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Essential oils and volatile emission of eight South African species of *Helichrysum* grown in uniform environmental conditions

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ABSTRACT

This paper reported for the first time the aroma profile and essential oil composition of eight species of *Helichrysum* endemic in South Africa but grown in Italy (CREA-Sanremo collection). The volatiles of all the studied *Helichrysum* were dominated by monoterpene hydrocarbons, except for *H. basalticum*, where sesquiterpene hydrocarbons was the main class of compounds with β-caryophyllene (13.7%) and α-guaiene (11.9%) as major components.

Despite the great diversity of compounds obtained in the volatile emission (more than 130), only four compounds were responsible for at least 34% of the identified fraction. These compounds were: sabinene (16.0%), β-caryophyllene and α-guaiene in *H. basalticum*; sabinene (57.7%) in *H. foetidum*; (Z)-β-ocimene (34.6%), tricyclene (16.2%) and myrcene (10.0%) in *H. incanatum*; myrcene (29.3%), tricyclene (21.5%) and limonene in *H. indicum*; tricyclene (32.1%) and (E)-3-hexanol acetate (20.1%) in *H. montanum*; limonene (10.3%), sabinene (8.9%), 1-decene (7.6%) and 1-hexanol (7.0%) in *H. mutabile*; α- and β-pinene in *H. patulum* (27.6% and 44.9%, respectively) and α-pinene (36.3%) and α-fanchene(15.6%) in *H. setosum*.

The EOs composition of these species was also different from each other with sesquiterpene compounds as the prevalent class. Valerianol (16.3%, os, in *H. basalticum*), ledol (16.3%, os, in *H. foetidum*), β-caryophyllene (11.0% and 13.4%, sh, in *H. indicum* and *H. patulum*, respectively), viridiflorol (18.3%, os, in *H. montanum*) and valerianol (30.1%, os in *H. setosum*) were found to be the main constituents. It is worthy to highlighting that the *H. incarnatum* EO showed a similar behaviour to that of the spontaneous emission with a predominance of monoterpene hydrocarbons (60.7% in EO vs 81.4% in VOC), both represented by (Z)-β-ocimene as main constituent.

1. Introduction

Helichrysum Mill. genus (Asteraceae family, tribe Inuleae and subtribe Gnaphaliinae) (Lourens et al., 2008) consists of about 600 species widespread through Eurasia and Australia (Ghassemi-Dehkordi et al., 2015). Most of the species are distributed throughout South Africa (245 species), Africa and Madagascar (Bougatsos et al., 2003). *Helichrysum* species display great morphological diversity which has enabled them to divide into 30 groups. They are annual, biennial, perennial, sub-shrub or shrubby plants which are generally erect, sometimes prostrate, usually woolly often with glandules, and rarely hairless. They are sometimes dwarf and cushion forming. The flower heads are solitary or occur in compact or spreading inflorescences, and are often aromatic (Pooley, 2003). They grow in sandy grasslands or open woodlands (Bougatsos et al., 2003).

Helichrysum species are well recognised for their medicinal properties by the indigenous people of Africa, and traditionally used as diuretic, anti-inflammatory, antiallergic agents (Al-Rehaily et al., 2008), for wound healing, infections, respiratory issues (Lourens et al., 2004), as well as hepatoprotective and anti-psoriasis remedies (Satta et al., 1999). Recently, different authors have confirmed their antimicrobial (Antunes Viegas et al., 2014), anti-inflammatory (Legoalea et al., 2013), and antioxidant activities (Bigovic et al., 2011). Phytochemical studies have evidenced different compounds in *Helichrysum* extracts such as: phenolics (e.g. flavonoids and chalcones, phthalides), α-pyron derivatives, terpenoids, and fatty acids (Czinner et al., 2000). Lourens and collaborators (2008), reported the phytochemistry of *H. foetidum*, *H. indicum* and *H. patulum* as well as *H. montanum* and they found flavonoids derivatives, phloroglucinols, terpenes, diterpenes, sesquiterpenes and coumarins. Several papers have illustrated the EO composition of some species of *Helichrysum* collected from around the word, even though the number of studied species was limited in comparison with the large biodiversity of this genus.

