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Chemical Diversity in Volatiles of *Helichrysum plicatum* DC. Subspecies in Turkey

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Abstract: In the present work three subspecies of *Helichrysum plicatum* DC. (*Helichrysum plicatum* DC. subsp. *plicatum, Helichrysum plicatum* DC. subsp. *polyphillum* (Ledeb) P.H.Davis & Kupicha and *Helichrysum plicatum* DC. subsp. *isauricum* Parolly) were investigated for the essential oil chemical compositions. The volatiles were obtained by conventional hydrodistillation of aerial parts and microdistillation of inflorescences. Subsequent gas chromatography (GC-FID) and gas chromatography coupled to mass spectrometry (GC/MS) revealed chemical diversity in compositions of the volatiles analyzed.

A total of 199 compounds were identified representing 73.9-98.3% of the volatiles compositions. High abundance of fatty acids and their esters (24.9-70.8%) was detected in the herb volatiles of *H. plicatum* subsp. *polyphyllum* and *H. plicatum* subsp. *isauricum*.

The inflorescences of *Helichrysum* subspecies were found to be rich in monoterpenes (15.0-93.1%), fatty acids (0.1-36.3%) and sesquiterpenes (1.1-25.5%). The inflorescence volatiles of *H. plicatum* subsp. *isauricum* were distinguished by predomination of monoterpene hydrocarbons (93.1%) with fenchene (88.3%) as the major constituent

Keywords: *Helichrysum plicatum;* GC/MS*;* volatiles; biodiversity; Turkish flora.

1. Introduction

The tribe Gnaphalieae of Asteraceae comprises 185 genera and more than 1240 species. *Helichrysum* Mill. is the largest genus of this tribe, including approximately 600 species occurring in Europe, Asia, Africa and Madagascar [1]. This genus which is represented by 24 species, 30 taxa of which, 17 are endemic, has been recorded in the Flora of Turkey [2]. A high level of anatomical and morphological polymorphism among *Helichrysum* species was noted by Jahn et al. [3]. Chemical and genetic diversity of the genus *Helichrysum* has been reported in a number papers [4-6]. Some species demonstrated a high level of intraspecific differences in the essential oil composition due to different environmental factors [7]. Even though this large genus has been extensively investigated, the chemical variations and the genetic relationships within the same species still remain unclear [8].

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H. plicatum species generally known as "ölmez çiçek, altın otu and mantuvar" in Anatolian folk medicine and have been used for treatment of wound, burn, otitis, nephritis, to pass kidney stone and against stomach ulcer [9-11]. Previous investigations of *H. plicatum* subspecies are mostly concerned with biological activity of the extracts obtained by different solvents. *H. plicatum* methanol extract was reported to have weak antioxidant and antineoplastic activities [12, 13]. Eroğlu et al. estimated about genotoxic and mutagenic effects of *H. plicatum* DC. subsp. *plicatum* (*Hppl*) methanol extract [14]. The extracts obtained by acetone, ethanol and chloroform from *Hppl* demonstrated antifungal, antimutagenic, hypoglycaemic, antioxidant and antimicrobial activities [15-19]. The water-ethanol extract may be a potential drug for urolithiasis treatment [20]. *In vitro* antioxidant, radical scavenging and antimicrobial activities of the methanol extracts of three subspecies of *H. plicatum* have been reported before[21, 22]. Küçükoğlu et al. suggested that the methanol extract of *Hppl,* manifested a considerable inhibition on the mammalian DNA topoisomerase-I enzyme with in vitro supercoil relaxation assays in a dose dependent manner [23]. Moreover experimental studies *in vivo* revealed antidiabetic and antioxidant potential of *Hppl* capitulums in streptozotocin-induced-diabetic rats [24].

In the past few decades, several *Helichrysum* species have been investigated from a phytochemical point of view. Reported chemical constituents from the genus *Helichrysum* include terpenes, acetophenones, fatty acids, flavonoids and related phenolic compounds [8, 24, 25-30]. Even though essential oils of this large genus have been extensively investigated, the chemical composition of many species still remains unrecorded. This is especially important for the differentiation of taxa belonging to the same species as well as different subspecies. The volatile constituents, particularly monoterpenes, have been extensively studied since it has been demonstrated that the monoterpene composition, besides some environmental variability, is dependent upon the plant's genotype and can be used for taxonomic purpose [31-32]. To this aim, we have carried out comparative study the chemical composition of the volatiles obtained from inflorescences and aerial parts of *H. plicatum* DC. subsp. *plicatum (Hppl), H. plicatum* DC. subsp. *polyphillum* (Ledeb) P.H.Davis & Kupicha *(Hppo)*, and *H. plicatum* DC. subsp. *isauricum* Parolly *(Hpi)*. To the best of our knowledge, the present work is the first report about the volatiles of *Hppo* and *Hpi.* However there is only one local paper about essential oil composition of *Hppl* [33].

2. Materials and Methods

2.1. Plant materials

Inflorescences and aerial parts of plants were collected in flowering stage from west and south parts of Turkey. Voucher specimen numbers, local names, collection sites, and yields of the oils are presented in Table 1. The research materials were collected and identified by one of us (Dr. Bintug Özturk) and stored in IZEF Herbarium (Ege University). Distribution of the studied species in Turkey is indicated on map (Figure 1).

