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Original Article

The chemical composition of the essential oil of *Dracocephalum moldavica* L. from Vojvodina Province (Serbia)

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Abstract:
Dracocephalum moldavica L., also called Moldavian balm or Moldavian dragonhead, is native to temperate climate of Asia, but it was naturalized in Eastern and Central Europe, North Africa, China and north-eastern United States. This is an annual plant, with numerous stems (up to 6), 22-45 cm high, and blue flowers arranged in pseudo-whorls growing in leaf axils. Essential oil accumulates in exogenous oil-containing cells at the dorsal sides of the leaves, and in the inflorescence. Because of this, the entire plant has a citrus-like flavor, resembling that of lemon balm and catnip. This plant is extensively used as a spice and for composition of tea blends, in food aromatization (canned fish, jams, candies, syrups), perfumery, alcohol industry, soaps and detergents. *Dracocephalum moldavica* from Vojvodina Province, Serbia contains geranial (29.6%), geranyl acetate (27.2%) and neral (19.4%) as the most abundant compounds. Further investigations will be focused on the influence of weather conditions on essential oil composition, as well as on bioactive potential of this essential oil.

Key words:
Moldavian balm, Moldavian dragonhead, GC-MS analysis, geranial, geranyl acetate, neral

Apstract:
Hemijski sastav etarskog ulja *Dragocephalum moldavica* L. iz Vojvodine (Srbija)
Biljka *Dracocephalum moldavica* L., poznata je kao Moldavska melisa ili Moldavska zmajeglavka. Poreklom je iz umerenog klimata Azije, ali je naturalizovana u Istočnoj i Centralnoj Evropi, Severnoj Africi, Kini i severo-istočnom delu SAD. Iz korena ove jednogodišnje biljke izbija veći broj stabljika (do 6), koje su 22-45 cm visine, sa plavim cvetovima raspoređenim u lažnim pršljenastim cvastima u pazusima listova. Etarsko ulje se akumulira u egzogenim uljanim ćelijama sa donje strane listova i u cvetovima. Zbog etarskog ulja koje ima citrusnu notu ova biljka podseća na matičnjak i macinu travu. Biljka se intenzivno koristi kao začin i kao dodatak čajnim mešavinama, za aromatizaciju hrane (konzervirana riba, džemovi, slatkiši, sirupi), parfimeriji, alkoholnoj industriji, proizvodnji sapuna i deterdženata. Etarsko ulje *D. moldavica* iz AP Vojvodine, Srbije sadrži geranial (29,6%), geranil-acetat (27,2%) i neral (19,4%) kao najzastupljenije komponente. Dalja istraživanja biće usmerena na uticaj vremenskih uslova na sastav etarskog ulja, kao i na njegov biološki potencijal.

Ključne reči:
Moldavska melisa, Moldavska zmajeglavka, GC-MS analiza, geranial, geranil acetat, neral

Introduction

Dracocephalum moldavica L., also called Moldavian balm or Moldavian dragonhead, is native to temperate climate of Asia, but it was naturalized in Eastern and Central Europe, North Africa, China and north-eastern United States. This species belongs to the subtribe Nepetinae, tribe Menthae of Lamiaceae family. The genus contains 71 species, widespread across Northern Hemisphere regions (Naderifar et al., 2015; Amirnia et al., 2017).

This annual plant is very beautiful, with numerous stems (up to 6), 22-45 cm high, and blue flowers arranged in pseudo-whorls growing in leaf axils.

Essential oil accumulates in exogenous oil-containing cells at the dorsal sides of the leaves, and in the inflorescence. Because of this, the entire plant has a citrus-like flavor, resembling that of lemon balm (*Melissa officinalis* L.) and catnip (*Nepeta cataria* L.). Apart from essential oil, the plant produces nectar, due to which it is grown as a honey-bearing plant and cultivated in gardens and parks as an ornamental plant (Aćimović et al., 2019).

This plant with its citrus like flavor is extensively used as a spice and for composition of tea blends, because of neral and geranial as major constituents of essential oil. *Dracocephalum moldavica* is used in food aromatization (canned fish, jams, candies, syrups), perfumery, alcohol industry, soaps and detergents. Apart from this, dried leaves have the potential of being used as a functional additive for extruded crisps with high nutritional value, especially because of the dietary fiber and rosmarinic acid content, strong antioxidant potential and acceptable sensory properties (Wojtowicz et al., 2017).

