.8	0.0	6.8	0.0	6.8	83.5
VIII	18.4	18.3	15.6	17.4	17.1	73.0	79.7	45.6	66.1	68.6



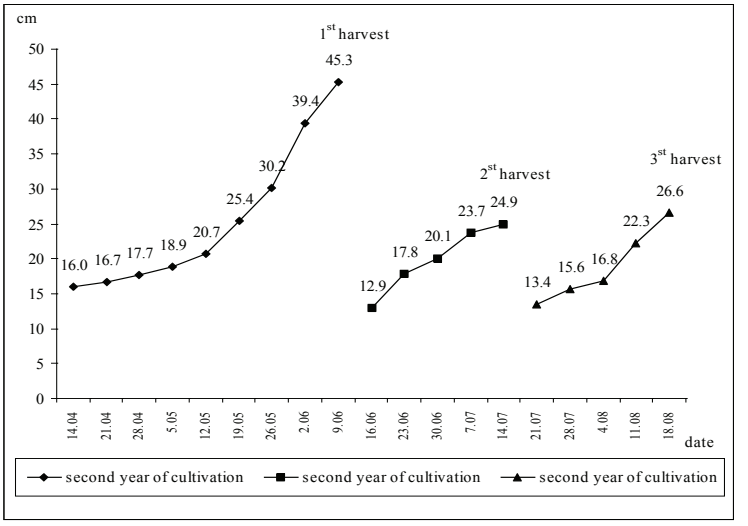


Fig. 1. Height of *Marrubium vulgare* L. in the first and second year of cultivation

Weather conditions affected the content of D-germacrene in essential oil from biennial plants (Fig. 2). The drought (mid of June, beginning and end of July) along with poor atmospheric precipitation (end of June, mid of July) contributed to doubling the D-germacrene content in essential oil from herb harvested in the mid of July (second harvest). Content of the component on oil from plants collected during the third harvest (18th August) maintained at similar level, despite that no water deficiency was recorded on the plantation in August.