Table 1. Collection data for the *Helichrysum* species studied

Plant name	Abbrev.	Voucher Specimen No	Locality	Studied plant part	Oil yield, %
H. plicatum subsp. *polyphyllum*	*Hppo*	IZEF 5555	Antalya, Elmalı-Finike road, Cedar research forest, 1600 m	herb inflorescences	0.02 -*
H. plicatum subsp. *isauricum*	*Hpi*	IZEF 5643	Antalya, Gündoğmuş, Oğuz district, North of Sobiçimen plateau, 1990 m	herb inflorescences	0.006 -*
H. plicatum subsp *plicatum*	*Hppl*	IZEF 5556	Bursa**,** Uludağ, 1875m	herb inflorescences	0.014 -*

* Percentage was calculated only for the oils hydrodistilled from the herbs in Clevenger type apparatus.

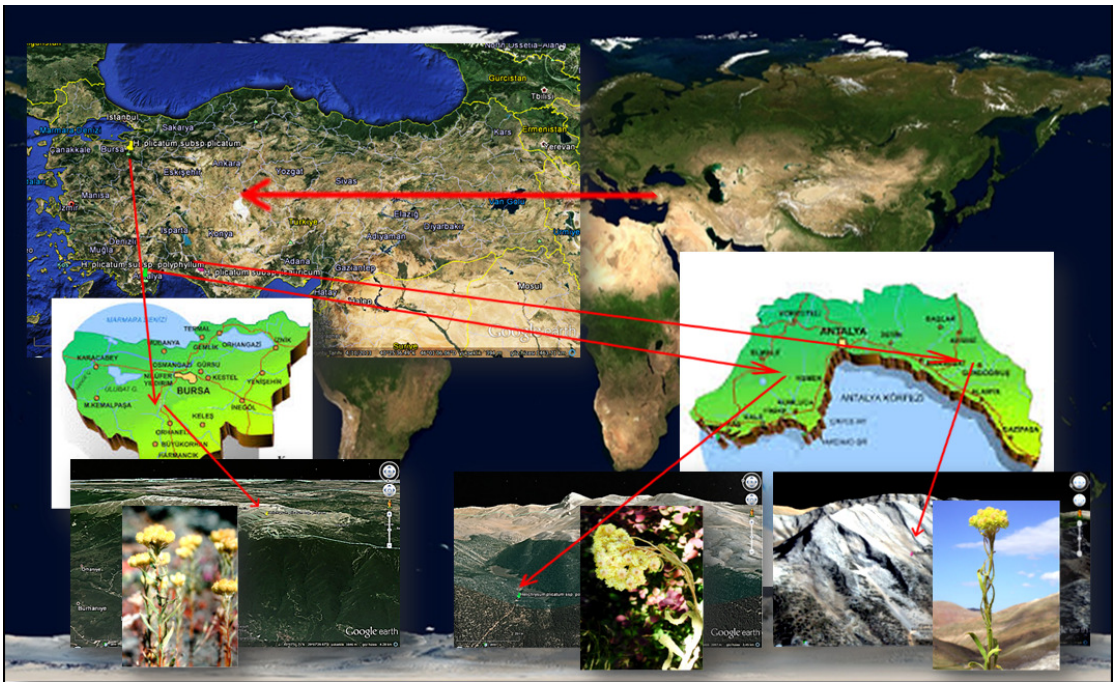

Figure 1. Distribution of the studied *Helichrysum plicatum* DC. subspecies in Turkey

2.2. Chemicals

Anhydrous sodium sulfate (ACS-ISO, for analysis) and *n*-hexane (ACS, for analysis) were purchased from Carlo Erba (Italy). For retention index (RI) determination, a mixture of *n*-alkanes (C_9-C_{30}) was used and run under the experimental conditions reported below. All compounds were of analytical standard grade.

2.3. Isolation of essential oils

The essential oils were isolated by hydrodistillation (HD) of aerial parts according to published procedure in the European Pharmacopoeia [34] and stored as previously reported [35]. The oil yields are presented in Table 2. The volatiles of the inflorescences were obtained by microdistillation (MD) technique using MicroDistiller device (Eppendorf-Netheler-Hinz, Hamburg, Germany).

For MD, the dried inflorescences (0.5 gr) of *H. plicatum* subspecies were placed in a sample vial together with 10 mL of water. The vials (20 mL for the sample vials, 10 mL for the collection vials), capillary columns, crimp caps and septa were original accessories from the manufacturer. Sodium chloride (2.0 g), water (1.0 mL) and *n*-hexane (0.3 mL) were placed in the collecting vial to trap volatile compounds. The microdistiller was operated according to stepwise heating programme. After completing the distillation, the organic layer in the collection vial was separated from the water phase and injected directly into GC/FID and GC/MS.

2.4. Gas Chromatography – Mass Spectrometry (GC/MS)

The oils were analyzed by capillary GC-FID and GC/MS techniques using an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). The same column and analytical conditions were used for both GC/MS and GC-FID. HP-Innowax FSC column (60m × 0.25mm, 0.25 µm film thickness, Agilent, Walt & Jennings Scientific, Wilmington, Delaware, USA) was used with helium as a carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then

programmed to 240 °C at a rate of 1 °C/min. The split ratio was adjusted at 40:1. Mass spectrums were taken at 70 eV. Mass range was from *m/z* 35 to 450.

2.5. Gas Chromatography (GC-FID)

The GC-FID analysis was carried out using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). FID detector temperature was 300 °C. In order to obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions.

