THE CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *MARRUBIUM VULGARE* L. FROM POLAND

GRAŻYNA ZAWIŚLAK*

University of Life Science in Lublin, Department of Vegetable and Medicinal Plants, 58 Leszczyński Street, 20-068 Lublin, Poland
**corresponding author: grazyna.zawislak@up.lublin.pl*

Abstract

Marrubium vulgare L. is a plant cultivated in Poland for medicinal purposes. The study presents the qualitative and quantitative composition of the essential oil of *Marrubium vulgare* L. The main components of the oil were as follows: E-caryophyllene, germacrene D, bicyclogermacrene, and α-humulene. The oil from the flowering plants was shown to have the highest contents of germacrene D (43.36%). Other constituents (E-caryophyllene – 44.54%, bicyclogermacrene -20.6%, and α-humulene – 5.79%) were found in higher amounts in the oil from vegetative stage plants.

Rezumat

Studiul prezinta analiza calitativa si cantitativa a uleiului esential extras din produsul vegetal *Marrubium vulgare* L, o planta medicinala cultivata pe scara larga in Polonia. Rezultatele au aratat ca uleiul esential extras din acest produs vegetal, are un continut crescut de: E-cariofilena, germacren D, biciclogermacren si alfa-humulen. Studiul prezinta de asemenea variatiile cantitative ale acestor compusi atunci cand produsu vegetal a fost recoltat in perioada de inflorire, comparativ cu etapa vegetativa.

Keywords: *Marrubium vulgare* L., E-caryophyllene, germacrene D, bicyclogermacrene and α-humulene

Introduction

The genus *Marrubium* sp. comprises about 40 plant species widespread in Europe, Asia, America, and also in Australia [12]. White horehound (*Marrubium vulgare* L.) belongs to the *Lamiaceae*. It is native to the Mediterranean Sea region [21]. In Poland, white horehound is found in the wild, but the herb is collected only from cultivated plants [9,16].

Under Polish climatic conditions, *Marrubium vulgare* L. is a perennial plant. Medicinal properties of horehound have been long known and the origin of its use goes back to ancient Egypt. The medicinal raw material is the herb of horehound (*Marrubi herba*) [19]. The herb consists of whole or crushed flowering aerial parts of *Marrubium vulgare* L. [14], and it shows multiple effects on human organism [6,7,9]. The essential oil of *Marrubium vulgare* L. has a relaxant and expectorant effect as well as a

vasodilator [21]. It also exhibits antioxidant [8], antimicrobial [20], and immunotropic properties [10]. Herbal raw material and herbal remedies obtained from horehound are used in laryngitis and bronchitis [19]. In folk medicine, horehound is used externally on skin abrasions and wounds [5]. Horehound can also be used as an aromatic agent [3].

Materials and Methods

Plant material

Herbal raw material (herb) came from a one-year plantation, located in an experimental section of the Department of Vegetable Crops and Medicinal Plants of the University of Life Sciences in Lublin (Poland) ($51^{o}14^{'}$N $22^{o}34^{'}$E). The plants were grown in 2009. The plantation was established by planting seedlings produced in a greenhouse. The plants were planted in the field in the 1st decade of May at a spacing of 30x40 cm. The herb was harvested at two times: at the end of June (the plants were at the vegetative stage) and at the end of July (the plants were at full bloom). The plants were cut at a height of 5 cm above ground. The herb was dried in a drying oven at a temperature of 30^{o}C. The raw material was dried for 6 days.

Isolation of the essential oil

The essential oil of *Marrubium vulgare* L. was obtained through steam distillation in a Deryng apparatus, according to the Polish Pharmacopoeia VII (2006) [13]. 40 g of dry herb and 400 mL of distilled water were used for distillation and the distillation time was 3 hours.

