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Essential oil quality of chamomile grown in Province of Vojvodina

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ABSTRACT
Chamomile (*Matricaria chamomilla* L., Asteraceae) is one of the most popular medicinal plants used as a herb infusion for treating numerous ailments, including sleep disorders, anxiety, digestion and intestinal conditions etc. Chamomile essential oil is used in a wide variety of consumer goods such as detergents, soaps, toiletries, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard liquor) and as insecticide. According to the European Pharmacopoeia, there are two types of essential oils, one rich in bisabolol oxides and the other in *α*-bisabolol, which are preferred for tea brewing because of its sweet, grassy, and slightly fruity aroma. From the other side, varieties rich in chamazulene and *β*-farnesene have bitter taste, and because of that they are low valuable raw material. According to the results of the essential oil composition obtained from three different chamomile samples grown in Province of Vojvodina, it could be concluded that domestic cultivars "Banatska" and "Tetraploidna" contains *β*-farnesene as dominant compound, while the content of bisabolol oxides and *α*-bisabolol was lower than required standards in European Pharmacopoeia. Because of that they could not be classified as quality raw material. The German cultivar "Mabamille" grown in agroecological conditions of Vojvodina region, with 37.5% of *α*-bisabolol can be classified as a bisabolol rich type, and as high quality row material.

KEY WORDS: Essential oil, *Matricaria chamomilla*, *β*-farnesene, *α*-bisabolol, bisabolol oxides

Introduction

Chamomile or *Matricaria chamomilla L.* (syn. *Matricaria recutita* L.; *Chamomilla recutita* L. Rauschert) is an annual plant that belongs to Asteraceae family. It is one of the most popular medicinal plants used as a herb infusion i.e. herbal tea beverage (Formisano et al., 2015), because of its sweet, grassy, and slightly fruity aroma (Zadeh et al., 2014). It is used for treating numerous ailments, including sleep disorders, anxiety, digestion and intestinal conditions (McKay and Blumberg, 2006). Over 120 constituents have been recognized in chamomile essential oil, out of which α-bisabolol, chamazulene, *β*-farnesene, bisabolol oxides and α-bisabolone oxide are the most important ones (Ghasemi et al., 2016). According to the European Pharmacopoeia (Ph. Eur 7.0), there are two types of essential oils, one rich in bisabolol oxides (between 29 and 81%) and the other in *α*-bisabolol (between 10-65%). Chamomile varieties rich in bisabolol oxides and *α*-bisabolol, but with low chamazulene and *β*-farnesene content as they cause the bitterness, are preferred for tea brewing (Mezaka et al., 2020).

Chamomile essential oil is a clear, intensely blue, viscous liquid with an intense characteristic odor (Ph. Eur 7.0). Chamomile essential oil is used in a wide variety of consumer goods such as detergents, soaps, toiletries, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard liquor) and insecticides (Yadav and Yadav, 2016).

The chamomile essential oil is blue due to the presence of chamazulene, however, it can slowly oxidize to green and finally brown (Satyal et al., 2015). Chamazulene is considered to be the most valuable constituent, as it demonstrates anti-inflammatory (Salamon, 2004) and antimicrobial activities (Owlia et al., 2007). Furthermore, *α*-bisabolol is a naturally occurring sesquiterpene alcohol widely used as an anti-inflammatory agent but it also exhibits several other pharmacological properties such as analgesic, antibiotic and anticancer activities (Kamatou and Viljoen, 2010). In addition, bisabolol oxides possess antimicrobial and antioxidant properties (Kazemi, 2015). Additionally, as *E*-*β*-

farnesene possesses an insect attracting activity (Satyal et al., 2015) this lead to its potential use in crop protection strategies (Mauchamp and Pickett, 1987).

Gathering and cultivating of medicinal plants in Serbia has a long tradition. Chamomile was considered to be the most valuable raw material. However, after the breakup of Yugoslavia, other countries from the region took over the lead on the market. In recent years, Serbia has once again become a source of high-quality raw material on the European market. The aim of our investigation was to compare the differences in chemical compositions between different chamomile cultivars grown in North part of Serbia, Province of Vojvodina, by different medicinal plant producers.

