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Essential Oil and Glycosidically Bound Volatiles of *Thymus pulegioides* L. growing Wild in Croatia

Ani Radonić* and Josip Mastelić

Department of Organic Chemistry, Faculty of Chemistry and Technology, University of Split,
N. Tesle 10/V, 21000 Split, Croatia

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The present paper represents the continuation of our research of free (essential oil) and glycosidically bound volatile compounds from Croatian wild-growing aromatic plants. The essential oil and glycosidically bound volatile compounds were isolated from the fresh and dried plant material of *Thymus pulegioides* L. at three stages of plant development and analysed by gas chromatography-mass spectrometry (GC-MS). The most abundant constituents in essential oils were geraniol and linalool followed by thymol, γ -terpinene, thymol methyl ether, borneol and geranyl acetate, all of which showed quantitative variations during the plant development stages and whether fresh or dried plants were used for essential oil isolation. After enzymatic hydrolysis of glycosidic fractions, the GC-MS analysis of volatile aglycones revealed eugenol, geraniol and 1-octen-3-ol as the main aglycones in all samples. Other important aglycones were thymoquinone, 2-phenylethanol, 3-hexenol, 3-octanol, benzyl alcohol and linalool. Quantitative variations were observed when the aglycones were analysed at three different stages of plant development. Because of the high geraniol content in the essential oils and in the aglycone fractions this *Thymus pulegioides* L. could be interesting as a fragrant material for perfume industry or for food flavouring.

Keywords:
Thymus pulegioides L.
essential oil
aglycones
GC-MS
geraniol
eugenol

INTRODUCTION

The chemical polymorphism of essential oils characteristic for the plants belonging to the genus *Thymus* (Lamiaceae) has been reviewed by Stahl-Biskup.¹ The most important compounds in the essential oils of this genus are terpene phenols thymol and carvacrol followed by linalool, *p*-cymene, γ -terpinene, borneol, terpinen-4-ol and 1,8-cineole.

Thymus pulegioides L., studied in this paper, is one of the three most common *Thymus* species growing wild

in Croatia which belongs to the taxon *Thymus serpyllum*. It comprises a number of forms or subspecies occurring all over Europe, including Croatia. *T. pulegioides* is a low, creeping and perennial aromatic plant which grows on dry, stony and gravelly soils, from lowland to the mountains and often covers large areas of grasslands on the sunny hillsides of continental Croatia. The flowering period lasts from early May to September.² The whole plant has a pleasant odour reminiscent of geraniol. It is interesting that the odour of individual plants growing at only one square meter is different. The plant and its extracts are

* Author to whom correspondence should be addressed. (E-mail: radonic@ktf-split.hr)

used in traditional medicine in the treatment of coughs, headaches and various gastroenterologic disorders.

The essential oil composition of *Thymus pulegioides* originating from different parts of Europe has been reported before. Essential oils of the Italian wild growing *Thymus pulegioides* were defined as thymol chemotype according to the high content of γ -terpinene, *p*-cymene, thymol and carvacrol.³ In the Vilnius district of Lithuania three chemotypes (citral/geraniol, carvacrol and α -terpenyl acetate) of *Thymus pulegioides* were identified at one location.⁴ The same authors collected *Thymus pulegioides* samples in Vilnius district according to their odour and defined five different chemotypes.⁵ Ložienė *et al.*, after intensive investigations of the chemical composition of the essential oil of different varieties of *Thymus pulegioides*, concluded that there is no clear relation between the varieties and chemotypes. They confirmed once more the chemical polymorphism of the essential oil of *Thymus pulegioides*.⁶ Oil samples of *Thymus pulegioides* L. of Croatian origin were rich in *p*-cymene (6 %–15 %), thymol (1 %–22 %) and carvacrol (10 %–30 %), while one of the analysed samples contained an almost 50 % of linalool.⁷ Geraniol was the major component of another Croatian *Thymus pulegioides* essential oils (38.4 %–44.7 %), followed by linalool (28.2 %–28.6 %); thymol in this case constituted only 2.2 %–6.2 %, while carvacrol was absent in those samples.⁸

