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# Chemical composition of essential oils of *Marrubium vulgare* L. and *Marrubium incanum* Desr. grown in Poland

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*Marrubium vulgare* L. is a plant that is grown in Poland for medicinal purposes. *Marrubium incanum* Desr. grows primarily in plant collections in Polish botanical gardens. The present study compared the essential oil content in *Marrubium vulgare* L. and *Marrubium incanum* Desr. as well as the qualitative and quantitative composition of their essential oils. The essential oil content in the dried herb of *Marrubium vulgare* L. was 0.05%, while in the herb of *Marrubium incanum* Desr. it was 0.04%. This study showed the presence of 34 compounds in the oil of *Marrubium vulgare* L. and 20 compounds in the oil of *Marrubium incanum* Desr. The main components of the oil of *Marrubium vulgare* L. were as follows: E-caryophyllene (25.91–32.06%), germacrene D (20.23–31.14%) and δ-amorphene (8.38–10.22%), while in the oil of *Marrubium incanum* Desr. the following compounds were predominant: germacrene D (32.46–37.87%), E-caryophyllene (22.49–30.79%) and α-cadinol (14.36–17.87%).

**Key words:** *Marrubium vulgare* L., *Marrubium incanum* Desr., essential oil, E-caryophyllene, germacrene D, δ-amorphene

## INTRODUCTION

The genus *Marrubium* sp. comprises about 40 plant species [1]. These plants contain essential oil, and the composition of essential oils of various plant species of the genus *Marrubium* sp. has been the subject of research for many researchers across the world [2–11]. *Marrubium vulgare* L. and *Marrubium incanum* Desr. belong to the most known species of this genus [1].

*Marrubium vulgare* L. grows in the wild in Poland, but this herb is collected only from plantations [12]. *Marrubium incanum* Desr. is rarely cultivated in our country and it usually grows in botanical gardens.

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Herbal raw material from *Marrubium vulgare* L. is the herb [12]. Many researchers have indicated multifaceted therapeutic activity of *Marrubium vulgare* L. [12–15]. In the dried herb of *Marrubium vulgare* L., essential oil is found at an amount of about 0.05% [16]. The oil has expectorant [15], antioxidant [3, 11], and antimicrobial effects [17]. The oil of *Marrubium incanum* Desr. also exhibits antimicrobial properties [18]. Sometimes fake *Marrubium vulgare* L. raw material is marketed, with the addition of plant material from other plant species of the genus *Marrubium* sp., including *Marrubium incanum* Desr. [19].

The aim of this study was to compare the content as well as the qualitative and quantitative composition of the oil from the herb of two horehound species: *Marrubium vulgare* L. and *Marrubium incanum* Desr.

## MATERIALS AND METHODS

This study was conducted during the period 2008–2009.

### 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°14'N 22°34'E). Seeds of *Marrubium vulgare* L. and *Marrubium incanum* Desr. came from the Botanical Garden of the Maria Curie-Skłodowska University in Lublin. The plantation was established by planting seedlings produced in a greenhouse. Plants were planted at a spacing of 30 × 40 cm at the end of May. The herb was harvested at the flowering stage at the end of July. 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 °C.

### Isolation of essential oil

Essential oils of *Marrubium vulgare* L. and *Marrubium incanum* Desr. were obtained through steam distillation using a Deryng apparatus, according to Polish Pharmacopoeia VII [20]. 40 g of dried herb and 400 ml of distilled water were used for distillation, and the distillation time was 3 hours.

### GC-MS

A 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 40–1 000 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 [21] and with data available in the literature [22]. The identity of the compounds was confirmed by their retention indices, taken from the literature [22].

## RESULTS AND DISCUSSION

The two-year study showed that the essential oil content in the dried herb of *Marrubium vulgare* L. was on average 0.05%. A similar content of oil was found in the dried herb of *Marrubium incanum* Desr. (Table 1). The essential oil content in the herb *Marrubium vulgare* L. is in agreement with the reports of several authors [13, 16, 23]. In the study of other researchers, the oil content in the herb *Marrubium vulgare* L. was higher and it was 0.09% [7].

The present study showed the presence of 34 compounds in the oil of *Marrubium vulgare* L. and 20 compounds in the oil of *Marrubium incanum* Desr. (Tables 2 and 3). The presence of 30 compounds was found in the oil obtained from *Marrubium vulgare* L. growing in natural stands in Algeria [8]. There were 34 compounds in the oil from Iran [7], whereas the oil of *Marrubium vulgare* L. growing in Lithuania contained 47 compounds [3]. A few studies on the oil of *Marrubium incanum* Desr. show the presence of 47 compounds [18].

The main components of the oil of *Marrubium vulgare* L. were as follows: E-caryophyllene, germacrene D and δ-amorphene. The following constituents were predominant in the oil of *Marrubium incanum* Desr.: germacrene D, E-caryophyllene and α-cadinol (Tables 2 and 3).

