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FREE AND GLYCOSIDICALLY BOUND VOLATILES OF *Mentha longifolia* GROWING IN CROATIA

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The glycosides of volatile compounds and the essential oil were isolated from wild Mentha longifolia. *After the enzymatic hydrolysis of glycosides, fourteen volatile aglycones were identified by GC/MS. The main aglycones were eugenol, 2-phenylethanol, benzyl alcohol, lavandulol, trans- and cis-carveol, 3-octanol, and 3-hexen-1-ol. The content of aglycones was 40.85 mg kg^{-1} of dried plant material. The main components of the essential oil (yield 0.93 w/w) were carvone, piperitenone oxide, limonene, and* β*-caryophyllene. Eugenol, carveol, 3-octanol, and* α*-terpineol were identified in the aglycones and in the essential oil.*

Key words: *Mentha longifolia* L. Hudson, essential oil, glycosidically bound volatiles, GC/MS analysis.

Essential oil and glycosides were isolated from dried plant material (leaves, flowers, and apical stalks) *Mentha longifolia* L. Hudson by simultaneous water distillation-water extraction. The yield of the essential oil obtained by hydrodistillation was 0.93% w/w. The content and chemical composition of the essential oil is given in Table 1. Twenty-two compounds were identified representing 93.95% of the total oil. In Table 1 the components are listed in the order of their elution on an HP-20M column. The oil was characterized by a high content of oxygenated monoterpenes, approximately 69.0%. The main oxygenated components were carvone (33.48%; s = 0.99) and piperitenone oxide (28.95%; s = 1.57). The rest of the oil, approximately 31.0%, was composed of monoterpene and sesquiterpene hydrocarbons with limonene (10.29%; s = 0.78) and β-caryophyllene (5.84%; s = 0.01) as the major components. Limonene is a key component of Mentha essential oils since it was shown that limonene is the first cycle precursor in the biosynthesis of all oxygenated *p*-menthane C-2 and C-3-substituted compounds [1, 2]. The essential oil also contained smaller quantities of myrcene, borneol, and α-pinene. This chemotype of *M. longifolia* is rich in carvone and piperitenone oxide. Carvone and carvone drugs (caraway and dill seeds and their essential oils) are used as carminative in folk medicine. Piperitenone oxide is a relaxant of intestinal smooth muscle whose activity may be mediated at least in part by an intracellular effect [3].

M. longifolia is a very variable plant, both morphologically and chemically. It grows wild in many parts of the world. The majority of the chemotypes and subspecies of *M. longifolia* contain piperitenone oxide, piperitone oxide, carvone, menthone, and 1,8-cineol as the major components of their essential oils. Maffei identified the chemotype of *M. longifolia* from Piedemont Valley (Italy) with piperitenone oxide (77.4%) in its essential oil [4]. Similar chemotypes were identified in Jordan and Lithuania [5, 6]. Piperitenone oxide, β-caryophyllene, piperitone oxide, and germacrene D are the major components of *M. longifolia* from Iran [7]. Z. Fleisher and A. Fleisher identified several new chemotypes of *M. longifolia* growing in Israel [8, 9]. Some of these are with or without small amounts of piperitenone oxide and piperitone oxide. Their main components are 1,8-cineol and menthone. *trans*-Piperitone oxide is the main component of the essential oil of *M. longifolia ssp. petiolata* which grows on the island of Crete [10]. On the other hand, the same authors identified a chemotype with carvone as the main component of the essential oil [11].

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TABLE 1. Composition of the Essential Oil of *Mentha longifolia* L.