2.6. Identification and quantification of compounds

Most of the volatile constituents were identified by parallel comparison of their retention indices and mass spectra with those of authentic compounds available in our laboratory and with those of the literature [35]. The retention indices (RI) were determined in relation to a homologous series of *n*-alkanes (C_9-C_{30}) under the same operating conditions. Further identification was made by comparison of their mass-spectra on HP Innowax column with those data stored in the Wiley GC/MS Library (Wiley, New York, NY, USA), MassFinder software 4.0 (Dr. Hochmuth Scientific Consulting, Hamburg) [36], Adams Library [37], NIST Library [38] and the in-house "Başer Library of Essential Oil Constituents". Quantification of volatile components was performed on the basis of their GC-FID peak areas using integration data.

3. Results and Discussion

In scope of the present work, we have studied the composition of the volatile metabolites of three subspecies of *H. plicatum* collected at flowering period in the west and south part of Turkey. Results of hydrodistillation of aerial parts and microdistillation of infloresence of *Hppl, Hppo* and *Hpi* with subsequent GC-FID and GC/MS simultaneous analysis of the volatiles are given in Table 2. The detected compounds with their relative percentages, retention indices and percentages of compound classes are listed in order to elution on the HP-Innowax FSC column.

Table 2. Chemical composition of the volatiles of *Helichrysum plicatum* subspecies

			Aerial parts			**Infloresence**		
No	**RRI**	**Compound**	**Hppo**	**Hpi**	**Hppl**	**Hppo**	**Hpi**	**Hppl**
1	1032	α-Pinene				**8.4**	1.0	**10.4**
2	1035	α-Thujene				0.1		
3	1072	α-Fenchene					**88.3**	**18.2**
4	1076	Camphene				0.1		
5	1093	Hexanal		0.1		0.3		
6	1118	β-Pinene						0.1
7	1174	Sylvestrene			t			
8	1175	Myrcene						0.2
9	1192	2-Heptanone			0.1			
10	1202	3-Hexanol					0.6	
11	1203	Limonene	t	0.1		0.8	1.5	1.6
12	1218	β-Phellandrene			t			0.2
13	1220	*cis*-Anhydrolinalool oxide	t					
14	1225	2-Hexanol					1.1	
15	1244	Amyl furan (=*2-Pentyl furan*)	t		t			0.1
16	1246	(*Z*)-β-Ocimene						0.1
17	1255	γ-Terpinene			0.1	0.3	0.2	0.1
18	1266	(*E*)-β-Ocimene	t					
19	1280	*p*-Cymene			t		0.5	0.5
20	1290	Terpinolene			t		t	0.1
21	1296	Octanal	0.1	0.2	0.1	0.3		
22	1300	Tridecane					t	
23	1348	6-Methyl-5-hepten-2-one	0.3	1.1	0.1	2.6		0.2

			Aerial parts			Infloresence		
No	**RRI**	**Compound**	**Hppo**	**Hpi**	**Hppl**	**Hppo**	**Hpi**	**Hppl**
24	1400	Tetradecane					0.5	
25	1400	Nonanal	0.1	0.1	0.2	0.6		0.3
26	1448	*trans*-Linalool oxide (*Furanoid*)				0.7		0.1
27	1463	Theaspirane A	0.5		0.1	0.3		
28	1478	*cis*-Linalool oxide (*Furanoid*)				0.4		0.1
29	1490	Siphin-1-ene			0.1			0.1
30	1493	α-Ylangene			0.3			0.3
31	1495	Dihydroedulane I	0.1	0.5	0.3			
32	1496	2-Ethyl hexanol				0.3		
33	1497	α-Copaene						0.1
34	1500	Pentadecane					0.1	
35	1503	Vitispirane	0.1					
36	1505	Dihydroedulane II *	0.1	0.5				
37	1506	Decanal	0.1	0.4	t	0.4		0.1
38	1512	Theaspirane B	0.4	0.4		0.7		
39	1532	Camphor						0.3
40	1541	Benzaldehyde				0.3		0.2
41	1553	Linalool						1.0
42	1555	α-Isocomene			0.1			
43	1562	Octanol				0.3		
44	1581	Terpinen-1-ol					0.9	
45	1583	Isopulegol				0.2		
46	1587	6-Methyl-3,5-heptadiene-2-one				0.4		
47	1589	Bornyl acetate	0.1	0.2				
48	1593	Fenchyl alcohol				0.3		0.2
49	1610	Calarene (=*β-Gurjunene*)						t
50	1611	Terpinen-4-ol				0.4	0.4	0.1
51	1612	β-Caryophyllene			1.0	0.3		1.3
52		Hexadecene			0.1			
53	1617	6,9-Guaiadiene			0.9			1.8
54	1625	Cadina-3,5-diene						1.2
55	1644	Widdrene (=*Thujopsene*)	0.1					
56	1658	β-Guaiene			0.3			1.7
57	1661	Alloaromadendrene			0.6			
58	1668	(*Z*)-β-Farnesene			0.1			
59	1671	Acetophenone		1.9		12.4	0.4	
60	1681	2,6,6-Trimethyl-2-cyclohexen-1,4-dione* (=6-Oxoisophorone,Oxopholone)				t		
61	1686	4,6-Guaiadiene (= γ-*Guaiene*)			0.2			***0.1***
62	1687	α-Humulene				0.5		0.1
63	1689	Eremophila-1(10),7-diene* MassFinder						0.4
64	1693	Neral				0.2		
65	1700	Heptadecane					0.2	0.1
66	1707	Zizanene			0.5			0.2
67	1709	γ-Muurolene	0.1					
68	1710	α-Terpineol	t	0.1		0.6		0.3
69	1711	δ-Selinene			1.0			2.5
70	1712	Ledene			0.1			
71	1719	Borneol		0.1		0.3		0.1
72	1722	Dodecanal	0.1					