GC-MS (Gas chromatography completed with mass spectroscopy)

The GC-MS instrument ITMS Varian 40000 GC-MS/MS (Varian, USA) was used, equipped with a CP-8410 auto-injector and a 30 m x 0.25 mm i.d. VF-5ms column (Varian, USA), film thickness 0.25μm; carrier gas, helium at a rate of 0.5 mL/min; injector and detector temperature, 220^{o}C and 200^{o}C, respectively; split ratio, 1:20; injection volume, 1μL. A temperature gradient was applied (60^{o}C for 0.5 min, then incremented by 3^{o}C/min to 246^{o}C and maintained at this temperature for 10 min); ionization energy, 70 eV; mass range, 40-1000 Da; scan time, 0.80 s.

GC-FID (Gas chromatography completed with the detector FID)

A Varian3800 Series (Varian, USA) instrument with a DB-5 column (J&W, USA) was used, operated under the same conditions as GC-MS; FID, 256^{o}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 [11] and with

data available in the literature [1]. The identity of the compounds was confirmed by their retention indices, taken from the literature [1].

Results and Discussion

Weather conditions were favourable for the growth and development of white horehound (Table I). Air temperatures during planting and during the growth of the plants were similar to the long-term averages, which created favourable conditions for plant growth. Low rainfall in the 1st decade and very abundant rainfall in the 2nd and 3rd decades of May did not have a negative effect on plant survival and the initial growth. Large amounts of rainfall in June were not observed to affect negatively the growth of horehound (and the essential oil content in dry herb of *Marrubium vulgare* L., either. The essential oil content in the fruits of *Foeniculum vulgare* Mill. was the most susceptible features to be affected by climatic condition (temperature and rainfall) [2]).

Table I

Air temperature and total precipitation in 2009 compared to a background of many year averages

	Month	Decade			Mean* Σ**	1995-2005
		I	II	III		
Temperature (°C)	May	13.6	13.1	14.2	13.6	13.0
	June	15.3	14.9	19.1	16.4	16.2
	July	19.9	20.5	19.3	19.9	17.8
Precipitations (mm)	May	3.6	34.7	32.9	71.2	57.7
	June	28.2	32.7	64.6	125.5	65.7
	July	15.6	9.8	31.7	57.1	83.5

* Temperature

** Precipitations

The study showed that the essential oil content in the dry herb of *Marrubium vulgare* L. was on average 0.05% [22].

On the basis of the study, the presence of 23 compounds was found in the oil of *Marrubium vulgare* L. obtained from plants in the vegetative stage (Table II). 17 compounds were found in trace amounts (below 0.05%). In the oil extracted from flowering horehound, the number of identified compounds was twice higher (50), including 36 compounds that were present in amounts below 0.05%. The dominant components of the oil were as follows: E-caryophyllene, germacrene D, bicyclogermacrene, and α-humulene. The oil obtained from plants in the vegetative stage contained more E-caryophyllene (44.54%), bicyclogermacrene (20.06%), α-humulene (5.79%), and caryophyllene oxide (5.56%) than the oil from flowering plants. The highest amount of germacrene D (43.36%) was found in

flowering plants. The content of germacrene D in the oil from plants harvested in the vegetative stage was 23.85% (Table II). Considering previous studies cited in the literature, the oil obtained in Algeria contained small amounts of this constituent [4]. The content of germacrene D in the oil from vegetative stage plants was higher than in the oil from flowering plants; it was 2.3% and 0.3%, respectively. The main constituents of the oil of *Marrubium vulgare* L. obtained in Algeria were as follows: eugenol (16.2% - the oil from plants in the vegetative stage, and 50.1% - the oil from flowering plants), and β-bisabolene (10.9% - the oil from plants in flowering stage, and 28.8% in the oil from plants in the vegetative stage) [4]. Studies of other authors have also shown a lower content of germacrene D in the oil of *Marrubium vulgare* L. than in that obtained in the present experiment. The content of germacrene D in the oil of *Marrubium vulgare* L. grown in Tunisia was 9.37% [8]. The oil from Lithuania contained 4.71% of germacrene D [18]. The main components of the oil obtained in Tunisia were as follows: γ-eudesmol, β-citronellal, citronellyl formate, and germacrene D [8]. The oil from Lithuania had the following compounds as main components: (Z)-β-farnesene β-caryophyllene, (E)-hex-2-enal, α-humulene, and germacrene D [18]. Germacrene D was one of the dominant components of the oil obtained from *Thymus pulegioides* L., either [15].