Glycosidically bound volatile compounds were detected in many aromatic plants of the *Lamiaceae* family.^{9–21} These water soluble, nonvolatile and odourless compounds can be considered as a source of potential aroma, since they are able to release volatile flavour compounds (aglycones) by acid or enzymatic hydrolysis. Therefore, the study of glycosidically bound volatile compounds may be of some interest for the food (food flavouring) and perfume (fragrant compounds) industries. As far as we know, there is only one report on glycosidically bound volatile compounds in *Thymus pulegioides* L. (our preliminary investigation).⁸ Mastelić *et al.* identified four aglycones after enzymatic hydrolysis of the glycosidic fraction isolated from Croatian *Thymus pulegioides* with geraniol (37.6 %–48.6 %) and eugenol (11.2 %–20.7 %) being the main ones.

Considering everything mentioned above, it is of interest to continue research on the composition of free and glycosidically bound volatile compounds from this species of *Thymus*. Therefore, our objective was to study the influence of the stage of development and drying of plant material on the content and composition of these compounds from *Thymus pulegioides* L. of Croatian origin. The qualitative and quantitative composition of the obtained free and glycosidically bound volatiles was also compared in order to establish eventually correlation between them.

EXPERIMENTAL

Chemicals

All the solvents employed (*p.a.* grade), β -glucosidase, α -pinene (*purum*), β -pinene (*purum*), limonene (*puriss. p.a.*), *p*-cymene (*puriss. p.a.*), 1-octen-3-ol (*purum*), carvacrol (*purum*), benzyl alcohol (*puriss. p.a.*), and 2-phenylethanol (*purum*) were purchased from Fluka Chemie, Buchs, Switzerland. Linalool (97 %) and geraniol (98 %) were purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany, and thymoquinone (99 %) from Aldrich Chem. Co., Milw. USA. Camphor (techn.), thymol (*puriss.*) and menthol (*puriss.*) were products of Kemika, Zagreb, Croatia, borneol (techn.) and nerol (techn.) of Chromos-Aroma, Zagreb, Croatia, and eugenol (techn.) of Galenika, Belgrade, Serbia. Silica gel for column chromatography (Kieselgel 60, 0.040 mm–0.063 mm), silica gel plates (Kieselgel 60, thickness 0.2 mm) for thin-layer chromatography, ammonia (25 % solution), calcium carbonate (98 %), anhydrous sodium sulphate (*puriss. p.a.*) were purchased from Merck, Darmstadt, Germany.

Plant Material

The plant material of wild-growing *Thymus pulegioides* L. was collected in the submediterranean region of Dalmatia (south Croatia) in 2004. The plants were harvested at three stages of development: (1) in May (spring), when the whole plant was in full vegetation and during the flowering period; (2) at the beginning of July (summer), when the flowering had ended; (3) at the end of September (early autumn). This investigation was carried out on the aerial parts of fresh and air-dried (performed at room temperature in a shaded place) plant material. The voucher specimens are deposited at Department of Organic Chemistry, Faculty of Chemistry and Technology, University of Split, Croatia.

Isolation of the Essential Oil and Glycosides

Upon the addition of powdered calcium carbonate (2 g) in order to neutralize of eventually existing plant acids, the plant material (70 g) was submitted to hydrodistillation using 500 mL of distilled water. This hydrodistillation was performed in a Clevenger-type apparatus for three hours. The obtained essential oil was separated from the aqueous layer, dried over anhydrous sodium sulphate and stored in a sealed vial at low temperature (–18 °C) until analysis.