			Aerial parts			Infloresence		
No	**RRI**	**Compound**	**Hppo**	**Hpi**	**Hppl**	**Hppo**	**Hpi**	**Hppl**
73	1725	Verbenone				0.8	0.1	
74	1740	Geranial				0.2		
75	1742	β-Selinene			1.0			1.1
76	1744	α-Selinene	0.3		0.3			
77	1763	Naphthalene	0.1	0.1		0.2	0.2	
78	1766	Decanol	0.1					
79	1773	δ-Cadinene	0.1		0.3			0.2
80	1776	γ-Cadinene	t		0.2			0.1
81	1780	1,2-Dihydro-1,1,6-trimethyl naphthalene	0.2		0.1			
82	1786	*ar*-Curcumene				t		
83	1796	Selina-3,7(11)-diene			0.3			0.4
84	1798	Nerol						0.1
85	1802	Cumin aldehyde				t		
86	1800	Octadecane		t				
87	1838	(*E*)-β-Damascenone	t	0.1		0.3		0.1
88	1840	*E*-Anethole				0.1		
89	1845	*trans*-Carveol						0.3
90	1852	1-Methylethyl dodecanoate		0.3				
91	1853	*cis*-Calamenene			0.3			0.2
92	1868	(*E*)-Geranyl acetone	0.7	0.9	0.5	0.2		0.1
93	1884	1-Isobutyl 4-isopropyl 3-isopropyl-2,2-dimethyl succinate		2.0	0.5	0.2	0.2	0.1
94	1895	5,11-Epoxy-1(10)-cadinene			0.1			
95	1900	Nonadecane		0.2		t		0.1
96	1901	Neophytadiene isomer I	0.1		0.5			
97	1918	β-Calacorene			0.1			
98	1933	Tetradecanal						0.1
99	1941	α-Calacorene	0.1	0.1	0.6			0.5
100	1958	(*E*)-β-Ionone	0.1	0.2	0.5			0.2
101	1972	Dodecanol	0.1		0.1	0.2		0.1
102	1973	*cis*-Jasmone				t		
103	1975	Methyl tetradecanoate	0.1			0.3		
104	1981	Anisaldehyde				0.1		
105	1983	γ-Nonalactone				0.3		
106	1984	γ-Calacorene			0.3			
107	1992	Neophytadiene			0.2			
108	1993	2-Pentadecanone	0.2	0.2				
109	2000	Eicosane						0.1
110	2008	Caryophyllene oxide	0.3		0.9			0.1
111	2010	Anisaldehyde						0.1
112	2024	Ethyl tetradecanoate	0.1					0.1
113	2030	Methyl eugenol	0.1			2.1		
114	2032	Octanoic acid						**5.6**
115	2035	Epiglobulol			0.2			
116	2041	Pentadecanal				1.9	0.2	0.2
117	2050	(*E*)-Nerolidol	0.1	0.2	0.5	2.6	0.9	
118	2071	Humulene epoxide-II	t					
119	2073	β-Caryophyllene alcohol	t	0.1	1.4			0.2
120	2075	Guaiyl acetate			0.9			0.5
121	2083	1,10-di-*epi*-Cubenol		0.2	0.1			
122	2086	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo* (WileyNIST)				3.5		
123	2088	1-*epi*-Cubenol			0.2			0.4
124	2098	Globulol			2.2			2.8