Material and method

Chamomile samples from three different locations in Vojvodina Province were collected during May 2020 from large scale medicinal plant producers. The sample from Đala (46° 09′ 09″ N; 20° 06′ 32″ E) belongs to the native cultivar “Banatska”, the sample from Bač (45° 23′ 29″ N; 19° 14′ 12″ E) is cultivar “Tetraploidna”, while the sample from Čoka (45° 56′ 22″ N; 20° 08′ 44″ E) is German cultivar called “Mabamille”.

Air-dried chamomile flowers were subjected to hydro-distillation using an all glass Clevenger-type apparatus to extract essential oil according to the method outlined by the European Pharmacopoeia (Ph. Eur 7.0). In order to extract essential oil, 30 g of the dry flowers were placed in 1000 ml conical flask and connected to the Clevenger apparatus. Distilled water (300 ml) was added to the flask and heated up to the boiling point. The steam, in combination with the essential oil, was distilled into a graduated cylinder for 4 h and then separated from the aqueous layer.

The GC-FID and GC-MS analyses were carried out with an Agilent 6890 apparatus equipped with an auto-injection system, an inert 5973C XL EI/ CI mass-selective detector (MSD) and a flame ionization detector (FID) connected by a cap. Flow technology 2-way splitter with make-up, and a HP-5 MS fused-silica cap. column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The GC oven temperature was programmed from 60°C to 285°C at a rate of 4.3°C/min. Helium was used as carrier gas; the inlet pressure was 25 kPa; the linear velocity was 1 ml/min at 210°C. Injector temperature: 250°C; injection mode: splitless. MS scan conditions: source temperature, 200°C; interface temperature, 250°C; energy, 70 eV; mass scan range, 40-350 amu. Identification of the components was done on the basis of retention index and the comparison with reference spectra (Wiley and NIST databases). Area percentages obtained by FID were used as a base for the purpose of quantitative analysis.

Results and discussion

Among 145 detected compounds in three chamomile samples (cultivars) from Province of Vojvodina (Table 1), 58 compounds were unidentified (NI). These NI compounds compromise 12.6%, 2.1% and 3.9% of the total for cultivar “Banatska”, “Tetraploidna” and “Mabamille”, respectively. Sesquiterpene hydrocarbons and oxygenated sesquiterpenes were the main class of compounds in all samples.

Table 1
Essential oil composition of analysed chamomile cultivars
Tabela 1
Komponente etarskog ulja ispitivanih sorti kamilice

No	Compound	Banatska		Tetraploidna		Mabamille	
		RI	%	RI	%	RI	%
1	Hexanal ^{O}	801	0.1	-	nd	-	nd
2	Butanoic acid, 2-methyl-, ethyl ester ^{O}	844	tr	842	0.5	841	0.3
3	Ethyl isovalerate ^{O}	-	nd	845	0.1	845	0.1
4	α-Pinene ^{MT}	931	0.2	930	0.1	931	0.1
5	Butanoic acid, 2-methyl-, propyl ester ^{O}	941	tr	940	0.1	940	0.1
6	2E-Heptenal ^{O}	952	0.1	-	nd	-	nd
7	Benzaldehyde ^{O}	958	0.1	-	nd	-	nd
8	Sabinene ^{MT}	970	0.1	970	0.5	970	0.9
9	1-Octen-3-ol ^{O}	975	0.1	-	nd	-	nd
10	6-methyl-5-Hepten-2-one ^{O}	984	0.2	983	tr	983	0.1
11	Myrcene ^{MT}	-	nd	987	0.1	-	nd
12	2-Pentyl furan ^{O}	989	0.4	992	tr	989	0.1
13	Yomogi alcohol ^{OMT}	997	0.1	-	nd	996	0.3