The extraction of water soluble glycosidically bound volatiles was performed simultaneously with hydrodistillation of the essential oil. After the hydrodistillation, the aqueous extract was separated by decantation, and the remaining plant material was extracted once more with 300 mL of boiling water. The combined aqueous extracts were concentrated to 30 mL in a rotary evaporator. Precipitation of ballast compounds was performed by addition of ethanol (300 mL) and, subsequently, with ammonia-absolute ethanol.^{19,22} Final purification of the glycosidic fraction was performed by chromatography on a silica gel column as described in previous paper.¹⁶ The obtained glycosidic fraction was fur-

ther concentrated to dryness and the residue dissolved in an acetate buffer (pH = 4.4, 5 mL). Prior to enzymatic hydrolysis, the remaining free volatile compounds were removed by means of extraction as described previously.^{16,23}

Enzymatic Hydrolysis

Enzyme β -glucosidase (20 mg) was added to the glycosidic extract together with pentane (\approx 3 mL) as a trap for liberated aglycones. After incubation at 37 °C for 72 hours, the hydrolysed volatile aglycones were extracted from the aqueous layer with pentane (4×5 mL). After drying over anhydrous sodium sulphate, the internal standard (an exact quantity of menthol) was added into combined pentane extracts. Then, pentane extracts were concentrated to 0.5 mL, of which 1 μ L was used for GC and GC-MS analysis.

Gas Chromatography

Gas chromatographic analysis was performed on a Hewlett-Packard gas chromatograph (model 5890 series II) equipped with the split/splitless injector, flame ionization detector (FID), and capillary HP-20M column (polyethylene glycol; 50 m \times 0.2 mm i.d., film thickness 0.2 μ m) Chromatographic conditions were as follows: helium as carrier gas at 1.0 mL min⁻¹; injector and detector temperatures, 250 °C and 280 °C, respectively. Oven temperature was programmed: 70 °C for 4 min, then to 180 °C at a rate of 4 °C min⁻¹ and held isothermal for 10 min. Volume injected was 1 μ L and split ratio 1:50.

Gas Chromatography-Mass Spectrometry (GC-MS)

The analyses of the free and glycosidically bound volatiles were also run on a Hewlett-Packard GC-MS system (GC model 5890 series II; MS model 5971A). Two fused-silica capillary columns of different polarity were used: HP-20M (polyethylene glycol; 50 m \times 0.2 mm i.d., film thickness 0.2 μ m) and HP-101 (dimethylpolysiloxane; 25 m \times 0.2 mm i.d., film thickness 0.2 μ m).

The oven temperature program for HP-20M column was as follows: 70 °C for 4 min, then to 180 °C at a rate of 4 °C min⁻¹ and held isothermal for 10 min. For HP-101 column the oven temperature was programmed: 70 °C for 2 min, then to 200 °C at a rate of 3 °C min⁻¹ and held isothermal for 10 min. Carrier gas was helium with flow rate of 1 mL min⁻¹. Injector temperature 250 °C; split ratio 1:50; detector temperature 280 °C; m/z was scanned from 30 to 300. The ionization of sample components was performed in electron impact mode (electron energy 70 eV).

Identification and Quantitative Determination of Components

Component identification was accomplished by comparing their retention indices with those of authentic compounds, as well as by comparing their mass spectra with spectra in the Wiley 6.0 and NIST98 (National Institute of Standards and Technology, Gaithersburg) MS database or reported in the literature.²⁴ The percentage composition of the samples

was computed from the GC peak areas without using correction factors. For the calculation of the mass fractions of the aglycones (mg kg⁻¹ respecting the masses of starting fresh or air-dried plant material) menthol was used as internal standard. Preliminary GC-MS analysis showed the absence of menthol in aglycone samples. The content of aglycones was calculated from GC peak areas related to the GC peak area of menthol. Quantitative results are means of the data derived from duplicate GC analyses.