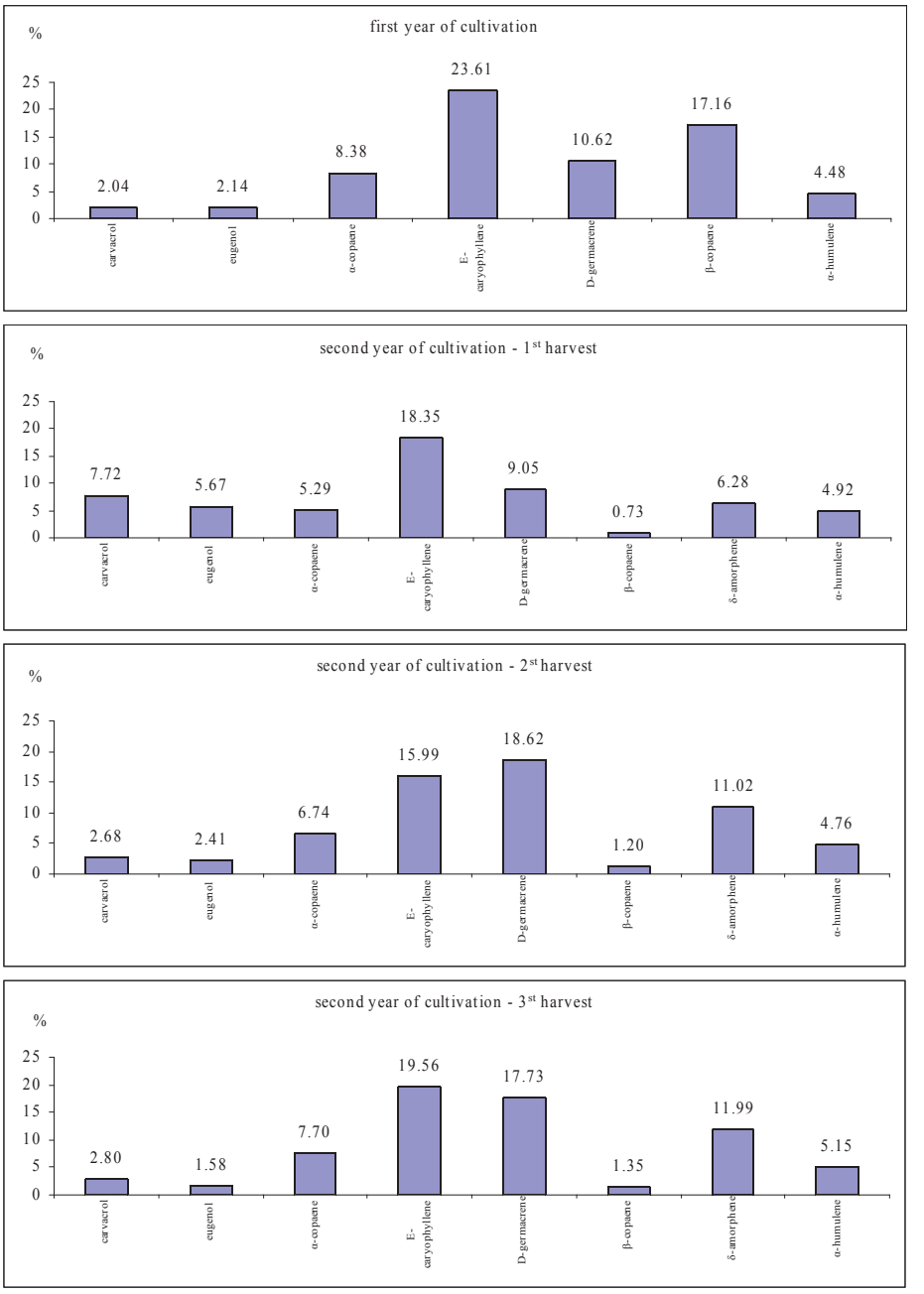


Fig. 2. The main composition of essential oil obtained from *Marrubium vulgare* L. in the first and second year of cultivation

Laboratory determination confirmed the presence of 79 compounds in annual horehound essential oil (Table 2). Fifteen of them were not identified. In essential oil from biennial horehound, number of chemicals ranged from 91 to 95, among which 15 to 18 were not identified.

Table 2. Percentage composition of essentials oil from the herb of *Marrubium vulgare* L.

No	Compound	IR	Percentage			
			First year of cultivation	Second year of cultivation		
				1st harvest	2st harvest	3st harvest
1	Octane	827	tr.	0.16	0.07	0.07
2	Hexanal	830	tr.	tr.	tr.	tr.
3	1,2-cyclopentadiene, 5-(1.1-dimethylethyl)	858	tr.	0.09	tr.	0.06
4	2(E)-Heenal	868	0.29	0.14	0.23	0.13
5	n.i.	877	tr.	tr.	tr.	tr.
6	n.i.	900	0.31	0.24	0.21	0.18
7	α-Pinene	934	-	tr.	0.08	-
8	Benzaldehyde	967	0.06	0.14	0.06	0.06
9	Sabinene	972	-	-	0.14	-
10	1-octen-3-ol	979	0.13	0.63	0.52	0.14
11	3-octanone	984	tr.	tr.	0.05	tr.
12	Furan	989	0.06	0.66	0.16	0.12
13	3-octanol	996	0.06	0.18	0.06	0.06
14	n.i.	998	0.05	0.39	0.09	0.11
15	p-Cymene	1025	tr.	tr.	tr.	tr.
16	Limonene	1029	tr.	0.06	0.06	tr.
17	1,8-Cineole	1033	0.05	0.06	1.70	tr.
18	E-Ocimene	1044	-	tr.	tr.	-
19	Terpinolene	1058	0.09	0.08	0.10	0.06
20	cis-Sabinene hydrate	1071	tr.	0.06	0.09	tr.
21	Linalool	1100	0.38	1.15	0.32	0.25
22	Nonanal	1105	0.08	0.11	0.15	0.13
23	n.i.	1111	0.09	0.07	0.07	0.10
24	α-Campholenal	1122	tr.	tr.	tr.	tr.
25	n.i.	1135	tr.	0.06	0.12	tr.
26	Geijerene	1143	0.10	0.14	0.73	0.08
27	trans-Pinocarvel	1145	-	-	0.09	-
28	2(E), 6(Z)-Nonadienal	1155	tr.	tr.	tr.	tr.
29	2(Z)-Nonen-1-al	1162	-	0.06	0.06	tr.
30	Pinocarvone	1167	-	tr.	0.11	tr.
31	Menthol	1177	tr.	-	tr.	0.05
32	Terpinen-4-ol	1184	tr.	-	tr.	tr.
33	n.i.	1191	-	0.12	tr.	tr.
34	Myrtenol	1199	-	0.09	0.25	0.10
35	Safranal	1204	0.06	0.14	tr.	tr.
36	Decanal	1207	-	tr.	tr.	tr.

