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Volatile Constituents of Different Plant Parts and Populations of *Malabaila aurea* Boiss. from Montenegro

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Abstract: The volatile constituents of different plant parts and populations of *Malabaila aurea* Boiss. from Montenegro were obtained by simultaneous distillation-extraction and analyzed by GC-FID and GC-MS. A total of 12 samples were examined and 45 compounds were identified. The volatile content of different *M. aurea* populations was very similar, while the volatile fractions obtained from different plant parts showed significant qualitative and quantitative differences. The most abundant compounds found in stems & leaves were apiole (51.0-56.3%), myristicin (16.3-25.4%), and falcarinol (4.1-10.7%). The roots showed the same major components, but with different relative abundances: 30.9-49.1% of apiole, 12.9-34.7% of falcarinol, and 9.9-31.1% of myristicin. The volatile constituents of fruits & flowers were remarkably different, containing up to 71.2-80.5% octyl butyrate, 11.4-18.0% octanol, and 2.7-6.8% octyl hexanoate. The results were discussed as possible indication of relatedness of *Malabaila aurea* and *Pastinaca sativa* (parsnip).

Keywords: *Malabaila aurea*; volatile constituents; octyl butyrate; apiole; myristicin; falcarinol.
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1. Introduction

Malabaila Hoffm. is a small genus of Apiaceae (Umbelliferae) family distributed from East Mediterranean to Central Asia and Iran. In European Flora genus *Malabaila* is represented by three species: *M. aurea* (Sibth. & Sm.) Boiss., *M. involucrata* Boiss. & Sprunger, and *M. graveolens* (Sprengel) Hoffm. [1]. Distribution area of *M. aurea* is restricted to Balkan Peninsula: Albania, Bulgaria, Greece, and ex Yugoslavia [1]. In ex Yugoslav republics it is reported for Montenegro [2], Macedonia [3], and Croatia [4]. In Montenegro *M. aurea* is distributed only in Mediterranean and Submediterranean part of the country, and it inhabits thermophilic and sunny habitats. This pubescens

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and somewhat viscid biennial reaches height up to 50 cm. Considering chromosome number, two different cases have been reported: $2n=22$ [5,6], and $2n=20$ [7,8].

According to Pimenov and Ostroumova [9], in genotype, as well as in all essential taxonomic characters including carpological, all species of *Malabaila* genus are closely related to *Pastinaca* L. species. Although *M. aurea* is slightly aromatic plant, a literature search did not reveal any reference to its local use in traditional medicine or culinary. Only the essential oil composition of the aerial parts (Greece collection) has previously been reported: 48 compounds were identified representing 90.9% of the total oil; the major constituents were octyl isobutyrate (40%), spathulenol (12.4%), and octanol (8.9%) [10]. The aim of this study was a complete analysis of the volatile constituents of different *M. aurea* populations from Montenegro, the comparison between the volatiles obtained from different plant parts, and the evaluation of their similarity with other *Malabaila* species, as well as related *Pastinaca* species.

2. Materials and Methods

2.1. Plant Material

Herbal material of *Malabaila aurea* Boiss. was collected in late spring 2010, at four different localities in Montenegro: *i* = hill Gorica in Podgorica (UTM CM 59, N 42° 26' 57", E 19° 16' 02"), *ii* = Vranjske njive near Podgorica (UTM CN 50, N 42° 28' 21", E 19° 15' 06"), *iii* = Bioče (UTM CN 60, N 42° 30' 57", E 19° 21' 04"), *iv* = Bušat (UTM CM 44, N 42° 00' 23", E 19° 08' 58") (Figure 1). The plant was identified by one of the authors (D. Stešević), and voucher specimens are deposited at herbarium collection of Faculty of Sciences (TGU), University of Montenegro (voucher codes 253790-253793).