			Aerial parts			Infloresence		
No	**RRI**	**Compound**	**Hppo**	**Hpi**	**Hppl**	**Hppo**	**Hpi**	**Hppl**
125	2100	Heneicosane	0.2					0.4
126	2104	Viridiflorol			0.3			
127	2131	Hexahydrofarnesyl acetone	3.0	1.9	2.6	1.1	0.2	0.5
128	2138	Selin-6-en-4-ol			0.8			0.3
129	2141	Clovenol		0.1	1.0			
130	2143	Rosifoliol	0.2		0.3			0.2
131	2150	Eremoligenol			0.7			
132	2153	Porosadienone						0.4
133	2156	6-*epi*-Cubenol			0.3			0.6
134	2157	Ambrox			t			
135	2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	0.8	0.4	t			t
136	2179	Tetradecanol	t	0.2			0.1	
137	2185	γ-Eudesmol	0.4					
138	2186	Eugenol				1.1		3.2
139	2187	T-Cadinol	0.2	0.1	**7.9**			**4.6**
140	2192	Nonanoic acid				1.2		0.8
141	2194	3,4-Dimethyl-5-pentyl-5H-furan-2-one				t		0.3
142	2196	Clovenol						0.1
143	2198	Thymol			t	1.2	0.2	0.3
144	2200	Docosane	0.1					
145	2209	T-Muurolol	0.3	0.1	0.3			0.1
146	2226	Methyl hexadecanoate (=*Methyl palmitate*)	0.2	0.3		0.5		
147	2240	Isophytol	0.2	0.3	0.5			
148	2250	α-Eudesmol	0.3		0.4	t		0.2
149	2255	α-Cadinol		0.1				
150	2256	Cadalene	t		0.9			0.1
151	2257	β-Eudesmol	1.0	0.2	3.3	2.0		2.0
152	2261	1-Methylethyl hexadecanoate (=*Isopropyl palmitate*)	t	t		**5.1**	0.1	0.3
153	2262	Ethyl hexadecanate (=*Ethyl palmitate*)	0.1	0.3				0.1
154	2271	Porosadienol			0.3			
155	2273	Selin-11-en-4α-ol	0.2					
156	2275	Galaxolide I	0.1	0.1				
157	2280	Galaxolide II	0.1					
158	2298	Decanoic acid	1.1	0.1		0.3		**6.4**
159	2300	Methyl dihydrojasmonate (=*Hedione*)				0.3		
160	2302	Tricosane	0.3	0.2	0.2	0.7		0.5
161	2310	Hexyl cinnamic aldehyde	0.2	0.2				
162	2324	Caryophylla-2(12), 6(13)-dien-5α-ol (=*Caryophylladienol II*)						0.1
163	2335	13-epi Manoyl oxide		0.3	0.7			
164	2375	α-Cyperone			0.7			0.1
165	2380	Dihydroactinidiolide						0.1
166	2384	Hexadecanol						0.3
167	2389	Caryophylla-2(12), 6-dien-5α-ol (=*Caryophyllenol I*)	0.2		0.5			0.3
168	2392	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)			0.4			
169		Farnesyl acetone	0.8	1.0	0.6			
170	2396	γ-Dodecalactone	0.2					0.1
171	2400	Tetracosane		0.1	0.1			0.2
172	2400	Undecanoic acid	0.1					

			Aerial parts			Infloresence		
No	**RRI**	**Compound**	**Hppo**	**Hpi**	**Hppl**	**Hppo**	**Hpi**	**Hppl**
173	2430	Ambrettolide						0.2
174	2490	Eicosanal				1.3		
175	2500	Pentacosane	1.1	2.1	1.6	0.8	0.4	2.4
176	2503	Dodecanoic acid	**9.6**	**10.4**	2.3	**9.6**	t	2.1
177	2509	(*Z,Z*)-9,12-methyl octadecadienoate (=*Methyl linoleate*)		0.2				
178	2524	Abietatriene			0.1			
179	2583	Methyl linolenate		0.3				
180	2589	γ-Tetradecanolide * MassFinder						0.2
181	2600	Hexacosane	0.2	0.3				
182	2607	1-Octadecanol		0.9		2.3		0.7
183	2608	δ-Tetradecalactone	0.5					
184	2610	Bis(2-ethylhexyl) maleate*				0.3		
185	2615	Manool	0.3					
186	2617	Tridecanoic acid	1.2	0.2				
187	2622	Phytol		0.8	2.5			
188	2655	Benzyl benzoate		0.1				
189	2680	4-Nonyl phenol isomer*	0.6					
190	2670	Tetradecanoic acid (= *Myristic acid*)	**27.8**	**37.9**	1.3	0.5		
191	2700	Heptacosane	1.5	1.7	1.2	2.0		0.2
192	2705	Nonyl phenol		1.0	2.2			
193	2765	γ-Palmitalactone	0.6					
194	2800	Methyl pimarate				3.6		
195	2805	1-Eicosanol		1.6		2.1		
196	2800	Octacosane	0.6					
197	2822	Pentadecanoic acid	2.5	0.3				
198	2900	Nonacosane	0.7	1.7		0.7		0.3
199	2931	Hexadecanoic acid	**28.0**	**15.4**	**21.3**	**15.2**	t	3.4
		Total Identified %	**90.5**	**91.2**	**73.9**	**97.4**	**98.3**	**90.4**

RRI: Relative retention indices calculated against *n*-alkanes (C_9-C_{30}); **%** calculated from FID data; **t** Trace (< 0.1 %); * tentatively identified.

The chemical class distribution of the volatile constituents detected in the studied subspecies is presented in Fig. 2. The compounds were classified as monoterpene ($C_{10}H_{16}$) and sesquiterpene ($C_{15}H_{24}$) hydrocarbons, and their oxygenated derivatives, fatty acids and esters, and *n*-alkanes. Quantification of volatiles components has led to the determination of 199 compounds, distributed among the three subspecies investigated.