14	(Z)-2-(2-Pentenyl)furan [O]	1001	0.1	-	nd	-	nd
15	δ-2-Carene [MT]	-	nd	995	0.2	-	nd
16	α-Terpinene [MT]	1015	tr	1007	tr	1014	0.1
17	p-Cymene [AMT]	1022	0.1	1020	0.3	1022	0.2
18	Limonene [MT]	1026	0.1	1025	0.1	1026	0.1
19	1,8-Cineole [OMT]	1029	tr	1028	0.3	1028	0.3
20	(Z)-β-Ocimene [MT]	1034	0.2	1032	0.3	1034	0.1
21	(E)-β-Ocimene [MT]	1044	1.6	1044	2.6	1044	0.7
22	NI-1	1050	0.1	-	nd	-	nd
23	γ-Terpinene [MT]	1055	0.2	1055	0.7	1055	0.5
24	Artemisia ketone [OMT]	1057	0.5	1057	1.8	1057	0.8
25	NI-2	1064	0.1	-	nd	-	nd
26	NI-3	1066	0.1	-	nd	-	nd
27	Artemisia alcohol [OMT]	1080	tr	1080	0.3	1080	0.2
28	Terpinolene [MT]	1086	tr	1086	0.2	1086	tr
29	Linalool [OMT]	1098	tr	1097	0.1	1098	tr
30	n-Nonanal [O]	1102	0.5	-	nd	-	nd
31	allo-Ocimene [MT]	1126	0.1	-	nd	-	nd
32	NI-4	1137	1.1	-	nd	-	nd
33	NI-5	1144	0.1	-	nd	-	nd
34	2E-Nonen-1-al [O]	1156	0.2	-	nd	-	nd
35	Z-Chrysanthenol [OMT]	1160	0.1	-	nd	-	nd
36	Borneol [OMT]	1163	0.1	-	nd	1163	0.1
37	NI-6	1167	tr	1165	0.1	1165	0.1
38	Terpinen-4-ol [OMT]	1175	0.1	1174	0.1	1174	0.3
39	α-Terpineol [OMT]	1189	tr	1188	0.1	1188	0.1
40	n-Decanal [O]	1203	0.1	-	nd	-	nd
41	Z-3-Octen-1-ol, acetate [O]	-	nd	1211	0.1	-	nd
42	β-Cyclocitral [OMT]	1219	0.1	-	nd	-	nd
43	3Z-Hexenyl 3-methyl butanoate [O]	1234	0.1	1234	0.1	1234	0.1
44	Z-Chrysanthenyl acetate [OMT]	1259	0.4	-	nd	-	nd
45	NI-7	1269	0.1	-	nd	-	nd
46	4,8-Dimethyl-nona-3,8-dien-2-one [O]	1272	0.1	1272	tr	-	nd
47	Tridecane [A]	-	nd	1297	0.1	-	nd
48	2E,4E-Decadienal [O]	1315	0.2	-	nd	-	nd
49	δ-Elemene [ST]	1336	0.5	1335	0.5	-	nd
50	7-epi-Silphiperfol-5-ene [ST]	1342	0.1	-	nd	-	nd
51	NI-8	1363	0.1	-	nd	-	nd
52	NI-9	1371	0.2	-	nd	-	nd
53	α-Copaene [ST]	1374	0.4	1373	tr	-	nd
54	NI-10	1377	0.1	-	nd	-	nd
55	Modheph-2-ene [OST]	1378	0.1	1378	tr	-	nd
56	E-β-Damascenone [O]	1383	0.1	-	nd	-	nd
57	α-Isocomene [ST]	1385	0.5	1384	0.2	1385	0.1
58	β-Elemene [ST]	1390	0.1	1390	0.1	1390	0.1
59	NI-11	-	nd	-	nd	1392	0.1
60	β-Isocomene [ST]	1404	0.1	-	nd	-	nd
61	NI-12	1414	0.1	-	nd	-	nd
62	NI-13	1417	0.4	-	nd	-	nd
63	E-Caryophyllene [ST]	-	nd	1417	0.4	1417	0.4
64	β-Copaene [ST]	1428	0.1	1427	0.1	-	nd
65	NI-14	1443	0.1	1442	tr	-	nd
66	NI-15	1452	0.1	-	nd	-	nd
67	α-Humulene [ST]	-	nd	1452	0.1	1452	tr
68	E-β-Farnesene [ST]	1461	28.3	1458	36.0	1457	12.2
69	NI-16	1465	0.1	1464	tr	-	nd
70	dehydro-Sesquicineole [OST]	1470	0.4	1468	0.2	1468	0.2
71	γ-Muurolene [ST]	1478	0.1	1477	0.1	-	nd
72	Germacrene D [ST]	1483	2.3	1482	6.6	1481	3.0
73	E-β-Ionone [O]	1486	0.1	-	nd	-	nd
74	β-Selinene [ST]	1487	0.2	1486	0.3	1486	0.1
75	NI-17	1494	0.3	1494	0.7	-	nd
76	Bicyclogermacrene [ST]	1497	1.0	1496	3.0	1496	1.4