Seed is a good source of fatty oil with spicy taste and aromatic odor, rich in unsaturated fatty acids, principally the linolenic and linoleic acids. This categorizes *D. moldavica* seed into the group of raw materials suitable for nutraceuticals, food supplements, and functional food applications (Aćimović et al., 2019). Moreover, the application of *D. moldavica* residues as bagasse waste (oilcake) collected after pressing and added to corn crisps could be an effective way of limiting the oil waste after pressing and increasing the sustainability of waste management. A new range of nutritionally valuable snacks could be introduced to the market (Oniszczuk et al., 2017).

Fig. 1. *Dracocephalum moldavica*

Furthermore, numerous investigations show that this plant possesses good antioxidative (Aprotosoaie et al., 2016; Aslanipour et al., 2017; Weremczuk-Jeżynaet al., 2017; Ehsani et al., 2017; Fallah et al., 2018), antimicrobial (Pak et al., 2016; Ehsani et al., 2017; Keikhaie et al., 2018) and insecticidal activity (Chu et al., 2011; Ding et al., 2015). It is also used as antinociceptive (Maham et al., 2013), sedative (Martínez-Vázquez et al., 2012), neuroprotective (Sun et al., 2014), as well as cardiotonic agent (Najafi et al., 2009; Zeng et al., 2018), and for treating chronic mountain sickness (Maimaitiyiming et al., 2014).

This species is not well known in Serbia. It was introduced in Serbia in the collection garden of Institute of Field and Vegetable Crops, from Romania (Kišgeci et al., 1982). The aim of this paper is to analyze the essential oil obtained from *D. moldavica* grown in agroecological conditions of Vojvodina Province, Serbia.

Materials and methods

Plant material

The plant (**Fig. 1**) grown at Institute of Field and Vegetable Crops Novi Sad, during 2018, were confirmed by M. Rat and deposited at the Herbarium of Biology and Ecology (BUNS herbarium), Faculty of Natural Sciences, University of Novi Sad, as *D. moldavica*, Voucher specimens 2-1468. During the flowering stage (June), the aboveground parts were cut, dried and used for essential oil extraction.

Essential oil extraction

The dried aboveground parts of *D. moldavica* were subjected to hydro-distillation using an all glass Clevenger-type apparatus to extract essential oils. The samples were ground, homogenized and made into a fine powder. In order to extract the essential oils, 100 g of the powder was placed in 1 l conical flask and connected to the Clevenger apparatus. 500 ml of distilled water was added to the flask and heated to the boiling point. The steam in combination with the essential oils was distilled into a graduated cylinder for 4 h and then separated from aqueous layer. The yield essential oil was very low, so it is extracted with n-hexane, dried over anhydrous sodium sulfate and evaporated. The obtained oil was kept refrigerated at +4 °C until required for further analysis.