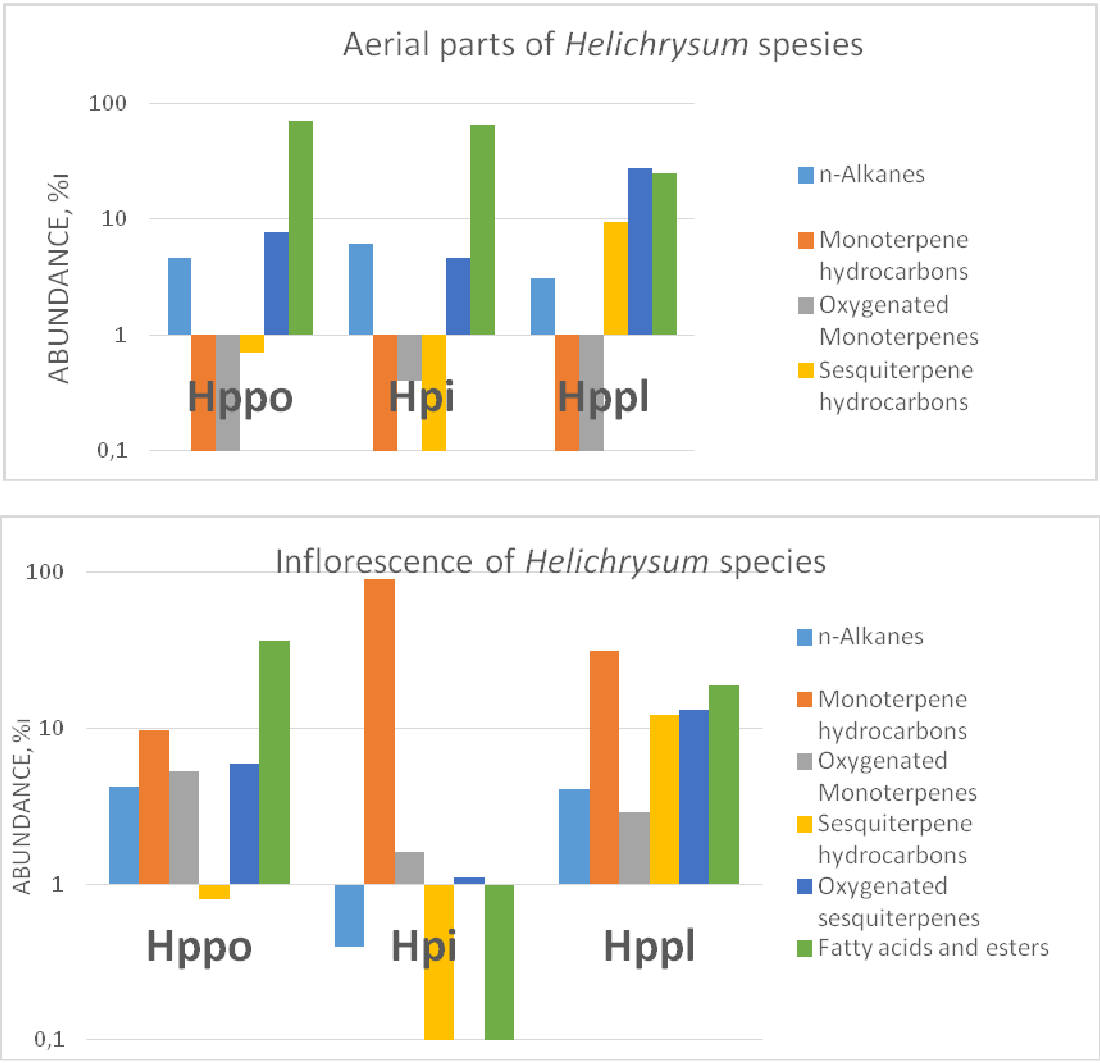

Figure 2. Distribution of main volatile compounds groups detected in the studied *Helichrysum plicatum* subspecies

Major compounds of aerial parts were presented by fatty acids with hexadecanoic acid (15.4-28.0%), tetradecanoic acid (1.3-37.9%) and dodecanoic acid (2.3-10.4%) as the major representatives of all oils. Especially *Hppo* and *Hpi* were characterized by high percentage of fatty acids and fatty acid esters (70.8% and 65.5% respectively). The sesquiterpenes were mostly detected in the aerial parts of *Hppl* (36.8%) with T- cadinol (7.9%) and β-eudesmol (3.3%) as major constituents.

The inflorescences of *Helichrysum* subspecies were found to be rich in monoterpenes (15.0-93.1%), fatty acids (0.1-36.3%) and sesquiterpenes (1.1-25.5%). The inflorescence volatiles of subsp. *isauricum* were distinguished by predomination of monoterpene hydrocarbons (93.1%) with fenchene (88.3%) as the major constituent. This compound has been reported from different *Helichrysum* species before [39-40] but this is the first report for including such as a high percentage.

The inflorescence of *Hppo* were characterized by monoterpenes (up to 15.0 %), with α-pinene (8.4%) as major constituent. The volatiles of *Hppl* inflorescence were rich in monoterpenes (up to 34.0%) with α-fenchene (18.2%) and α-pinene (10.4%) as major representatives. The sesquiterpenes were mostly presented in the inflorescence of *Hppl* and *Hppo* (25.5% and 6.7%, respectively) with T-cadinol (4.6%) and (E)-nerolidol (2.6%) as major constituents, respectively.

Fatty acids (36.3% and 18.8%) were presented by hexadecanoic acid (15.2%), dodecanoic acid (9.6%) in *Hppo,* decanoic acid (6.4%) and octanoic acid (5.6%) in *Hppl*. In a previous study

concerning with the essential oil composition of capitulums of *H. plicatum* subsp. *plicatum* sourced from east Turkey, fifty-four components representing 66% of the total oil were determined [33]. The major components were found as palmitic acid (11.8%), tetradecanoic acid (9.3%) and decanoic acid (6.7%) and α-pinene (2.5%) as major monoterpene. Variation in the percentages of fatty acids and monoterpenes between two volatiles may be due to the low percentage of identification of previous research and effect of ecological factors on essential oil composition.

This work represents the phytochemical approach to discriminate between three *H. plicatum* taxa from Turkey. To the best of our knowledge, this is the first chemical study on essential oils of aerial parts of all three *Helichrysum plicatum* subspecies and capitulums of *H. plicatum* subsp. *podophylum* and *H. plicatum* subsp. *isauricum.* Qualitative difference between essential oils allows differentiation between the taxa in agreement with the morphological and genetic observations described in Flora of Turkey for each taxon studied. The occurrence of volatile chemicals in aromatic plants is not only an indication of chemical diversity but may also help in solving taxonomical problems in comprehensively studied general.