The oil of *Marrubium vulgare* L. contained from 25.91 to 32.06% of E-caryophyllene, similarly to the oil of *Marrubium incanum* Desr. – 22.49–30.79% (Tables 2 and 3). The available literature does not indicate E-caryophyllene as the main component of the oil of *Marrubium vulgare* L. in Algeria [8], Iran [24] or Tunisia [11]. E-caryophyllene was the dominant compound in the oil of *Marrubium incanum* Desr., and its content was 27.0% [18].

In the present study, the content of germacrene D ranged from 20.23 to 31.14% in *Marrubium vulgare* L. and 32.46–37.87% in the oil of *Marrubium incanum* Desr. (Tables 2 and 3). The literature gives a lower value of germacrene D content in the oil of *Marrubium incanum* Desr. (26.2%) [18]. A lower content of germacrene D, compared to this study, was also found in the oil of *Marrubium vulgare* L. from Iran (9.7%) [7] and Lithuania (4.71%) [3].

Germacrene D is one of the dominant components of the oils obtained from other plant species of the genus *Marrubium* sp.: *Marrubium cuneatum* Russell from Iran (24.1%) [6] and Lebanon (15.6%) [25], *Marrubium parviflorum* (21.5%) [7], *Marrubium bourgaei* ssp. *caricum* P. H. Davis (10.3%) [5], *Marrubium peregrinum* L. (6.79–9.05%) [10].

The content of δ-amorphene in the oil of *Marrubium vulgare* L. was 8.38–10.22%. The oil of *Marrubium incanum*

Table 1. **Percentage of essential oil in the herb *Marrubium* sp.**

| Species | Essential oil content in dried herb, % | | |
|---|---|---|---|
| | 2008 | 2009 | Mean |
| *Marrubium vulgare* L. | 0.06 | 0.05 | 0.05 |
| *Marrubium incanum* Desr. | 0.05 | 0.03 | 0.04 |

Table 2. **Percentage composition of *Marrubium vulgare* L. essential oil**

| Compound | RI | Percentage | |
|---|---|---|---|
| | | 2008 | 2009 |
| Limonene | 1 032 | t | 1.53 ± 0.06 |
| γ-Terpinene | 1 060 | t | 1.75 ± 0.07 |
| Geijerene | 1 143 | t | t |
| trans-Pinocamphone | 1 167 | t | t |
| cis-Pinocamphone | 1 174 | t | t |
| Thymol | 1 290 | t | 0.42 ± 0.59 |
| Carvacrol | 1 298 | 1.16 ± 0.07 | 14.98 ± 0.07 |
| α-Cubebene | 1 352 | T | 0.28 ± 0.54 |
| α-Copaene | 1 377 | 7.56 ± 0.09 | 6.95 ± 0.12 |
| β-Bourbonene | 1 389 | T | 2.18 ± 0.01 |
| β-Elemene | 1 391 | 1.47 ± 0.01 | 1.37 ± 0.02 |
| β-Longipinene | 1 401 | t | t |
| α-cis-Bergamotene | 1 413 | t | t |
| E-Caryophyllene | 1 419 | 32.06 ± 0.28 | 25.91 ± 0.61 |
| β-Copaene | 1 432 | t | t |
| α-Guaiene | 1 440 | t | t |
| (Z)-β-Farnesene | 1 445 | 3.95 ± 0.04 | 1.10 ± 0.00 |
| α-Humulene | 1 455 | 6.15 ± 0.06 | 4.84 ± 0.11 |
| γ-Muurolene | 1 482 | t | t |
| Germacrene D | 1 484 | 31.14 ± 0.16 | 20.23 ± 0.55 |
| Bicyclogermacrene | 1 500 | 0.67 ± 0.11 | 0.30 ± 0.42 |
| β-Bisabolene | 1 506 | t | t |
| δ-Amorphene | 1 512 | 10.22 ± 0.09 | 8.38 ± 0.14 |
| γ-Cadinene | 1 514 | t | t |
| β-Sesquiphellandrene | 1 523 | t | t |
| trans-Cadina-1(2),4-diene | 1 535 | t | t |
| α-Cadinene | 1 539 | t | t |
| E-Nerolidol | 1 563 | 3.04 ± 0.06 | 4.52 ± 0.13 |
| Germacrene D-4-ol | 1 576 | 1.27 ± 0.02 | t |
| Caryophyllene oxide | 1 583 | 0.35 ± 0.57 | 2.33 ± 0.10 |
| Humulene epoxide II | 1 608 | t | t |
| 1-epi-Cubenol | 1 629 | t | t |
| epi-α-Muurolol | 1 642 | t | t |
| α-Cadinol | 1 654 | 0.80 ± 0.01 | 2.63 ± 0.50 |
| Total | | 99.84 | 99.7 |
| Monoterpene hydrocarbons | | – | 3.21 |
| Oxygenated monoterpenes | | 1.16 | 15.40 |
| Sesquiterpene hydrocarbons | | 93.22 | 71.54 |
| Oxygenated sesquiterpenes | | 5.46 | 9.48 |

t – trace (<0.05%).