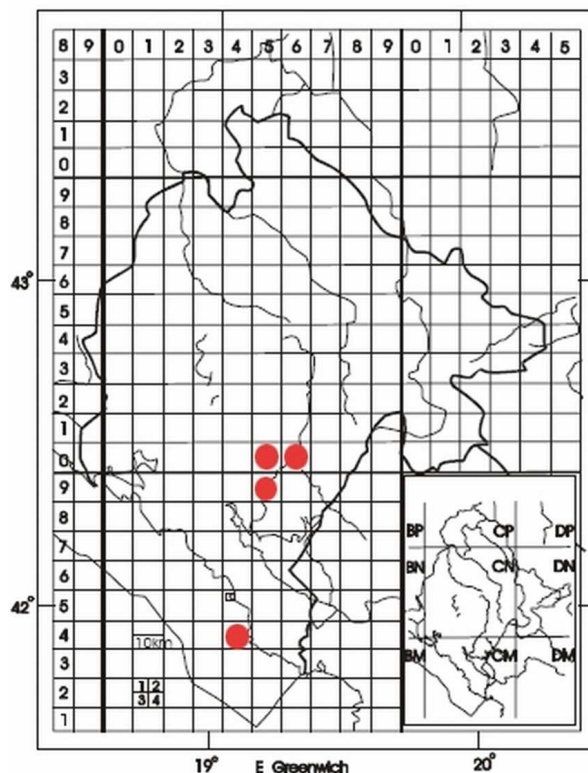


Figure 1. Sampling sites of *Malabaila aurea* in Montenegro: hill Gorica in Podgorica (UTM CM 59); Vranjske njive near Podgorica (UTM CN 50); Bioče (UTM CN 60); Bušat (UTM CM 44).

2.2. Likens-Nickerson distillation-extraction

Herbal material was air-dried in shade. Thereafter, the organs were separated (roots, stems & leaves, and fruits & flowers) and cut in pieces. Twelve samples (4 populations \times 3 plant parts) were subjected to simultaneous distillation-extraction on a *Likens-Nickerson* type glass apparatus. Approximately 100 g of each sample was soaked in 250 mL of water and distilled for 2 h, while methylene chloride (10 mL) was used as trap solvent. After distillation, the methylene chloride layer was separated and dried over Na_2SO_4 . An aliquot of the extract (1 mL) was then transferred into the vial for qualitative and quantitative analysis using GC-MS and GC-FID.

2.3. Gas chromatography-mass spectrometry

GC and GC-MS analyses were performed on an Agilent 7890A GC system equipped with a 5975C inert XL EI/CI MSD and a FID detector connected by capillary flow technology through a two-way splitter with make-up gas. An HP-5 MSI capillary column (Agilent Technologies, 25 mm i.d., 30 m length, 0.25 μm film thickness) was used. The injector temperature was 250°C and the injection volume was 1 μL . Each sample was injected for several times using different injection modes (in the range from splitless to 1:250 split ratio), in order to get the optimal intensities of the peaks in GC chromatogram. The carrier gas (He) flow rate was 1.1 mL/min, whereas the column temperature was programmed linearly in the range of 60–315°C at the rate of 3°C/min, with final 15-min hold. EI mass spectra (70 eV) were acquired in m/z range of 35–550, and the ion source temperature was 230°C. A library search and mass spectral deconvolution and extraction were performed by using NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software, version 2.64. We used retention index (RI) calibration data analysis based on the retention times of n-alkanes, which were injected after the sample under the same chromatographic conditions. The search was performed against NIST05 and Willey07 libraries and confirmed by retention time lock (RTL) method and RTL Adams database [11]. Percentages (relative) of the identified compounds were computed from the corresponding GC-FID peak areas.

2.4. NMR analysis

The volatile constituents of the selected sample (stems & leaves of *M. aurea* from sampling site *i* = Gorica) were analysed by NMR spectroscopy. An aliquot (5 mL) of the methylene chloride extract obtained by Likens-Nickerson distillation-extraction was evaporated to dryness under mild nitrogen stream. The residue was dissolved in 0.5 mL of CDCl_3 and transferred into NMR tube. ^1H NMR spectrum was recorded on Varian Gemini 2000 spectrometer at 200 MHz, with TMS as internal standard.