77	NI-18	1500	0.2	-	nd	-	nd
78	α-Muurolene ST	-	nd	1500	0.1	-	nd
79	NI-19	1507	tr	-	nd	1507	1.6
80	E,E-α-Farnesene ST	1508	2.7	1508	7.4	-	nd
81	NI-20	-	nd	-	nd	1513	0.1
82	γ-Cadinene ST	-	nd	1514	0.1	-	nd
83	δ-Cadinene ST	-	nd	1523	0.2	1523	0.2
84	NI-21	1523	0.2	-	nd	-	nd
85	NI-22	1527	1.2	1526	0.1	-	nd
86	NI-23	1531	0.1	-	nd	-	nd
87	NI-24	1536	0.1	-	nd	-	nd
88	NI-25	1553	0.2	1552	0.1	1551	0.1
89	NI-26	-	nd	-	nd	1557	0.2
90	NI-27	1562	0.9	1562	0.1	1561	0.1
91	NI-28	1566	0.4	-	nd	-	nd
92	NI-29	1569	0.2	1568	tr	-	nd
93	Dendrolasin AMT	1572	0.4	-	nd	-	nd
94	Spathulenol OST	1577	2.8	1576	0.2	1575	0.4
95	Caryophyllene oxide ST	1582	0.6	1581	0.3	1581	0.1
96	NI-30	1583	0.2	-	nd	1583	0.3
97	NI-31	1590	0.1	1589	0.1	-	nd
98	Salvial-4(14)-en-1-one OST	1592	0.2	-	nd	-	nd
99	NI-32	1595	0.2	-	nd	-	nd
100	NI-33	1600	0.1	1597	0.1	-	nd
101	NI-34	1610	0.4	-	nd	1609	0.1
102	NI-35	1616	0.9	1615	0.1	1616	0.1
103	NI-36	1621	0.1	-	nd	-	nd
104	NI-37	1629	1.1	1628	0.1	1628	0.1
105	NI-38	-	nd	-	nd	1636	0.1
106	NI-39	1638	0.8	-	nd	-	nd
107	epi-α-Murrolol (=tau-muurolol) OST	-	nd	-	nd	1640	0.1
108	NI-40	1647	0.3	-	nd	-	nd
109	α-Bisabolol oxide B OST	1658	10.9	1655	9.3	1654	3.9
110	NI-41	1661	0.4	1660	0.2	1660	0.2
111	α-Bisabolol OST	1685	6.7	1683	4.6	1688	37.5
112	NI-42	1687	0.5	-	nd	-	nd
113	NI-43	-	nd	1692	0.1	-	nd
114	2Z,6Z-Farnesol OST	1697	0.3	-	nd	-	nd
115	NI-44	-	nd	1699	0.1	1698	tr
116	NI-45	-	nd	-	nd	1706	0.1
117	NI-46	1711	0.1	1710	tr	-	nd
118	Chamazulene ST	1730	1.4	1730	6.7	1733	21.1
119	Isobicyclogermacrenal OST	1734	0.1	-	nd	-	nd
120	α-Bisabolol oxide A OST	1750	10.2	1746	7.6	1746	4.2
121	NI-47	1757	0.1	-	nd	-	nd
122	NI-48	1766	0.1	-	nd	1767	0.1
123	NI-49	1775	tr	-	nd	1775	0.1
124	NI-50	1788	0.2	1788	tr	1788	0.1
125	NI-51	1814	0.1	-	nd	-	nd
126	NI-52	1829	0.1	-	nd	-	nd
127	NI-53	1835	0.1	-	nd	-	nd
128	6,10,14-trimethyl-2-Pentadecanone O	1842	0.3	-	nd	-	nd
129	Z-Spiroether D	-	nd	1882	4.0	1883	4.8
130	E-Spiroether D	1887	7.4	1896	0.3	1895	0.1
131	Nonadecane (C19) A	1896	0.3	-	nd	-	nd
132	NI-54	1920	0.2	1920	tr	-	nd
133	NI-55	-	nd	1944	0.2	-	nd
134	NI-56	1952	0.8	-	nd	1951	0.3
135	Eicosane (C20) A	1994	0.1	-	nd	-	nd
136	Heneicosane A	2098	0.1	-	nd	-	nd
137	NI-57	2113	0.1	-	nd	-	nd
138	NI-58	-	nd	-	nd	2147	0.1
139	Docosane A	2197	0.1	-	nd	-	nd