37	Myrcenone	1214	tr.	tr.	0.05	tr.
38	Z-Myroxide	1218	-	tr.	0.10	tr.
39	1-Cyclohexene--1-carboxaldehyde, 2,6,6-trimethyl	1225	0.08	0.20	0.13	0.12
40	n.i.	1229	0.11	0.07	0.10	0.09
41	2E-Hexenyl isovalerate	1235	0.07	tr.	0.07	tr.
42	n.i.	1243	-	-	-	0.06
43	Geraniol	1253	tr.	0.15	0.09	tr.
44	n.i.	1273	0.06	0.10	0.06	0.06
45	n.i.	1276	tr.	0.26	tr.	0.11
46	Thymol	1297	0.13	0.26	0.20	0.19
47	Carvacrol	1308	2.04	7.72	2.68	2.80
48	3-methoxy-acetophenone	1318	tr.	0.13	0.08	0.06
49	2(E), 4(E)-decadienal	1325	-	tr.	0.08	0.09
50	δ-Elemene	1335	0.29	0.16	0.22	0.24
51	α-Cubebene	1349	0.28	0.19	0.23	0.23
52	Eugenol	1361	2.14	5.67	2.41	1.58
53	α-Copaene	1382	8.38	5.29	6.74	7.70
54	β-Bourbonene	1389	5.92	2.42	1.95	2.60
55	B-Elemene	1390	-	0.77	1.54	2.08
56	Z-Caryophyllene	1408	tr.	0.29	0.20	0.61
57	E-Caryophyllene	1435	23.61	18.35	15.99	19.56
58	β-Copaene	1438	17.16	0.73	1.20	1.35
59	γ-Cadinene	1444	1.20	-	-	-
60	Isoledene	1454	0.71	-	-	-
61	β-Funebrene	1461	1.16	0.79	2.02	2.41
62	α-Humulene	1467	4.48	4.92	4.76	5.15
63	γ-Muurolene	1480	0.62	0.19	0.07	0.10
64	(E)-β-Lonone	1483	-	0.89	tr.	tr.
65	D-Germacrene	1489	10.62	9.05	18.62	17.73
66	Eicosane	1498	-	0.27	0.18	0.23
67	Cubebol	1503	-	0.29	1.27	0.87
68	Bicyclogermacrene	1510	1.08	-	-	-
69	β-Bisabolene	1518	-	0.70	0.16	0.13
70	n.i.	1521	-	0.26	0.42	0.50
71	Myristicin	1529	-	2.11	-	-
72	δ-Amorphene	1530	-	6.28	11.02	11.99
73	trans-Cadina-1,4-diene	1540	0.11	-	0.10	0.12
74	α-Cadinene	1542	0.10	0.08	0.11	0.12