3. Results and Discussion

Twelve samples of volatile constituents were obtained by simultaneous distillation-extraction from three separated plant parts (stems & leaves, roots, and fruits & flowers) of four different populations of *Malabaila aurea*. Qualitative and quantitative analysis of the samples was performed by GC-MS and GC-FID techniques. The results are presented in Table 1. as relative percentages of components in GC-FID chromatograms (Supplementary Figures S1-S12).

A total of 45 compounds were identified: in each sample between 20 and 31 compounds, representing more than 97% of the volatile fraction. Regarding the volatile content of *M. aurea*, two main features can be observed: *i*) the volatiles from different populations were similar with some variations in quantities of major compounds; and *ii*) the volatile fractions from different parts of the plant showed significant qualitative and quantitative differences. Phenylpropanoids were the dominant class of compounds in the volatile fraction of stems & leaves and the roots, while major fruits & flowers volatiles were aliphatic esters.

Table 1. Volatile constituents of different plant parts and populations of *Malabaila aurea* from Montenegro

Compound	RI ^a	Roots				Stems&Leaves				Fruits&Flowers			
		<i>i</i> ^b	<i>ii</i>	<i>iii</i>	<i>iv</i>	<i>i</i>	<i>ii</i>	<i>iii</i>	<i>iv</i>	<i>i</i>	<i>ii</i>	<i>iii</i>	<i>iv</i>
1 Hexanal	804	0.7	0.6	0.1	0.9	0.4	0.2	0.1	0.3	t	t	t	t
2 (<i>E</i>)-2-Hexenal	840	-	-	-	-	0.2	0.2	-	0.2	-	-	-	-
3 Hexanol	850	-	-	-	-	-	-	-	-	0.1	0.1	t	t
4 Heptanal	884	0.2	0.3	0.2	0.4	0.1	-	-	t	t	t	t	-
5 α -Pinene	915	t	-	-	-	0.1	-	-	-	-	-	-	-
6 (<i>E</i>)-2-Heptenal	943	-	-	-	-	0.1	-	-	0.1	-	-	-	-
7 β -Pinene	968	0.5	1.3	0.3	0.9	0.1	-	t	0.1	-	-	-	-
8 2-Pentylfuran	987	0.2	0.3	t	0.2	0.1	t	t	t	-	-	-	-
9 Octanal	1003	0.7	0.8	0.6	1.4	0.4	0.2	t	0.3	0.8	0.5	0.4	0.6
10 2-Phenylacetaldehyde	1039	-	-	-	-	0.3	0.2	-	0.2	t	t	t	-
11 Octanol	1065	1.8	1.2	0.4	0.6	1.8	0.6	-	0.7	16.7	18.0	12.9	11.4
12 Nonanal	1096	t	0.1	t	0.2	0.1	t	t	0.1	t	t	-	-
13 Octanoic acid	1182	0.2	0.3	t	0.4	-	-	-	-	t	t	t	0.1
14 Hexyl butyrate	1197	-	-	-	-	-	-	-	-	1.1	1.1	0.7	0.7
15 Decanal	1209	-	-	-	-	-	-	-	-	0.2	0.2	0.2	0.1
16 Octyl acetate	1214	-	-	-	-	-	-	-	-	0.1	t	t	0.1
17 Decanol	1279	-	-	-	-	-	-	-	-	t	t	t	t
18 δ -Elemene	1335	-	-	-	-	0.3	0.3	t	0.3	-	-	-	-
19 α -Copaene	1373	0.2	0.3	0.3	0.3	0.6	0.9	0.4	0.6	t	-	t	-
20 Octyl butyrate	1389	0.5	0.4	0.4	0.2	1.0	1.1	0.4	t	71.2	74.3	78.0	80.5
21 (<i>E</i>)-Caryophyllene	1417	-	-	-	-	0.2	0.3	0.2	0.3	t	t	t	t
22 β -Copaene	1426	-	-	-	-	-	0.2	0.1	-	-	-	-	-
23 α -Humulene	1451	-	-	-	-	0.8	0.6	0.3	0.5	0.2	0.2	0.3	0.2