Table II

Chemical composition of essential oil from *Marrubium vulgare* L. during the vegetative and flowering phases

No	Compound	RI	%	
			Vegetative phase	Flowering phase
1.	α-thujene	857	-	t
2.	α-pinene	862	-	t
3.	α-terpinene	933	-	t
4.	limonene	942	-	0.72
5.	γ-terpinene	966	-	t
6.	n.i.	1039	-	t
7.	geijerene	1048	t	0.65
8.	trans-pinocamphone	1081	-	t
9.	safranol	1124	-	t
10.	1-cyclohexene-1-carboxaldehyde,2,6,6-trimethyl	1138	-	t
11.	carvacrol methyl ether	1153	-	t
12.	lonone	1167	-	t
13.	thymol	1197	-	t
14.	pregeijerene	1200	-	t
15.	carvacrol	1204	-	9.48
16.	n.i.	1219	-	t
17.	δ-elemene	1235	-	t
18.	α-cubebene	1253	-	t
19.	α-ylangene	1280	-	t
20.	isoledene	1282	t	-
21.	α-copaene	1287	t	1.14

No	Compound	RI	%	
			Vegetative phase	Flowering phase
22.	β-bourbonene	1297	t	0.93
23.	β-elemene	1302	t	1.54
24.	α-gurjunene	1318	-	t
25.	E-β-damascone	1322	-	t
26.	E- caryophyllene	1330	44.54	24.79
27.	β-copaene	1339	t	t
28.	β-gurjunene	1342	t	t
29.	aromadendrene	1346	t	t
30.	α-humulene	1361	5.79	3.26
31.	cis-muurola-4(14),5-diene	1366	t	t
32.	γ-muurolene	1376	t	0.56
33.	germacrene D	1382	23.85	43.36
34.	viridiflorene	1390	t	t
35.	bicyclogermacrene	1394	20.06	9.86
36.	α- muurolene	1400	-	t
37.	germacrene A	1406	t	t
38.	γ-cadinene	1412	t	t
39.	δ-amorphene	1416	t	1.17
40.	trans cadina-1,4-diene	1434	-	t
41.	α-cadinene	1438	-	t
42.	epi-longipinanol	1457	t	-
43.	1-nor-bourbonanone	1468	-	t
44.	ledol	1475	-	t
45.	spathulenol	1486	t	0.69
46.	caryophyllene oxide	1490	5.56	1.63
47.	globulol	1494	t	t
48.	viridiflorol	1503	-	t
49.	humulene epoxide II	1518	t	t
50.	epi-α-cadinol	1548	-	t
51.	epi-α-muurolol	1559	-	t
52.	epoxy allo-allomadendrene	1564	-	t

n.i.-no identified
t-trace (<0.05%)
RI, non –isothermal Kováts retention indices (from temperature-programming , using definition of Van den Dool and Kratz) [17] for series of n-alkanes C_6-C_{40}

Conclusion

The climatic conditions of Poland are appropriate for growing white horehound. According to Polish Pharmacopoeia VIII [14], the horehound herb should be harvested during flowering. This study showed that the oil from flowering plants contained twice more identified compounds than the oil extracted from plants in the vegetative stage. The dominant components of the oil of *Marrubium vulgare* L. were the following: E-caryophyllene, germacrene D, bicyclogermacrene, and α-humulene. The oil from flowering plants was shown to have the highest proportion of germacrene D (43.36%). Other constituents (E-caryophyllene, bicyclogermacrene, and α-humulene) were found at higher amounts in the oil from vegetative stage plants.