75	α-Calacorene	1550	0.15	0.15	0.13	0.14
76	E-Nerolidol	1567	3.64	3.34	2.09	3.52
77	D-Germacrene-4-ol	1584	1.01	0.80	1.62	1.51
78	Caryophyllene oxide	1590	1.22	1.64	2.32	1.65
79	Humulene epoxide II	1619	0.40	0.34	0.38	0.38
80	n.i.	1624	0.23	0.18	0.25	0.26
81	n.i	1661	0.68	0.81	0.29	0.18
82	α-Cadinol	1664	1.01	0.62	1.20	1.19
83	Apiole	1683	0.50	5.12	1.15	0.79
84	Heptadecane	1700	0.90	0.42	0.92	1.10
85	Mint sulfide	1752	0.19	0.16	0.23	0.26
86	n.i.	1755	-	0.17	0.20	0.16
87	Octadecane	1779	0.19	0.17	0.31	0.24
88	2-Pentadecanone-,6,10,14-trimethyl	1840	0.32	1.63	1.05	0.65
89	Dibutyl phthalate	1864	0.47	0.93	1.00	0.37
90	n.i.	1895	0.49	0.64	0.94	0.72
91	n.i.	1959	0.81	0.35	0.30	0.19
92	n.i	2024	0.79	0.64	0.74	1.04
93	Z-Falcarinol	2043	-	0.35	0.26	0.16
94	Heneicosane	2097	0.14	0.90	0.42	0.18
95	Phytol acetate	2111	0.72	2.27	0.85	0.82
96	n.i.	2114	-	0.22	0.17	0.14
97	9 cis-Retinal	2122	0.22	0.23	0.36	0.29
98	n.i	2174	0.27	-	-	-
99	n.i	2183	0.27	-	-	-
100	Isopimarol	2202	1.27	1.04	1.17	1.13
101	Tricosane	2296	0.33	1.01	1.23	0.33
102	n.i.	2305	0.99	1.70	1.36	1.33
Total			99.37	99.25	99.31	99.31

n.i. – not identified; tr – trace (<0.05)

Number of identified compounds reached to 34 in essential oil from horehound grown in Iran and Tunisia [9], while that from Lithuania contained 47 compounds [19].

Studies upon essential oil from *Marrubium vulgare* L. and other species from Marrubium sp. genus growing worldwide showed great diversity of chemicals contents depending on the localization of the plants [2,4,5,7,9,12,14,15,19].

The qualitative and quantitative composition of essential oil from horehound plants grown in south-eastern Poland depended on the plantation's age (Table 2). Following were the main components of annual horehound: E-caryophyllene (23.61%), β-copaene (17.16%), and D-germacrene (10.62%) (Figure 2). In herbs harvested in the second year of cultivation, regardless of the harvest date, the

following compounds dominated: E-caryophyllene (15.99 – 19.56%) and D-germacrene (9.05 – 18.62%). High content of carvacrole in oil from biennial horehound harvested at the beginning of its flowering (first date of harvest), as compared to those collected during the second and third dates of harvest, was some kind of interesting. Comparison of the contents of main essential oil constituents at annual and biennial plants revealed high level of β-copaene at annual horehound oil (17.16%), whereas small quantities in oil from biennial horehound plants (0.73 – 1.35%). δ-amorphene was absent in essential oil from annual horehound, while its content at oil from two-year plantation ranged from 6.28 up to 11.99%.

Studies upon essential oil from horehound harvested during the flowering stage in Algeria revealed that eugenol was the main component (50.1% - oil from plants collected at flowering stage and 16.2% - oil from horehound cut at vegetative stage) [2]. Oil from horehound grown in south-eastern Poland contained much less eugenol (1.58-5.67%) (Figure 2).