24	β -Farnesene	1456	0.2	t	0.2	0.2	0.3	0.2	0.5	0.6	-	-	-	-
25	γ -Decalactone	1470	0.6	t	t	0.6	-	-	-	-	-	-	-	-
26	Bicyclogermacrene	1496	0.2	0.3	t	0.2	2.4	2.5	3.2	1.7	t	-	t	-
27	β -Bisabolene	1508	3.2	4.9	4.1	2.8	4.4	6.6	4.4	3.8	t	0.1	t	t
28	Myristicin	1524	18.5	31.1	23.8	9.9	25.4	16.3	18.1	21.7	0.3	0.3	0.2	0.1
29	Dihydroactinidiolide	1542	t	-	-	-	0.1	0.1	-	0.1	-	-	-	-
30	Elemicin	1557	0.5	0.4	0.7	t	0.2	0.2	0.2	t	-	-	-	-
31	Spathulenol	1576	1.3	1.3	1.7	2.0	2.4	1.8	2.0	2.5	t	t	t	t
32	Octyl hexanoate	1583	-	-	-	-	-	-	-	-	6.8	2.7	4.7	3.3
33	Decyl butanoate	1587	-	-	-	-	-	-	-	-	0.4	0.4	0.5	0.5
34	Humulene epoxide	1607	0.4	0.3	0.3	0.4	0.5	0.5	0.5	0.7	t	t	t	t
35	Apiole	1689	30.9	41.3	49.1	48.1	51.0	51.0	56.3	54.4	0.8	1.1	0.6	0.6
36	Tetradecanoic acid	1765	-	-	0.2	-	0.1	t	t	0.2	-	-	-	-
37	Angelicin	1778	0.7	0.4	0.5	t	-	-	-	-	-	-	-	-
38	Octyl octanoate	1782	-	-	-	-	-	-	-	-	0.3	0.1	0.9	1.2
39	Hexadecanoic acid	1963	1.5	0.9	1.1	1.4	0.4	0.6	-	0.3	t	-	t	-
40	Falcarinol	2037	34.7	12.9	15.4	25.5	4.1	10.4	10.7	7.9	-	-	-	-
41	Phytol	2116	-	-	-	-	-	1.2	-	-	-	-	-	-
42	Linoleic acid	2142	0.2	0.2	0.1	0.1	-	-	-	-	-	-	-	-
43	Pentacosane	2487	t	t	t	t	0.2	0.2	0.2	0.2	t	-	-	-
44	Heptacosane	2684	t	t	t	0.2	0.3	0.5	0.4	0.3	-	-	-	-
45	Nonacosane	2882	t	t	t	0.1	0.3	0.5	0.5	0.2	-	-	-	-
Total			98.6	99.1	98.5	99.3	98.0	98.5	98.6	99.4	98.2	99.1	97.4	98.9

^aRI - Retention index (on HP-5MS column)

^b*i-iv* - Different populations of *Malabaila aurea* (*i* = Gorica, *ii* = Vranjske njive, *iii* = Bioče, *iv* = Bušat; see Section 2.1.)

t - traces (less than 0.1%)

The most abundant volatile compounds found in steams & leaves were apiole (51.0-56.3%), myristicin (16.3-25.4%), and fatty alcohol falcarinol (4.1-10.7%). The principal volatile components of the roots were the same, but with different relative abundances: 30.9-49.1% of apiole, 12.9-34.7% of falcarinol, and 9.9-31.1% of myristicin. The steams & leaves comprised more sesquiterpenes (9.9-12.5%) in comparison with roots (5.9-7.4%), though a participation of sesquiterpenes is moderately low in all plant parts. A significant difference between the analyzed samples and the one previously investigated from Greece [10] was in the content of sesquiterpene spathulenol. Although this guaiene derivative was one of the most abundant constituent of Greece's essential oil (12.4%), in our samples it is represented with 1.3-2.0% in the roots and 1.8-2.5% in stems & leaves.