140	Tricosane [A]	2298	0.2	2297	0.1	2298	0.1
141	Tetracosane [A]	2394	0.1	-	nd	2394	tr
142	Pentacosane [A]	2496	0.2	2497	0.1	2497	0.1
143	Hexacosane [A]	2596	tr	-	nd	-	nd
144	Heptacosane [A]	2695	0.1	2694	tr	-	nd
145	Nonacosane [A]	2892	tr	2895	0.1	-	nd
Monoterpene hydrocarbons [MT]			**2.5**		**4.8**		**2.5**
Aromatic monoterpene hydrocarbons [AMT]			**0.5**		**0.3**		**-**
Oxygenated monoterpene [OMT]			**1.4**		**2.7**		**2.1**
Sesquiterpene hydrocarbons [ST]			**38.4**		**61.9**		**40.2**
Oxygenated sesquiterpenes [OST]			**31.7**		**22.2**		**46.4**
Other [O]			**2.8**		**0.9**		**0.8**
Diacetylenes [D]			**7.4**		**4.3**		**4.9**
Alkanes [A]			**1.2**		**0.4**		**0.2**
NI			**12.6**		**2.1**		**3.9**
TOTAL			**99.5**		**99.7**		**99.7**

RI – Retention Index; NI – Not Identified compound; nd – not detected; tr – trace (less than 0.01%)

The selected chamomile cultivar "Banatska" is the most common variety of diploid chamomile in Serbia and in the region (Tsivelika et al., 2018). The dominant compounds in the essential oil from plants grown in Đala (Banat region, from which this cultivar originates) were *E*-*β*-farnesene (28.3%), *α*-bisabolol oxides A and B (10.2% and 10.9%, respectively), *α*-bisabolol (6.7%) and chamazulene (1.4%) (Table 1; Figure 1). Cultivated form of tetraploid chamomile mostly grown in Serbia is "Tetraploidna". The dominant compounds in its essential oil were *E*-*β*-farnesene (36.0%), α-bisabolol oxides A and B (7.6% and 9.3%, respectively), chamazulene (6.7%) and *α*-bisabolol (4.6%) (Table 1; Figure 2).

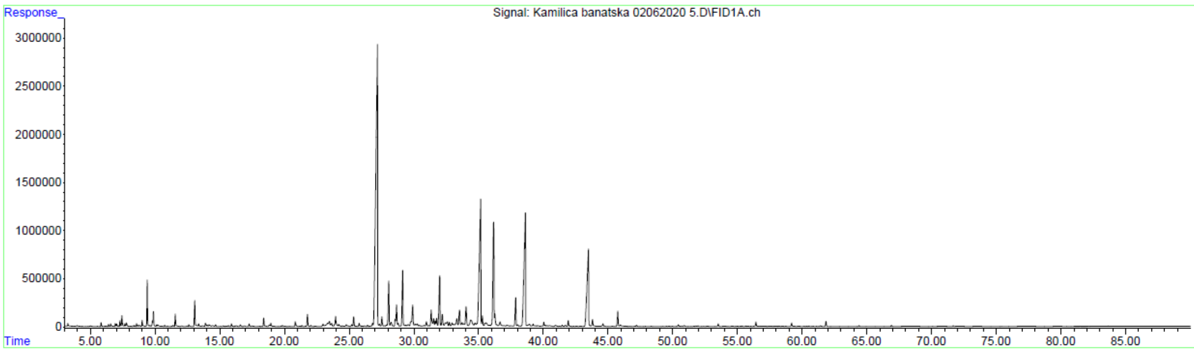

Figure 1. GC-FID chromatogram of chamomile essential oil cultivar "Banatska"
Slika 1. GC-FID hromatogram etarskog ulja kamilice sorta "Banatska"