Table 1. Chemical composition of *D. moldavica*

No	Compound	RI	Rt	%	No	Compound	RI	Rt	%
1	1-Octen-3-ol	982	7.14	0.2	46	*trans*-α-Farnesene	1514	29.23	tr
2	3-Octanone	990	7.38	0.2	47	Italicene ether	1540	30.28	tr
3	dehydro-1,8-Cineole	997	7.59	tr	48	Spathulenol	1582	32.032	0.2
4	3-Octanol	1000	7.65	0.1	49	Caryophyllene oxide	1587	32.25	0.4
5	Bergamal	1057	9.70	0.1	50	Viridiflorol	1596	32.60	tr
6	*cis*-Linalool oxide	1076	10.42	tr	51	Salvial-4(14)-en-1-one	1598	32.67	tr
7	*trans*-Linalool oxide	1093	11.04	tr	52	NI-12	1602	32.88	0.1
8	Linalool	1104	11.47	0.4	53	NI-13	1610	33.17	0.1
9	NI-1	1109	11.65	0.1	54	Humulene epoxide II	1613	33.28	0.1
10	1-Octen-3-yl acetate	1116	11.98	tr	55	1,10-di-*epi*-Cubenol	1619	33.51	tr
11	NI-2	1142	13.10	0.1	56	NI-14	1630	33.93	0.1
12	*exo*-Isocitral	1148	13.34	0.1	57	NI-15	1634	34.09	0.1
13	*trans*-Chrysanthemal	1152	13.54	0.3	58	NI-16	1648	34.63	0.1
14	Nerol oxide	1156	13.76	0.1	59	Vulgarone B	1654	34.87	1.6
15	*cis*-Isocitral	1166	14.18	0.6	60	NI-17	1659	35.02	0.1
16	NI-3	1175	14.59	0.2	61	*cis*-Calamenen-10-ol	1663	35.20	0.1
17	Rosefuran epoxide	1177	14.62	0.1	62	NI-18	1668	35.37	0.1
18	*trans*-Isocitral	1184	14.96	0.9	63	NI-19	1675	35.67	0.2
19	α-Terpineol	1193	15.34	tr	64	Germacra-4(15),5,10(14)-trien-1-α-ol	1690	36.25	0.1
20	NI-4	1196	15.50	0.2	65	Cyclocolorenone	1764	38.92	0.1
21	NI-5	1209	16.01	0.2	66	NI-20	1829	41.28	0.1
22	NI-6	1222	16.57	0.1	67	6,10,14-Trimethyl-2-pentadecanone	1851	42.02	0.3
23	NI-7	1226	16.77	0.1	68	NI-21	1926	44.59	0.1
24	Nerol	1232	17.04	0.4	69	NI-22	2011	47.47	0.1
25	NI-8	1236	17.22	0.2	70	NI-23	2038	48.29	0.1
26	Neral	1248	17.76	19.4	71	Manool	2068	49.18	0.1
27	Piperitone	1258	18.21	1.8	72	NI-24	2082	49.60	0.1
28	Geraniol	1262	18.38	5.4	73	NI-25	1997	50.02	0.1
29	NI-9	1264	18.48	0.2	74	NI-26	2116	50.62	0.1
30	Geranial	1280	19.18	29.6	75	NI-27	2126	50.91	0.3
31	*trans*-Anethole	1291	19.65	tr	76	NI-28	2156	51.80	0.2
32	Thymol	1298	19.96	tr	77	NI-29	2164	52.05	0.2
33	Carvacrol	1307	20.35	0.7	78	NI-30	2218	53.65	0.1
34	Methyl geranate	1328	21.26	0.2	79	NI-31	2238	54.26	0.1
35	Neryl acetate	1369	23.08	3.0	80	Tricosane	2307	56.54	0.1
36	α-Copaene	1380	23.58	0.1	81	Pentacosane	2503	62.04	0.2
37	Geranyl acetate	1393	24.15	27.2	82	Hexacosane	2603	64.65	tr
38	Decyl acetate	1412	25.07	0.1	83	Heptacosane	2706	67.19	0.3
39	*trans*-Caryophyllene	1425	25.49	0.2	84	2-methyloctacosane	2780	68.96	tr
40	α-Humulene	1459	26.91	0.1	85	Octacosane	2806	69.60	tr
41	NI-10	1464	27.11	0.1	86	Nonacosane	2906	71.96	0.3
42	NI-11	1469	27.35	0.1	87	Triacontane	3056	74.23	tr
43	Germacrene D	1488	28.10	0.1	88	Untriacontane	3105	76.45	0.1
44	*ar*-Curcumene	1489	28.15	0.1				TOTAL IDENTIFIED	95.5
45	*trans*-β-Ionone	1491	28.24	tr				TOTAL NI	4.1

RI – retention Index, Rt – Retention time, NI – not identified compounds (mass spectrum of these compounds, *m/z* (intensity) are shown at figures 2-32), tr – compound presented in traces (less than 0.1%)

GC/MS analysis

Gas chromatographic-mass spectrometric analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD) (both Agilent, Santa Clara, USA), in positive ion electron impact (EI) mode. The separation was effected using Agilent 19091S-433 HP-5MS fused silica capillary column with 30 m × 0.25 mm i.d., 0.25 µm film thickness. The GC oven temperature was programmed from 60 °C to 285 °C at a rate of 3 °C/min. Helium was used as carrier gas; inlet pressure was 20.3 kPa; linear velocity was 1 ml/min at 210 °C. Injector temperature: 250 °C; injection mode: splitless. MS scan conditions: MS source temperature, 230 °C; MS Quad temperature, 150 °C; energy, 70 eV; mass scan range, 40–550 amu.

Identification of volatile compounds

The identification of components was carried out on the basis of Kovats retention index and by comparison with reference mass spectra (Wiley and NIST databases).