The research proves the β-bisabolene being one of the most dominating components of horehound essential oil [2,9]. The study performed hererevealed very low levels of the compound in oil from biennial plants (0.13-0.70%), while its presence was not confirmed in essential oil from annual horehound (Table 2).

β-caryophyllene, as a dominating compound, occurs in essential oil from horehound grown in Lithuania (8.5%) and Iran (11.6%) [9,19], meanwhile D-germacrene is also the main component of Lithuanian (4.71%), Tunesian (9.37%), and Iranian (9.7%) oils [7,9,19].

Numerous studies upon other species of horehound revealed that β-caryophyllene is the main constituent of the essential oil from *Marrubium velutinum* Sm. (24.25%), *Marrubium astracanicum* Jacq (13.1%), *Marrubium bourgaei* ssp. caricum P.H. Davis, Marrubium globosum Montbr. et Auch. ex Benth. ssp. *libanoticum* (Boiss) Davis (5.2-12.4%), and *Marrubium peregrinum* L. (13.20-17.99%) [4,5,12,14]. D-germacrene is one of the most dominating chemicals in oil from *Marrubium bourgaei* ssp. caricum P.H. Davis (10.3%), *Marrubium peregrinum* L. (6.79-9.05%), *Marrubium parviflorum* Fisch & C.A.Mey (21.5), and *Marrubium cuneatum* Russell (21.1%) [4,8,9,15].

CONCLUSIONS

The horehound is grown for its medicinal properties. In Poland, annual and biennial plantations of the plant species are established. The qualitative and quantitative composition of essential oil from horehound depends on the age of a plantation. Weather conditions may also modify the oil composition. Literature references indicate the diversity of qualitative and quantitative composition of oil from *Marrubium vulgare* L. depending on the localization the plant grow [2,4,5,7,9,12,14,15,19]. In south-eastern Poland, the main components of essential oil from *Marrubium vulgare* L. are: E-caryophyllene, β-copaene, and D-germacrene (oil from annual plants) and E-caryophyllene and D-germacrene (oil from biennial plants).

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SUMMARY

Horehound (*Marrubium vulgare* L.) is native to Mediterranean Sea shore, but can be also found as a road-side weed. In Poland it is cultivated. The essential oil contributes to diastolic, expectorating, and blood-vessel dilatingactions. The presented study aims at evaluating the qualitative and quantitative composition of essential oil from horehound herb depending on plantation age and raw material harvest date. The oil was obtained by steam distillation and subsequently its composition was analysed using the GC/MS method. In south-eastern Poland, the main components of essential oil from *Marrubium vulgare* L. are: E-caryophyllene, β-copaene, and D-germacrene (oil from annual plants) and E-caryophyllene and D-germacrene (oil from biennial plants).

Keywords: Marrubium vulgare L., essential oil, E-caryophyllene, β-copaene, D-germacrene, GC/MS

STRESZCZENIE

Szanta zwyczajna (Marubium vulgare L.) pochodzi z okolic Morza Śródziemnego, ale często występuje jako przydrożny chwast. W Polsce jest uprawiana. Olejek eteryczny przyczynia się do działania rozkurczającego, wykrztuśnego i rozszerzającego naczynia krwionośne. Przeprowadzone badania miały na celu ocenę składu jakościowego i ilościowego olejku z ziela szanty w zależności od wieku plantacji i terminu zbioru surowca. Olejek otrzymano poprzez destylację z parą wodną, a następnie analizowano jego skład metodą GC/MS. W Polsce południowo – wschodniej głównymi składnikami olejku z *Marrubium vulgare* L. były: E-caryophyllene , β-copaene i D-germacrene (olejek z roślin jednorocznych) oraz E-caryophyllene i D-germacrene (olejek z roślin dwuletnich).

Słowa kluczowe: Marrubium vulgare L., olejek eteryczny, E-caryophyllene, β-copaene, D-germacrene, GC/MS