The volatile fraction from fruits & flowers of *M. aurea* was obtained in higher yields (estimated from peak intensities in GC chromatograms), and its content was completely different from the other parts of the plant. The major compounds were octyl esters and octanol, representing more than 95% in each sample. In the previous investigation of the essential oil of the aerial parts of the plant, Tzakou *et al.* [10] reported octyl isobutyrate as major component (40%). Using GC-MS technique, the major component in our samples could be identified as either octyl butyrate or octyl isobutyrate, since these compounds give almost identical EI MS spectra. The identification based on RT indices was also uncertain. To solve the dilemma we recorded NMR spectrum of the volatile fraction from fruits & flowers of representative sample (*i* = Gorica). The ¹H NMR spectrum (Supplementary Figure S13) showed unambiguously that the major component of fruits & flowers was octyl butyrate. The key signal at δ 2.28 ppm (2H, triplet) belongs to CH₂ group of butyric acid residue. The remaining signals of octyl butyrate were in good agreement with literature data [12]. Additional signals in ¹H NMR spectrum originated from octanol, the second most abundant component found in fruits & flowers, and were in agreement with the data from SDBS database [13].

The dominance of the aliphatic esters in the essential oils from the fruits and flowers was previously recorded for *Malabaila* species, but they were mainly hexyl esters. The major components of the oil from *M. suaveolens* fruits were hexyl butyrate (41.4%), *n*-octyl acetate (15.9%), and hexyl-2-methylbutyrate (7.6%) [14], while the oil from *M. secacul* flowers was rich in hexyl-3-methylbutyrate (26.3%), hexyl 2-methylbutyrate (14.9%) and hexyl butyrate (10.6%) [15]. Appreciable biosynthesis of octyl acetate, as well as octyl butyrate, which is the most abundant component in *M. aurea*, was taxonomically restricted to only a few genera of apiaceous plants including *Pastinaca* and *Heracleum* [16,17]. In fact, the most similar to *M. aurea* fruits & flowers volatiles was the essential oil of crushed seeds of *Pastinaca sativa* L. subsp. *urens*, whose major constituents were octyl butyrate and octyl hexanoate (79.5% and 5.3%, respectively) [18].

Pastinaca and *Heracleum* are the principal host plants of the parsnip webworm *Depressaria pastinacella* [19]. In these plants, octyl acetate and octyl butyrate are the only essential oil components found exclusively within tissues consumed by the webworm (reproductive tissues), whereas other essential oil components are distributed throughout the plant [20]. Octyl butyrate is both an olfactory and feeding deterrent to webworms, and its content correlates with co-occurring linear furocoumarins, which are phototoxic [21,22]. Octyl esters in these plants possibly serve as solubilizing agents that enhance penetration of these furanocoumarins into herbivore integuments and gut walls [23]. High similarity of *Pastinaca sativa* and *Malabaila aurea* in the essential oils composition, especially oils of the reproductive tissues, could indicate that both plants have developed similar defense mechanism. However, any connection of *M. aurea* and the webworm *D. pastinacella* is unknown.

Previous phylogenetic analysis of combined *rpoC1* and *rpl16* intron data supported 12 major clades within subfamily Apioideae (family Apiaceae) [24]. Within the "*Angelica*" clade four major groups of taxa were distinguished, however, not all molecular studies supported these groups as distinct. The "*Heracleum*" group comprises the genera *Heracleum*, *Malabaila*, *Pastinaca*, *Tordylium*, and *Zosima*, whose belonging species are characterized generally by fruits with thickened wing margins and a rich diversity of furanocoumarins [24]. The volatile constituents of 23 taxa belonging to the Apioideae subfamily from the Central Balkan were studied in detail [25], though *M. aurea* from Montenegro hasn't been analyzed before. Given data about the composition of the volatiles of *M. aurea*, and particularly a fact about the prevalence of octyl butyrate (confirmed by NMR), could

evidently support relatedness of *Malabaila* and *Pastinaca* species, and contribute to the better understanding of the genus position within subfamily Apiaceae.

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Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/RNP>

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