RESULTS AND DISCUSSION

Essential Oil

To calculate the percentage yields of the essential oil obtained by hydrodistillation of fresh and dried plant material the mass (g) of the produced oil was divided by the initial mass (g) of the plant material. The results varied depending on the plant development stage from 0.28 %–0.60 % for fresh and 0.60 %–1.31% for dried plant material. The plants harvested in May and at the beginning of July produced similar yields of essential oil, 0.46 % and 0.60 % for fresh and 1.31 % and 1.21 % for dried plants. The yield of the essential oil obtained from autumnal plants was about half of the quantity obtained from the plants gathered in spring and summer.

The variations of the yield and chemical composition of the essential oils isolated from fresh and dried *Thymus pulegioides* at three different stages of plant development are shown in Table I. The number of identified compounds in investigated oils was different regarding the stage of plant development and whether fresh or dried plant material was used. For fresh plant material of *Thymus pulegioides* that number decreased from 24 for springtime plants to 18 for autumnal plants, representing 94.8 % and 97.2 % of the total oil, respectively. Twenty-one compounds were identified in essential oil from dried springtime plants and 19 compounds in essential oils from dried summer and autumnal plants, which represented 97.0 %, 98.4 % and 97.5 % of the total oil, respectively. The most abundant constituents in these oils were geraniol and linalool followed by thymol, γ -terpinene, thymol methyl ether, borneol and geranyl acetate, all of which showed variations during the plant development stages and whether fresh or dried plants were used for essential oil production. The quantitative variations of the main oil constituents for fresh plant material were: geraniol (22.5 %–25.5 %), linalool (8.0 %–31.9 %), thymol (1.7 %–20.9 %), γ -terpinene (3.1 %–9.6 %), thymol methyl ether (4.1 %–8.5 %), borneol (2.2 %–8.3 %) and geranyl acetate (4.2 %–6.1 %). Other compounds of a certain importance were β -bisabolene (2.0 %–4.2 %), β -caryophyllene (1.6 %–3.8 %) and *p*-cymene (1.3 %–4.2 %). Geraniol (18.4 %–31.9 %) and linalool (11.3 %–37.4 %) were also quantitatively the most important constituents in the essential oils obtained from the dried plant mate-

Table I. Yield and percentage composition of *Thymus pulegioides* L. essential oil at three stages of plant development

No.	Compound ^(a)	RI ^(b) HP-20M/HP-101	Percentage composition					
			May		July		September	
			A	B	A	B	A	B
1.	α -Thujene	1031/935	0.2	–	–	–	–	–
2.	α -Pinene	1038/937	0.3	–	–	–	1.2	0.7
3.	Camphene	1062/951	–	–	0.6	0.3	2.4	1.1
4.	β -Pinene	1097/976	0.7	t	t	t	t	t
5.	Myrcene	1151/994	1.6	1.0	1.1	1.2	–	–
6.	γ -Terpinene	1165/–	2.2	1.6	–	–	–	–
7.	Limonene	1198/1023	–	–	0.5	t	–	–
8.	γ -Terpinene	1231/1063	9.6	7.4	3.2	3.1	3.1	3.1
9.	<i>p</i> -Cymene	1252/1026	4.2	2.6	1.3	1.6	1.8	2.0
10.	3-Octanol	1361/–	0.3	0.4	0.5	t	t	0.6
11.	1-Octen-3-ol	1414/979	1.4	1.5	1.8	1.8	0.9	0.7
12.	Camphor	1475/1122	0.6	0.6	–	–	–	–
13.	Linalool	1514/1112	8.0	11.3	31.9	36.7	30.3	37.4
14.	Bornyl acetate	1550/1266	–	–	0.6	0.6	1.0	0.9
15.	Thymol methyl ether	1562/1232	4.1	4.0	5.4	6.0	8.5	9.3
16.	Carvacrol methyl ether	1576/1242	–	–	2.0	2.3	1.2	1.2
17.	β -Caryophyllene	1578/1418	3.8	4.2	3.2	2.8	1.6	2.0
18.	α -Humulene	1634/1450	0.8	1.0	0.9	0.6	–	–
19.	Neral	1640/–	–	–	–	–	0.6	0.5
20.	Borneol	1652/1175	2.2	2.8	6.0	5.5	8.3	8.9
21.	β -Cubebene ^(c)	1672/1476	0.8	0.7	0.7	0.7	–	–
22.	Geranial	1680/1263	0.5	0.5	0.6	0.4	0.6	0.5
23.	β -Bisabolene	1694/1508	2.7	2.7	4.2	4.0	2.0	2.5
24.	Geranyl acetate	1718/1385	4.2	5.4	4.4	3.2	6.1	5.2
25.	δ -Cadinene	1725/1517	0.2	0.2	–	–	–	–
26.	Nerol	1750/1247	0.7	1.1	0.9	0.7	0.4	0.5
27.	Geraniol	1802/1286	22.5	31.9	25.0	24.4	25.5	18.4
28.	Thymol	2123/1374	20.9	14.8	3.6	2.5	1.7	2.0
29.	Carvacrol	2147/1381	2.0	1.3	0.2	–	–	–
Identified components / %			94.8	97.0	98.6	98.4	97.2	97.5
Oil yield / %			0.46	1.31	0.60	1.21	0.28	0.60