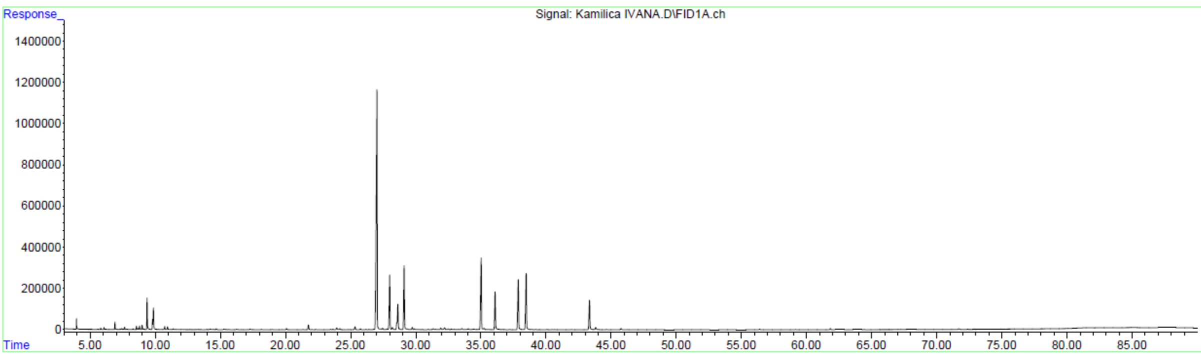

Figure 2. GC-FID chromatogram of chamomile essential oil cultivar "Tetraploidna"
Slika 2. GC-FID hromatogram etarskog ulja kamilice sorta "Tetraploidna"

Similar results from Serbia were also reported by Hoferl et al. (2020), where the dominant constituent was E-β-farnesene with 38.4%, followed by α-bisabolol oxides A and B with 6.3% and 5.8%, respectively, as well as α-bisabolol (11.5%) and chamazulene (8.0%). Chamomile with *E-β*-farnesene (29.8%) as the dominant compound is also grown in the neighboring region (Bosnia and Herzegovina) (Stanojevic et al., 2016). However, investigation conducted by Savikin et al. (2011) proved that chamomile essential oil obtained from cultivar "Banatska" from Serbia had the highest content of *E-β*-farnesene, while the *α*-bisabolol and its oxides, azulene and chamazulene were found to be more than 1%. It was stated that the chemical profile of the oil is not accountable to Ph Eur 7.0. Because of this, some chamomile producers grow foreign cultivars which satisfied Ph Eur 7.0 standards. Previous research conducted in Serbia with this cultivar (10 different samples) shows that the most abundant constituents in oils were *E-β*-farnesene (variation between 29.6 and 46.7%), followed by bisabolol oxide A (5.4-10.3%), bisabolol oxide B (5.7-9.5%) and chamazulene (5.4-12.0%) (Ristic et al., 2007).

German tetraploid cultivar "Mabamille" in Serbian agroecological conditions had essential oil with 37.5% *α*-bisabolol and 21.1% chamazulene, while the content of *E-β*-farnesene (12.2%), and *α*-bisabolol oxides A and B (4.2% and 3.9%, respectively) was significantly lower than in the domestic varieties "Banatska" and "Tetraploidna" (Table 1; Figure 3). Apart from this German cultivar, Polish "Zloty Lan", Slovak cultivar "Lutea" and Austrian "Manzana" are also grown in Serbia. The latter provides essential oil with *α*-bisabolol as the dominant compound (34.8%) in Serbian agroecological conditions (Acimovic et al., 2018). However, there are other *α*-bisabolol rich cultivars known worldwide such as: "Lianka" (Fejer and Salamon, 2016), "Robumille" (Singh et al., 2011), "Bona", "Novbona" and "Goral" (Salamon, 2004).