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References

[1] R.J. Bayer, I. Breitwieaser, J. Ward and C.F. Puttock (2007). Tribe Gnaphalieae (Cass.) Lecoq & Juillet (1831).- In the families and genera of vascular plants. Vol VIII: Asterales, ed:J.W. Kadereit and C. Jeffrey. Springer Verlag, Berlin&Heidelberg, pp: 246-284

[2] A. Guner (2012). Turkiye Bitkileri Listesi (The List of Turkish Plants), Namas Matbaacilik San. Tic. A.Ş., Istanbul. 163-165.

[3] R. Jahn and P. Schönfelder (1995). Exkursionflora fur Kreta, Verlag Eugen Ulmer, Stuttgart, Germany.

[4] S.G. Senol, O. Secmen, B. Ozturk and M. Galbany-Casals (2011). *Helichrysum unicapitatum* (Asteraceae), a new species from Turkey, *Ann. Bot. Fennici,* **48(2)**, 145-154.

[5] K.H.C. Başer (2000). Index to Turkısh plant chemical contents, In: Flora of Turkey and the East Aegean Islands, ed: A. Guner, N. Ozhatay, T. Ekim and K.H.C. Baser. Edinburgh, University Press: Edinburgh. Vol. 11, pp: 513-616

[6] A. Scialabba, C. Agrimonti, G.M. Abbate and N. Marmiroli (2008). Assessment of genetic variation in Sicilian *Helichrysum* (Asteraceae) and implication to germplasm conservation, *Plant Biosyst*. **142(2),** 287-297.

[7] A. Bianchini, F. Santoni, J. Paolini, A.F. Bernardini, D. Mouillot, J. Costa (2009). Partitioning the relative contributions of inorganic plant composition and soil characteristics to the quality of *Helichrysum italicum* subsp. *italicum* (Roth) G. Don fil. essential oil. *Chem. Biodivers*. **6**, 1014-1033.

[8] I. Morone-Fortunato, C. Montemurro, C. Ruta, R. Perrini, W. Sabetta, A. Blanco, E. Lorusso, P. Avato (2010). Essential oils, genetic relationships and in vitro establishment of *Helichrysum italicum* (Roth) G. Don subsp. *italicum* from wild Mediterranean germplasm, *Ind. Crops Prod* **32**, 639-649.

[9] T. Fujita, E. Sezik, M. Tabata, E. Yesilada, G. Honda, Y. Takeda, T. Tanaka, and Y. Takaishi (1995). Traditional medicine in Turkey. VII. Folk medicine in middle and west Black Sea regions*, Econ. Bot.* **49**, 406-422.

[10] E. Sezik, M. Tabata, E. Yeşilada, G. Honda, K. Goto, Y. Ikeshiro (1991). Traditional medicine in Turkey. I. Folk medicine in northeast Anatolia, *J. Ethnopharmacol.* **35**, 191-196.

[11] E. Sezik, E. Yesilada, G. Honda, Y. Takaishi, Y. Takeda, T. Tanaka (2001). Traditional medicine in Turkey. X. Folk medicine in Central Anatolia. *J. Ethnopharmacol.* **75**, 95-115.

[12] M. Turan, A. Sokmen, K. Karadayı, Z. A. Polat, M. Şen (2010). Antineoplastic properties of some plant extracts which are endemic in Sivas, *Cumhuriyet, Med. J.* **32**, 9-18.