^(a) The compounds are ordered according to elution from HP-20M column.

^(b) RI, retention indices relative to C₈–C₂₂ alkanes on polar HP-20M and apolar HP-101 column.

^(c) Tentatively identification based on the mass spectra only.

A = fresh plant material; B = dried plant material; t = traces (< 0.1%); – = not detected

rial. The quantitative variations of other important constituents were: thymol (2.0 %–14.8 %), thymol methyl ether (4.0 %–9.3 %), borneol (2.8 %–8.9 %), γ -terpinene

(3.1 %–7.4 %), geranyl acetate (3.2 %–5.4 %), β -bisabolene (2.5 %–4.0 %), β -caryophyllene (2.0 %–4.2 %) and *p*-cymene (1.6 %–2.6 %).

Monoterpene alcohols geraniol or linalool are the main constituents of these oils regarding the stage of plant development. The content of geraniol was approximately constant during all investigated stages of development. On contrary, the content of linalool increased after the period of flowering, and then remained almost constant. It is interesting that the spring oil contains much more geraniol than linalool, while the situation is opposite for the summer and autumnal oils; these oils contain more linalool than geraniol. Monoterpene phenol thymol was the second most abundant compound in essential oil produced from plants harvested during the flowering period (May), whose content decreased significantly with the stage of plant development. On the other hand, the content of its methyl ether increased slightly with plant maturation. The main compound among the oil monoterpene hydrocarbons was γ -terpinene, whose content was the highest in spring oil, then decreased and remained the same in summer and autumnal oils. As regards monoterpene alcohol borneol, an increase is observed from flowering period till the autumn. Only one ester was identified in all investigated essential oils, geranyl acetate. The content of geranyl acetate differed only a little according to the stage of plant development.

Compared to the results reported by Stahl-Biskup *et al.*¹⁵ who identified 36 compounds in the essential oil of lemon-scented thyme (*Thymus \times citriodorus* Schreb.) and 18 glycosidically bound volatile compounds in the same plant, the number of constituents identified in the essential oil of *Thymus pulegioides* is rather low. However, our results are similar to those reported by Kuštrak *et al.*⁷ who identified 23 constituents in the oils of the same plant originating from continental region of Croatia. The number of compounds identified in the present study is also consistent with the number of 17 compounds identified in our previous investigation of *Thymus pulegioides* essential oil originating from submediterranean part of Croatia.⁸

The results concerning the main constituents of Croatian *Thymus pulegioides* essential oil were also reported earlier by Mastelić *et al.*⁸ and Kuštrak *et al.*⁷ While Mastelić *et al.* obtained similar results with geraniol and linalool as the main constituents of the oil, Kuštrak *et al.* identified carvacrol and/or thymol as the main constituents and reported absolute absence of geraniol. It is important to note that the plant material of *Thymus pulegioides* used for the essential oil production in these two papers originated from two very different regions of Croatia, the submediterranean⁸ and continental⁷ region. It is well known that environmental conditions, stage of plant development and, especially in the case of genus *Thymus*, chemotype of plant effects essential oil composition and content of oil constituents.