Compound	X peak area, %	σ	Methods of identification
α-Pinene	1.10	0.02	I_1, I_2, MS
β-Pinene	0.80	0.16	I_1, I_2, MS
Sabinene	0.57	0.12	I_1, I_2, MS
Myrcene	3.21	0.55	I_1, I_2, MS
Limonene	10.29	0.78	I_1, I_2, MS
δ-3-Carene	0.56	0.01	I_1, I_2, MS
3-Octanol	0.97	0.11	I_1, -, MS
α-Bourbonene	0.51	0.02	I_1, I_2, MS
Linalool	0.19	0.01	I_1, -, MS
Bornyl acetate	0.50	0.02	I_1, -, MS
β-Caryophyllene	5.84	0.01	I_1, I_2, MS
α-Humulene	0.40	0.10	-, I_2, MS
α-Terpineol	0.28	0.02	I_1, -, MS
Borneol	2.74	0.98	I_1, I_2, MS
Dihydrocarvone*	1.30	0.03	-, I_2, MS
Carvone	33.48	0.99	I_1, I_2, MS
δ-Cadinene	0.70	0.03	I_1, -, MS
cis-Carveol	0.34	0.02	I_1, -, MS
cis-Jasmone	0.96	0.01	-, I_2, MS
Piperitenone oxide	28.95	1.57	I_1, I_2, MS
Eugenol	0.22	0.04	I_1, -, MS
Carvacrol	1.00	0.01	I_1, -, MS
Total	93.95		

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I_1 - retention indices on HP-20M; I_2 - retention indices on HP-101; MS-mass spectra; -not detected; X - mean value (average values of percentages obtained for three samples on two different columns); σ - standard deviation for three samples on two columns; *Correct isomer not identified.

Carvone chemotype is also identified in Iran [12]. The essential oils of many subspecies and chemotypes of *M. longifolia* have been studied, but glycosidically bound volatiles have not been sufficiently investigated [13]. Volatile aglycones were detected in many aromatic and nonaromatic plants [14]. Aliphatic alcohols, terpenes, coumarin, and phenylpropane compounds were identified as volatile aglycones in plants of the *Lamiaceae* family [15-17]. The aim of this study was to determine the chemical composition of volatile aglycones from *M. longifolia* and to compare it with the essential oil composition.

The fraction of glycosides is isolated from the water extract (after hydrodistillation) and purified by flash chromatography. The obtained glycosides were hydrolyzed and the liberated aglycones were analyzed by GC/MS. The content of glycosidically bound volatile compounds in dried plant material was 40.85 mg kg^{-1}. Fourteen aglycones were identified representing 93.39% of the total aglycone fraction. Aliphatic alcohols, derivatives of phenylpropanes, and monoterpene alcohols were identified. The results are shown in Table 2. The main aglycones were eugenol (49.99%; s = 0.32), 2-phenylethanol (11.39%; s = 0.26), benzyl alcohol (6.45%; s = 0.71), lavandulol (4.85%; s = 0.13), *trans*- + *cis*-carveol (2.78%; s = 0.14 + 1.40%; s = 0.10), 3-octanol (3.93%; s = 0.62), and 3-hexen-1-ol (3.77 %; s = 0.03). Merks and Baerheim Svendsen [13] identified eugenol and 2- methoxy-4-vinylphenol as two aglycones of *M. longifolia*. We have not identified 2-methoxy-4-vinylphenol in the aglycone fraction. Comparing the chemical composition of the essential oil (Table 1) and aglycones (Table 2), four compounds were established to be identical: eugenol, *cis*-carveol, 3-octanol, and α-terpineol. Our results show moderate correlation in the chemical composition of free and glycosidically bound volatiles of this plant. The aglycones, such as aliphatic alcohols, 2-phenylethanol, benzyl alcohol, eugenol, linalool, geraniol, nerol, α-terpineol, and terpinen-4-o1 can, more or less,

TABLE 2. Composition of Volatile Aglycones of *Mentha longifolia* L.

Compound	X peak area, %	σ	Methods of identification
1-Hexanol	1.33	0.01	I_1, -, MS
3-Hexen-1-ol	3.77	0.03	I_1, I_2, MS
3-Octanol	3.93	0.62	I_1, I_2, MS
1-Octen-3-ol	1.79	0.02	I_1, I_2, MS
Lavandulol	4.85	0.13	I_1, I_2, MS
α-Terpineol	0.75	0.18	I_1, -, MS
Methyl salicylate	0.89	0.20	I_1, I_2, MS
Nerol	2.10	0.01	I_1, I_2, MS
trans-Carveol	2.78	0.14	I_1, I_2, MS
Geraniol	1.97	0.11	I_1, I_2, MS
cis-Carveol	1.40	0.10	-, I_2, MS
Benzyl alcohol	6.45	0.71	I_1, I_2, MS
2-Phenylethanol	11.39	0.26	I_1, I_2, MS
Eugenol	49.99	0.32	I_1, I_2, MS
Total	93.39		