[13] B. Tepe, M. Sokmen, H. A. Akpulat, A. Sokmen (2005). In vitro antioxidant activities of the methanol extracts of four *Helichrysum* species from Turkey, *Food Chem.* **90**(4), 685-689.
[14] H.E. Eroglu, A. Aksoy, E. Hamzaoglu, U. Budak, S. Albayrak (2009). Cytogenetic effects of nine *Helichrysum* taxa in human lymphocytes culture*, Cytotechnology* **59(1)**, 65-72.
[15] M.T. Uguz (2011). Farkli çözücülerdeki bitki ekstraktlarinin antifungal özellikleri, *Science J. Bingöl Univ.* **1(2)**, 1-3.
[16] M.T. Uguz, M. Digrak, and M. Celik (1998). Bazı bitki türlerinin antimikrobiyal aktivitelerinin incelenmesi, *II. International Kizilirmak Science Congress*, Kirikkale, Turkey, 206-215.
[17] A. Demir, B. Mercanoğlu Taban, M. Aslan, E. Yesilada, S.A. Aytaç (2009). Antimicrobial effect of *Helichrysum plicatum* subsp. *plicatum*, *Pharm. Biol.* **47**(4), 289-297.
[18] T. Özbek, M. Güllüce, A. Adiguzel, H. Özkan, F. Şahin, F. Orhan (2009). Antimutagenic activity of the methanol extract of *Helichrysum plicatum* ssp. *plicatum*, *As. J. Chem.* **21**(4), 2705-2710.
[19] M. Sezik, M. Aslan, D.D. Orhan, E. Erdemoglu, M. Pekcan, T. Mungan, E. Sezik (2010). Improved metabolic control and hepatic oxidative biomarkers with the periconception use of *Helichrysum plicatum* ssp. *plicatum*, *J. Obstetr. Gyn.* **30**(2), 127–131.
[20] Y. Bayir, Z. Halici, M.S. Keles, S. Colak, A. Cakir, Y. Kaya, F. Akcay (2011). *Helichrysum plicatum* DC. subsp. *plicatum* extract as a preventive agent in experimentally induced urolithiasis model, *J. Ethnopharmacol*. **138**, 408– 414.
[21] B. Ozturk, E. Kilinc, S. Konyalioglu, N.U. Karabay Yavasoglu, U. Zeybek (2008). Türkiye'deki *Helichrysum* Geartner taksonlarının morfolojik, kimyasal ve biyoaktivite potansiyelleri açısından karşılaştırılması" *XVIII. Bitkisel Ilaç Hammaddeleri Toplantısı*, Istanbul.
[22] S. Albayrak, A. Aksoy, O. Sagdıc and U. Budak (2010). Phenolic compounds and antioxidant and antimicrobial properties of *Helichrysum* species collected from eastern Anatolia, Turkey. *Turkish J. Biol*. **34(4)**, 463-473.
[23] O. Kucukoglu, B. Ozturk, T. Kamataki, and Z. Topcu (2006). Inhibitory activities of *Helichrysum* taxa on mammalian type I DNA topoisomerase, *Pharm.Biol*. **44(3)**, 189-193
[24] M. Aslan, D.D. Orhan, N. Orhan, E. Sezik, E. Yesilada (2007). In vivo antidiabetic and antioxidant potential of *Helichrysum plicatum* subsp. *plicatum* capitulums in streptozotocin-induced-diabetic rats. *J. Ethnopharmacol*. **109(1)**, 54-9.
[25] B. Cubukcu, A.H. Mericli, B. Damadyan, S. Bingol, V. Yuksel (1986). Yukari Firat bolgesinde yetisen *Helichrysum* turlerinin flavonoid bilesikleri yonunden incelenmesi, *Fırat Havzası Tıbbi ve Endustriyel Bitkiler Sempozyumu*: Elazig, Turkey. 179-187.
[26] J.C. Chalchat and M. Özcan (2006). Composition of the essential oil of *Helichrysum chasmolycicum* growing wild in Turkey, *J. Med. Food* **9**(2), 287-289.
[27] E. Czinner, É. Lemberkovics, E. Bihátsi-Karsai, G. Vithyi and L. Lelik (2000). Composition of the essential oil from inflorescence of *Helichrysum arenarium* (L.) Moench. *J. Essent. Oil Res.* **12**, 728-730.
[28] C. Formisano, E. Mignola, D. Rigano, F. Senatore, N.A. Arnold, M. Bruno and S. Rosselli. (2009). Constituents of leaves and flowers essential oils of *Helichrysum pallasii* (Spreng.) Ledeb. growing wild in Lebanon. *J. Med. Food* **12(1)**, 203-207.
[29] B. Cubukcu and A.H. Mericli (1977). Flavonoides *d'Helichrysum plicatum* DC. *Plantes Medicinales et Phytotherapie* **11**, 294-302.
[30] B. Cubukcu and A.H. Mericli (1979). Flavonoides *d'Helichrysum plicatum* DC. subsp. *polyphyllum* (Ledeb.) Davis-Kupicha*, Plantes Medicinales et Phytotherapie* **13**, 107-115.
[31] J. B. Harborne (1977). Chemosystematics and coevolution, *Pure Appl. Chem.* **49**, 1403-1421.
[32] A.J. Poulose and R. Croteau (1978). Biosynthesis of aromatic monoterpenes. *Arch. Biochem. Biophys.* **187**, 307-314.
[33] E. Sezik and M. Aslan (2000). Chemical composition of the volatile oil of *Helichrysum plicatum* D.C. ssp. *plicatum*. *J. Fac. Pharm. Gazi Univ* **17(2)**, 95-98
[34] European Pharmacopoeia (2005). Fourth Edition, Council of Europe, Strasburg
[35] G. Ozek, F. Demirci, T. Ozek, N. Tabanca, D.E. Wedge, S.I. Khan, K.H.C. Başer, A. Duran and E. Hamzaoğlu (2010). Gas chromatographic–mass spectrometric analysis of volatiles obtained by four different techniques from *Salvia rosifolia* Sm., and evaluation for biological activity. *J. Chrom. A*, **1217**,741-748.

[36] D.H. Hochmuth (2008). *MassFinder-4*, Hochmuth Scientific Consulting: Hamburg, Germany.
[37] R.P. Adams (2007). Identification of essential oil components by gas chromatography/mass spectroscopy. Fourth ed. Allured publishing Co. Carol Stream, Illinois.
[38] 08", N., Mass spectral library (NIST/EPA/NIH), (2008). National Institute of Standars and Technology, Gaithersburg, USA.
[39] L.B. Larijerson, B.J.R. Rabehaja, J.F. Rajaonarison and S.R. Urverg (2005). Comparison between the fresh and dry essential oil of *Helichrysum faradifani* Scott Elliot from Madagascar. *J. Essent. Oil. Res*.**17**, 597-600
[40] C. Bougatsos, O. Ngassapa, D.K.B. Runyoro and I.B. Chinnou (2004). Chemical composition and in vitro antimicrobial activity of the essential oils of two *Helichrysum* species from Tanzania, *Z. Naturforsch*. **59(c)**, 368-372