With regard to the chemical composition of investigated essential oils, the general similarity is obvious. On

the other hand, quantitative variations were observed according to the different stages of plant development.

Glycosidically Bound Volatiles

The mass fractions (mg kg^{-1}) of liberated volatile aglycones (after enzymatic hydrolysis) were calculated by using menthol as internal standard. The content of aglycones obtained from fresh plant material varied from 61.9 mg kg^{-1} to 76.9 mg kg^{-1} according to the stage of plant development. The content of these compounds in fresh *Thymus pulegioides* was the smallest during the flowering period of plant, and then slightly increased till the end of September. The highest content of aglycones was obtained from the plants gathered at the beginning of July (summer), and the smallest from the autumnal plants.

The results of the GC-MS analyses of volatile aglycones are shown in Table II. Seventeen compounds were identified in aglycone fraction isolated from fresh and dried spring plant material and 16 from fresh and dried summer plants. The smallest number of aglycones was isolated from fresh and dried autumnal plant material, 12 and 13, respectively. The chemical composition of glycosidically bound volatiles was shown to be more or less uniform. The main aglycones in all samples were the same: eugenol, geraniol and 1-octen-3-ol. On contrary, quantitative variations were observed when the aglycone samples were analysed at three different stages of development. The variations of the main aglycone contents from fresh plant material were: eugenol (13.4 mg kg^{-1} –46.4 mg kg^{-1}), 1-octen-3-ol (7.3 mg kg^{-1} –17.5 mg kg^{-1}) and geraniol (7.0 mg kg^{-1} –12.3 mg kg^{-1}). The mass fraction variations of other quantitatively important aglycone components were: thymoquinone (1.4 mg kg^{-1} –5.3 mg kg^{-1}), 2-phenylethanol (1.2 mg kg^{-1} –3.2 mg kg^{-1}), 3-hexen-1-ol (traces–3.6 mg kg^{-1}) and 3-octanol (1.8 mg kg^{-1} –3.0 mg kg^{-1}). Benzyl alcohol, linalool and *p*-cymen-8-ol were determined in smaller mass fractions. In the dried plant material mass fractions of the most important aglycones fluctuated as follows: eugenol (37.8 mg kg^{-1} –102.2 mg kg^{-1}), geraniol (11.8 mg kg^{-1} –47.4 mg kg^{-1}), 1-octen-3-ol (16.0 mg kg^{-1} –40.6 mg kg^{-1}), 3-hexen-1-ol (2.6 mg kg^{-1} –10.8 mg kg^{-1}), thymoquinone (2.2 mg kg^{-1} –9.4 mg kg^{-1}), 2-phenylethanol (3.0 mg kg^{-1} –8.6 mg kg^{-1}), linalool (1.6 mg kg^{-1} –6.1 mg kg^{-1}), 3-octanol (4.2 mg kg^{-1} –5.1 mg kg^{-1}) and benzyl alcohol (2.3 mg kg^{-1} –4.3 mg kg^{-1}).