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I_1- retention indices on HP-20M; I_2 - retention indices on HP-101, MS - mass spectra, X - mean value (average values of percentages obtained for three samples on two Different columns); σ - standard deviation for three samples on two different columns.

be considered as ubiquitous in aglycone fractions of *Lamiaceae* family. Eugenol and other *p*-hydroxyphenylpropanes which have been identified in many plants as the main aglycones can be connected with lignin biosynthesis via the peroxidase-hydrogen peroxide system according to Siegel [18]. The aliphatic volatiles (alcohols, carbonyls, esters) can originate from fatty acid catabolism, and aromatic volatiles (alcohols, acids, carbonyls) of cinnamic acid catabolism [19].

EXPERIMENTAL

Gas Chromatography-Mass Spectrometry. The essential oil and volatile aglycones were analyzed by gas chromatography-mass spectrometry (Hewlett-Packard, model 5890, with a mass selective detector, model 5971A). Two columns with different polarity of stationary phases were used. GC operating conditions were: column HP-20M, 50 m × 0.2 mm i.d., film thickness 0.2 mm, column temperature programmed from 70°C isothermal for 4 min, then increased to 180°C at a rate of 4°C min^{-1}; column HP-101, 25 m × 0.2 mm i.d., film thickness 0.2 mm, column temperature programmed from 70°C isothermal for 2 min, then increased to 200°C at a rate of 3°C min^{-1}; carrier gas helium, flow rate 1 mL min^{-1}, injector temperature 250°C, volume injected 1 mL, split ratio 1:50. MS conditions: ionization voltage 70 eV, ion source temperature 280°C, mass range 30-300 mass units.

Identification and Quantization. Individual peaks were identified by comparison of their retention indices with those of authentic samples, as well as by comparison of their mass spectra with those stored in the database (Wiley library) and library data [20]. The percentage composition of the samples was computed from the GC peak areas without using correction factors. The contents of aglycones were calculated from the GC-peak areas related to the GC-peak area of 1-octanol (liberated from octyl-*β-D*-glucoside). Preliminary GC-MS analysis showed the absence of 1-octanol as potential aglycone.

Plant Material. Aerial parts of fully flowered *M. longifolia* were collected in the region of central Dalmatia, August, 2001, near the river bed of Ruda. Dried plant material (leaves, flowers, and apical stalks) was used for investigation.

Treatment of Plant Material. Plant material, 100 g, and 500 mL of water were submitted to simultaneous extraction and hydrodistillation in a Clevenger type apparatus for 3 h. Octyl-*β-D*-glucoside, 500 mg, was added to water intended for hydrodistillation as internal standard. The essential oil was separated, dried over Na_2S0_4, and stored. The aqueous extract was separated and the residual plant material was extracted once more with 300 mL of boiling water. The pooled aqueous extracts were concentrated to 30 mL in a rotating evaporator. Ballast components were removed by precipitation with ethanol as well

as by precipitation with conc. ammonia in ethanol (for removing acidic ballast compounds). Finally, the purification was performed by flash chromatography on a silica gel column applying ethyl acetate: ethanol: ammonia 6:3:1 v/v/v.

The isolated glycosidic fraction was concentrated to dryness, and dissolved in 20 mL of citrate buffer (pH 5.5), and the remaining free terpenes and hydrophobic compounds were removed by extraction with penthane-dicloromethane. Prior to the enzymatic hydrolysis, the absence of volatile compounds was tested by TLC and GC/MS as described in our previous papers [15-17].

Enzymatic Hydrolysis. 13-Glucosidase from almonds (Fluka, 20 mg) was added to the glycosidic solution along with 5 mL pentane for the trapping of liberated aglycones. The hydrolysis was carried out for 72 h, at 30°C with the mixture being shaken occasionally. After the hydrolysis, the pentane layer was separated. The remaining aglycones were extracted from the aqueous layer with pentane (10×3 mL). The combined pentane extracts were dried over anhydrous sodium sulfate, concentrated to a final volume of 0.5 mL, and 0.5 mL was used for GC/MS analysis.

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