Desr. had a lower proportion of this constituent (3.05–3.76) (Tables 2 and 3).

This study showed that the oil of *Marrubium incanum* Desr. also contained α-cadinol as a dominant component (14.36–17.87%). This compound was also found in the oil of *Marrubium vulgare* L. in a much smaller proportion (0.80–2.63%) (Tables 2 and 3). The available literature does not indicate this compound as a dominant component in the oil of *Marrubium vulgare* L. and *Marrubium incanum* Desr. as well as in the oils of other plant species of the genus *Marrubium* sp. [2–11, 18].

The essential oil of *Marrubium vulgare* L. contained from 6.95 to 7.56% of α-copaene (Table 2). A twice lower content of this compound was found in the oil of *Marrubium incanum* Desr. (2.65–2.97%) (Table 3). The content of this component was at a similar level in the oil of *Marrubium vulgare* L. from Lithuania (3.08%) [3] and Iran (3.4%) [7]. A lower content of α-copaene was shown in the oil from Algeria [8].

A higher content of α-humulene was found in the oil of *Marrubium vulgare* L. (4.84–6.15%) than in the oil of *Marrubium incanum* Desr. (3.05–4.57%) (Tables 2 and 3). The oil of *Marrubium vulgare* L. from Lithuania [3] and Algeria [8] contained a smaller amount of this compound.

Table 3. **Percentage composition of *Marrubium incanum* Desr. essential oil**

| Compound | RI | Percentage | |
|---|---|---|---|
| | | 2008 | 2009 |
| Geijerene | 1 143 | 0.55 ± 0.02 | t |
| Carvacrol | 1 298 | 7.37 ± 0.05 | t |
| α-Ylangene | 1 375 | 0.48 ± 0.00 | t |
| α-Copaene | 1 377 | 2.97 ± 0.02 | 2.45 ± 1.11 |
| β-Bourbonene | 1 389 | 0.52 ± 0.08 | t |
| β-Elemene | 1 391 | 1.21 ± 0.04 | t |
| γ-Gurjunene | 1 410 | 0.14 ± 0.34 | t |
| E-Caryophyllene | 1 419 | 22.49 ± 0.16 | 30.79 ± 1.24 |
| β-Copaene | 1 432 | t | t |
| α-Humulene | 1 455 | 3.05 ± 0.06 | 4.57 ± 0.10 |
| γ-Muurolene | 1 482 | t | t |
| Germacrene D | 1 484 | 37.87 ± 0.07 | 32.46 ± 1.17 |
| Bicyclogermacrene | 1 500 | 3.22 ± 0.15 | 4.89 ± 0.49 |
| Germacrene A | 1 509 | t | t |
| δ-Amorphene | 1 512 | 3.05 ± 0.00 | 3.76 ± 0.40 |
| γ-Cadinene | 1 514 | t | t |
| Spathulenol | 1 578 | t | t |
| Caryophyllene oxide | 1 583 | 2.51 ± 0.18 | 3.01 ± 0.08 |
| epi-α-Muurolol | 1 642 | t | t |
| α-Cadinol | 1 654 | 14.36 ± 0.03 | 17.87 ± 0.27 |
| Total | | 99.79 | 99.80 |
| Monoterpene hydrocarbons | | 0.55 | – |
| Oxygenated monoterpenes | | 7.37 | – |
| Sesquiterpene hydrocarbons | | 75.00 | 78.92 |
| Oxygenated sesquiterpenes | | 16.87 | 20.88 |

t – trace (<0.05%).

## CONCLUSIONS

The herb *Marrubium vulgare* L. is collected only from plantations. *Marrubium incanum* Desr. is rarely cultivated in our country. The essential oil content in the dried herb of *Marrubium vulgare* L. was 0.05%. A similar content of oil was found in the dried herb of *Marrubium incanum* Desr. – 0.04%. The main components of the oil of *Marrubium vulgare* L. and *Marrubium incanum* Desr. were E-caryophyllene and germacrene D. Herbs *Marrubium vulgare* L. and *Marrubium incanum* Desr. were harvested at the flowering stage. According to Polish Pharmacopoeia VIII [26], the horehound herb should be harvested during flowering.

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## LENKIJOJE AUGINAMŲ *MARRUBIUM VULGARE* L. IR *MARRUBIUM INCANUM* DESR. ETERINIŲ ALIEJŲ CHEMINĖ SUDĖTIS

*S a n t r a u k a*

*Marrubium vulgare* L. Lenkijoje auginamas medicininiams tikslams. *Marrubium incanum* Desr. auginamas Lenkijos botanikos soduose, augalų kolekcijose. Darbe buvo tiriama abiejų augalų eterinių aliejų cheminė sudėtis.