It is interesting that the three main aglycones belong in different classes of organic compounds; eugenol is phenylpropane derivative, geraniol monoterpene alcohol and 1-octen-3-ol aliphatic alcohol. The dominating compound among aglycones was eugenol, which is considered to be connected with the lignin biosynthesis.²⁵ Eugenol was not present in the essential oils, but occurred in relatively large mass fractions in a glycosidically bound form. Furthermore, the mass fraction of eugenol

Table II. Volatile aglycones obtained by enzymatic hydrolysis of the glycosidic fractions of *Thymus pulegioides* L. at three stages of plant development

No.	Compound ^(a)	RI ^(b) HP-20M/HP-101	Aglycone mass fraction / mg kg ⁻¹					
			May		July		September	
			A	B	A	B	A	B
1.	3-Hexen-1-ol	1346/864	3.2	10.8	3.6	9.9	t	2.6
2.	3-Octanol	1361/–	1.7	5.1	1.8	4.2	3.0	4.7
3.	1-Octen-3-ol	1414/979	13.0	30.6	17.5	40.6	7.3	16.0
4.	6-Methyl-5-hepten-2-ol ^(c)	1423/–	0.6	1.5	t	1.0	–	–
5.	Linalool	1514/1112	0.4	1.6	2.3	6.1	1.0	2.8
6.	Thymoquinone	1688/1239	5.3	9.4	1.4	2.2	4.1	5.1
7.	Methyl salicylate	1716/1194	0.4	1.3	0.4	1.3	–	–
8.	Nerol	1750/1247	0.8	2.4	0.5	1.3	0.8	t
9.	Geraniol	1802/1286	11.7	47.4	12.3	32.6	7.0	11.8
10.	Benzyl alcohol	1811/1099	1.6	4.3	0.8	2.3	1.0	2.5
11.	2-Phenylethanol	1847/1153	3.2	8.6	1.2	3.0	3.1	6.1
12.	p-Cymen-8-ol	2029/1331	1.1	2.4	0.5	1.6	1.3	3.1
13.	Eugenol	2099/1377	13.4	37.8	25.0	74.3	46.4	102.2
14.	Thymol	2123/1374	3.2	6.9	t	t	t	1.3
15.	4-Hydroxy-3-methoxy acetophenone ^(c)	2136/1579	1.9	3.9	0.5	0.9	1.9	2.0
16.	Carvacrol	2147/1381	t	0.5	t	t	–	–
17.	Indole	–/1425	0.4	1.0	–	–	–	–
Total mass fraction / mg kg ⁻¹			61.9	175.5	67.8	181.3	76.9	160.2
Mass of dried material produced from 100 g of fresh plant material /g			35.6		42.5		46.0	

^(a) The compounds are ordered according to elution from HP-20M column.

^(b) RI, retention indices relative to C₈–C₂₂ alkanes on the polar HP-20M and apolar HP-101 column.

^(c) Tentatively identification based on the mass spectra only.

A – fresh plant material; B – dried plant material; t = traces (< 0.3 mg kg⁻¹); – = not detected

constantly increased with the plant development stage. In contrast, the other two aglycones mentioned above, geraniol and 1-octen-3-ol, were detected in corresponding essential oils, one of them being even the main constituent of the oils (geraniol). In the fresh plant material the geraniol content was almost the same in two stages of development (May and July), and then decreased. On contrary, the content of geraniol in the dried plant material constantly decreased with the stage of plant development. The maximum mass fraction of 1-octen-3-ol was found to be at the beginning of July. Monoterpene alcohol linalool, one of the main constituents of the *Thymus pulegioides* essential oils, was identified among the aglycone compounds but in much smaller mass fractions. One of the aglycones, which was absent in investigated essential oils, was thymoquinone. The maximum content of this compound was found to be at full flower-

ing period. Similar observations can be established for 2-phenylethanol and benzyl alcohol. Besides 1-octen-3-ol, other aliphatic alcohols found to be present were 3-hexen-1-ol and 3-octanol.