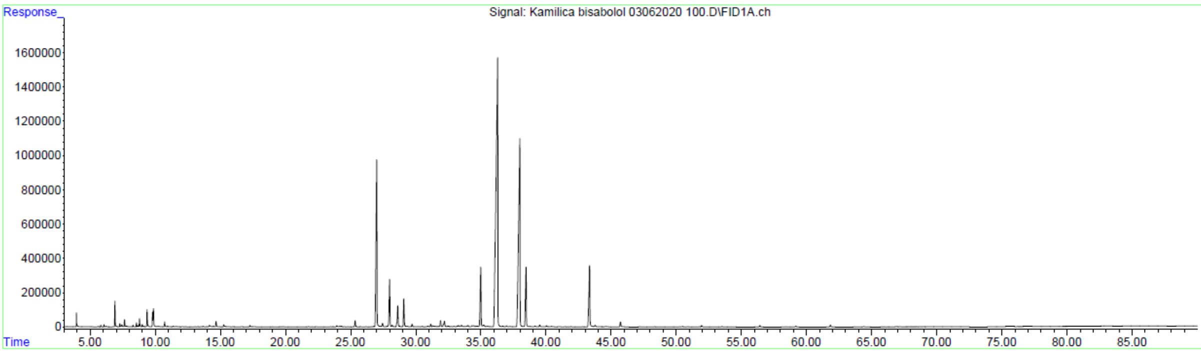

Figure 3. GC-FID chromatogram of chamomile essential oil cultivar "Mabamille"
Slika 3. GC-FID hromatogram etarskog ulja kamilice sorta "Mabamille"

Conclusions

According to the results of the essential oil composition obtained from three different chamomile cultivars grown in Vojvodina region, it could be concluded that in domestic cultivars "Banatska" and "Tetraploidna" the dominant compound was *E-β*-farnesene with 28.3% and 36%, respectively. Furthermore, these cultivars have high content of bisabolol oxides (21.1% and 16.9% respectively), and low content of α-bisabolol (6.7% and 4.6%, respectively). Furthermore, the German cultivar "Mabamille" grown in Vojvodina agroecological conditions, with 37.5% of α-bisabolol can be classified as a bisabolol rich type.

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Kvalitet etarskog ulja kamilice gajene u Vojvodini

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SAŽETAK
Kamilica (*Matricaria chamomilla* L., Asteraceae) je jedna od najpopularnijih lekovitih biljaka koja se upotrebljava u obliku infuza (čaja) za lečenje mnogih stanja i bolesti, uključujući poremećaje sna, napetost, probleme organa za varenje itd. Etarsko ulje kamilice se koristi u mnogim granama industrije: hemijskoj (kao dodatak deterdžentima), kozmetičkoj (u sapunima, sredstvima za ličnu higijenu), farmaceutskoj, parfimerijskoj i prehrambenoj industriji (u slatkišima, alkoholnim i bezalkoholnim pićima) i kao insekticid. Prema Evropskoj Farmakopeji, postoje dva tipa etarskog ulja kamilice, prvi koji je bogat bisabolol oksidima i drugi bogat *α*-bisabololom. Oba tipa su poželjna u industriji čajnih napitaka zbog njihovog slatkog, osvežavajućeg ukusa blago voćne arome. Sa druge strane, varijeteti bogati hamazulenom i *β*-farnezenom imaju gorak ukus i zbog toga predstavljaju manje vrednu biljnu sirovinu. Na osnovu rezultata hemijskog sastava etarskog ulja tri različita uzorka kamilice gajene u Pokrajini Vojvodini od strane individualnih poljoprivrednih proizvođača, može se zaključiti da domaće sorte "Banatska" i "Tetraploidna" sadrže *β*-farnezen kao dominantnu komponentu, dok je sadržaj bisabolol oksida i *α*-bisabolola niži nego što zahtevaju standardi u Evropskoj Farmakopeji, zbog čega se ne mogu kategorisati kao kvalitetni biljni materijal. Nemačka sorta "Mabamille" gajena u agroekološkim uslovima pokrajine Vojvodine, sa 37,5% *α*-bisabolola pripada bisabolol tipu kamilice i predstavlja visoko kvalitetnu sirovinu.

KLJUČNE REČI: Etarsko ulje, *Matricaria chamomilla*, *β*-farnezen, *α*-bisabolol, bisabolol oksidi

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