References

1. Adams RP., Identification of Essential Oil Compounds by Gas Chromatography/Quadrupole Mass Spectroscopy. *Allured Carol Stream*, IL 2001
2. Aprotosoaie A.C., Spac A., Hăncianu M., Miron A., Tănăsescu V.F., Dorneanu V., Stănescu U., The chemical profile of essential oils obtained from fennel fruits (*Foeniculum vulgare* Mill.), *Farmacia*, 2010, 58(1), 46-53
3. Barnes J., Anderson L.A., Phillipson J.D., Herbal Medicines, Pharmaceutical Press, London Chicago, 2002, 294-295
4. Belhattab R., Larous L., Essential Oil Composition and Glandular Trichomes of *Marrubium vulgare* L. Growing Wild in Algeria. *J. Essential. Oil Res*., 2006, 18, 369-373
5. Bisset N.G., Herbal Drugs and Phytopharmaceuticals, Medpharm Scientific Publishers Stuttgart, 2001, 317-318
6. Bradley P. R., British herbal compendium, vol. 1, British Herbal Medicine Association, 1992, 218-220
7. El Bardai S., Wibo M., Hamaide M-Ch., Lyoussi B., Quetn-Leclerq J., Morel N., Characterisation of marrubenol, a diterpene extracted from *Marrubium vulgare*, as an L-type calcium channel blocker, *British Journal of Pharmacology*, 2003, 140, 1211-1216
8. Kadri A., Zarai Z., Békir A., Gharsallah N., Damak M., Gdoura R., Chemical composition and antioxidant activity of *Marrubium vulgare* L. essential oil from Tunisia. *African Journal of Biotechnology*, 2011, 10(19), 3908-3914
9. Kohlmünzer S., Farmakognozja, Wydawnictwo Lekarskie PZWL Warszawa, 2007, 308
10. Lamer-Zarawska E., Kowal-Gierczak B., Niedworok J., Fitoterapia i leki roślinne, Wydawnictwo Lekarskie PZWL Warszawa, 2007, 40-41
11. Mass Spectral Library. NIST/EPA/NIH: USA, 2002
12. Meyre-Silva Ch., Cechinel-Filho V., A Review of Chemical and Pharmacological Aspects of the Genus Marrubium, Current Pharmaceutical Design, 2010, 16 (31), 3503-3518
13. Polish Pharmacopoeia VII. PTFarm., Warszawa, 2006, 325-326
14. Polish Pharmacopoeia VIII. PTFarm., Warszawa, 2008, 2249-2250
15. Rădulescu V., Nistoroiu M.M., Panait M.E., Ilieş D.-C., Tirifon E., Chemical composition and cancer chemopreventive effect of volatile oil isolated from *Thymus pulegioides* L. *Farmacia*, 2011, 59(2), 228-238
16. Senderski M.E., Prawie wszystko o ziołach, Leśna Podkowa, 2004, 603-605
17. Van den Dool H., Kratz D.P., A generalization the retention index system including liner temperature programmed gas-liquid partition chromatography. *J. Chromatography*, 1963, 11, 463-471
18. Weel K.G.C., Venskutonis P.R., Pukalskas A., Gruzdiene D., Linssen J.P.H., Antioxidant activity of horehound (*Marrubium vulgare* L.) grown in Lithuania. *Fett/ Lipid*, 1999, 101(10), 395-400
19. Wolski T., Matosiuk D., Baj T., Ziewiec A., Szanta zwyczajna (*Marrubium vulgare* L.) – roślina lecznicza o wielokierunkowym działaniu farmakologicznym, *Postępy Fitoterapii*, 2007, 1, 39- 45
20. Wren R.C., Potter's new cyclopaedia of botanical drugs and preparations, 1988, 146
21. Wyk B.E., Wink M., Rośliny lecznicze świata, MedPharm Polska, 2008, 198
22. Zawiślak G., Cropping evaluation of while horehound (*Marrubium vulgare* L.) grown from sowing and seedling. *Herba Pol*., 2009, 55(3), 63-68

Manuscript received: April 15th, 2011