The compounds present in these aglycone fractions such as aliphatic alcohols (1-octen-3-ol, 3-hexen-1-ol and 3-octanol), phenylpropane derivatives (eugenol, 2-phenylethanol, benzyl alcohol) and terpene compounds (geraniol, linalool, nerol) can be considered as very common among aglycones of many plant families.¹³ As regards various species of the *Lamiaceae* family, eugenol was identified as the main aglycone in nine plants,^{11,16,22} while 1-octen-3-ol was the main compound in aglycone fraction of two *Mentha* species, *Mentha citrata* and *M. aquatica*.^{18,19} We recently identified almost the same aglycones in evergreen candytuft (*Iberis sempervirens* L., *Brassicaceae*) with eugenol and 2-phenylethanol as the

main ones. This is the first investigation of such aglycones in cruciferous plants to date.²⁶

All the aglycones mentioned above, along with thymol and carvacrol, were also reported, by Stahl-Biskup *et al.*,¹³ to be present in three different species of genus *Thymus* (*T. x citriodorus*, *T. praecox* ssp. *arcticus* and *T. vulgaris*). By comparison of the results concerning the chemical and percentage composition of aglycones in two species of genus *Thymus* reported earlier (*Thymus pulegioides* and *T. x citriodorus*) and our results, the similarity can be observed. In our previous study⁸ only four volatile aglycones were detected in *Thymus pulegioides* aglycone fraction with a high content of geraniol (37.6 %) and eugenol (11.2 %), while 1-octen-3-ol amounted to 5.9 % and linalool only to 0.5 %. Geraniol was found to be the main aglycone (45.3 %) in the aglycone fraction of *Thymus x citriodorus* also.¹⁵ Other quantitatively important aglycone components were farnesol isomer (7.3 %), eugenol (6.3 %), 3-octanol (5.9 %) and 1-octen-3-ol (5.5 %). All of these compounds, except farnesol isomer, were found to be quantitatively important components of *T. pulegioides* aglycone fraction.

The comparison of *T. pulegioides* free and glycosidically bound volatile compounds (Table I and II, respectively) shows only a partial similarity between their chemical compositions. Seven compounds were found to be identical; five alcohols (aliphatic or terpene) and two phenols.

Regarding the high geraniol content in both the essential oil and the volatile aglycone fraction, especially at the flowering period, it can be suggested that this *Thymus* species could be interesting as a fragrant material for perfume industry or for food flavouring.

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SAŽETAK**Eterično ulje i glikozidno vezani hlapljivi spojevi samoniklog *Thymus pulegioides* L. iz Hrvatske****Ani Radonić i Josip Mastelić**

Ovaj rad predstavlja nastavak istraživanja slobodnih (eterična ulja) i glikozidno vezanih hlapljivih spojeva iz hrvatskog samoniklog aromatičnog bilja. Eterično ulje i glikozidno vezani hlapljivi spojevi izolirani su iz svježeg i suhog biljnog materijala *Thymus pulegioides* L. u tri stadija razvoja biljke i analizirani vezanim sustavom plinske kromatografije i spektrometrije masa (GC-MS). Najzastupljeniji sastojci eteričnih ulja bili su geraniol i linalol, zatim timol, γ -terpinen, timol-metil-eter, borneol i geranil-acetat. Zabilježene su kvantitativne promjene svih sastojaka u ovisnosti o stadiju razvoja biljke i biljnom materijalu upotrebljenom za izolaciju eteričnog ulja (svježi ili suhi). GC-MS analiza hlapljivih aglikona, dobivenih enzimskom hidrolizom glikozidnih frakcija, pokazala je da su eugenol, geraniol i 1-okten-3-ol glavni aglikoni u svim uzorcima. Ostali značajni aglikoni bili su timokinon, 2-feniletanol, 3-heksen-1-ol, 3-oktanol, benzilni alkohol i linalol. Analizom aglikona u tri različita stadija razvoja biljke uočene su kvantitativne promjene. Zbog visokog udjela geraniola u eteričnim uljima i aglikonskim frakcijama ovaj *Thymus pulegioides* L. mogao bi biti interesantan kao mirisna komponenta u industriji parfema ili kao aroma u prehrambenoj industriji.