Results and discussion

In *D. moldavica* essential oil 88 compounds were detected, among which geranial (29.6%), geranyl acetate (27.2%) and neral (19.4%) were the most abundant, comprising 76.2%. Other significant compounds were: geraniol (5.4%), neryl acetate (3.0%), piperitone (1.8%) and vulgarone B (1.6%). Other compounds were present in the amount less than 1.0%, among which 31 unidentified compounds

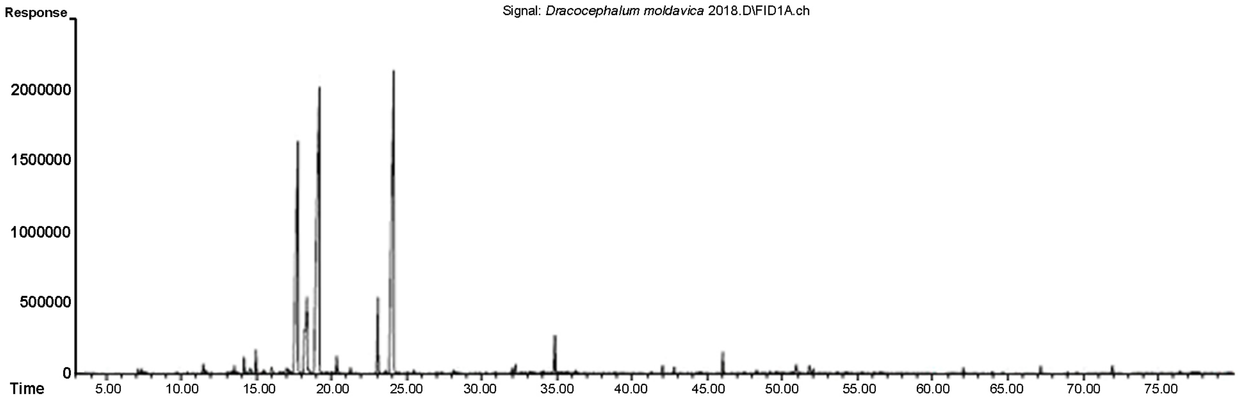

Fig. 2. A GC-FID chromatogram of *D. moldavica* essential oil

comprising 4.1% (**Tab. 1**). A GC-FID chromatogram of *D. moldavica* essential oil is shown at **Fig 2**.

Chemical composition of *D. moldavica* essential oil was previously studied in Egypt (Hussein et al., 2006; El-Baky and El-Bsroty, 2008; Aziz et al., 2013; Ahl et al., 2015; Hegazy et al., 2016), Iran (Omidbaigi et al., 2010; Maham et al., 2013, Golparvar et al., 2016, Ehsani et al., 2017; Janmohammadi et al., 2017; Fallah et al., 2018), Turkey (Eshan et al., 2014), Ukraine (Kotyuk and Rakhmetov, 2017) and China (Chu et al., 2011). It is established that chemical composition of essential oil from aerial parts of *D. moldavica* depends on many factors, among which origin, cropping system, fertilization, salt stress, weed management, etc. (Aziz et al., 2013; Janmohammadi et al., 2017; Fallah et al., 2018).

The principal compounds in almost all essential oils are neral, geranial, geranyl acetate and geraniol. However, the content of neral in essential oil varied between 10.25 and 43.49%, while geranial ranged between 9.10 and 42.45%. Furthermore, geranyl acetate and geraniol content varied in larger scale, between 0.20%-40.40%, and 0.50 and 28.14%, respectively (Aćimović et al., 2019).

Investigations show that the geranyl acetate, geranial and geraniol in essential oil reach their maximum levels during the flowering, while the content of neral, decreases during flowering. These observations indicate that the biosynthesis of geranyl acetate is dominant at the beginning of the vegetative period, but is superseded by biosynthesis of geranial and geraniol, from the early stage of flowering. It indicated that the optimal harvest time proved to be during the flowering stage, when the oil content is the highest and thus also the amount of the main terpenes is the highest (Holm et al., 1988).

Conclusion

Dracocephalum moldavica from Serbia contains geranial (29.6%), geranyl acetate (27.2%) and neral (19.4%) as the most abundant compounds. Further investigations will be focused on the influence of weather conditions on essential oil composition, as well as on bioactive potential of this essential oil.

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