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The EO composition of some *Helichrysum* spp. grown in CREA collection (Centro di Recerca Orticolture e Florovivaismo, Sanremo, Italy) has already been reported by different authors (Bandeira Reidel et al., 2017; Leonardi et al., 2018; Giovanelli et al., 2018)

Continuing our research on the aroma profile (SPME) and EO composition of *Helichrysum* spp. grown in Italy (CREA collection) eight new South African *Helichrysum* species have been studied for their ornamental use or as a source of bioactive compounds: *Helichrysum basalticum* Hilliard, *Helichrysum foetidum* (L.) Cass, *Helichrysum incarnatum* DC, *Helichrysum indicum* (L.) Grierson, *Helichrysum montanum* DC, *Helichrysum mutabile* Hilliard, *Helichrysum patulum* (L.) D.Don, and, *Helichrysum setosum* Harv. Most of these species had already been studied in their polar composition but, to the best of our knowledge, no data have been reported in the literature about their essential oil composition as well as their spontaneous emission.

2. Experimental procedures

2.1. Plant material

This study involved eight South African species of *Helichrysum* (See Fig. 1): *Helichrysum basalticum* (*H. bas.*), *Helichrysum foetidum* (*H. foe.*), *Helichrysum incarnatum* (*H. inc.*), *Helichrysum indicum* (*H. ind.*), *Helichrysum montanum* (*H. mon.*), *Helichrysum mutabile* (*H. mut.*), *Helichrysum patulum* (*H. pat.*), and *Helichrysum setosum* (*H. set.*) maintained in CREA collection (Sanremo, Italy). The plants were grown from seeds obtained by a seed company (Silver Hill – P.O. Box 53108, Kenilworth, 7745 Cape Town, South Africa and B & T World Seeds – Paguignan, 34210 Aigues Vives, France) and cultivated in pots under the same edaphic and climatic conditions. After the propagation period, the plants were grown in pots in open air with a periodic water supply. The agronomic conditions were reported in a previous paper (Leonardi et al., 2018). The final pot size was 12-liter and plants flowered after 1 year of cultivation.

Voucher specimens were deposited at the herbarium of Giardini Botanici Hanbury (La Mortola–Ventimiglia, Imperia, Italy). A complete list of their botanical characteristics and habitus, together with voucher number are reported in Table 1. The correct identification of the plant material was accomplished by Claudio Cervelli.

2.2. Essential oil extraction

Aerial parts were collected during the blossoming season (in 2017 and 2018) and hydro-distilled using Clevenger apparatus for 2 h. The obtained oils were stored in dark at 4 °C and successively analysed by GC-MS.

2.3. Volatile organic compounds analyses

Fresh plants of each species in pot were used for the analyses of spontaneous emission (SPME), which was performed with polydimethylsiloxane fiber (PDMS, 100 μm) and according to the previously described method (Giovanelli et al., 2018).

2.4. GC-MS analysis

GC-MS was performed to determine the composition of both VOCs and essential oils was performed using the method reported by Demasi et al. (2018) (see Table 2).

2.5. Statistical analyses

Results were submitted to multivariate statistical analysis using the Past 3 software package' ver. 3.1J. The Hierarchical Cluster Analysis (HCA) is a method in which samples are considered as lying in a *n*-dimensional space and distances between samples are calculated, joining the object with an agglomerative procedure. To carry out the PCA analysis, Ward's method was used basing a Euclidean distance measure of similarity.

3. Results and discussion

3.1. VOC emission

The aroma profile of the studied *Helichrysum* species revealed a total of 132 different compounds (Table 2.). The volatile composition differed from each other and only four compounds (sabinene, limonene, γ-terpinene and β-caryophyllene) were present in all the *Helichrysum* emissions, although the total number of constituents was higher and varied from 24 (in *H. indicum*, *H. patulum* and *H. incarnatum*) to 54 in the *H. foetidum* aroma profile. These latter species, along with *H. setosum*, were rich in monoterpene hydrocarbons: 89.8%, 85.7%, 81.4%, 72.6%, 75.8%, respectively. Monoterpene hydrocarbons was also the major class of volatile constituents in *H. mutabile* and *H. montanum* (45.5% and 45.3%, respectively). These last two species were characterised by a high amount of non-terpene derivatives which ranged from 28.2% in *H. mutabile* to 38.2% in *H. montanum*, while all the others contained percentages lower than 1.3%. Only *H. basalticum* was characterised by the highest percentage of sesquiterpene

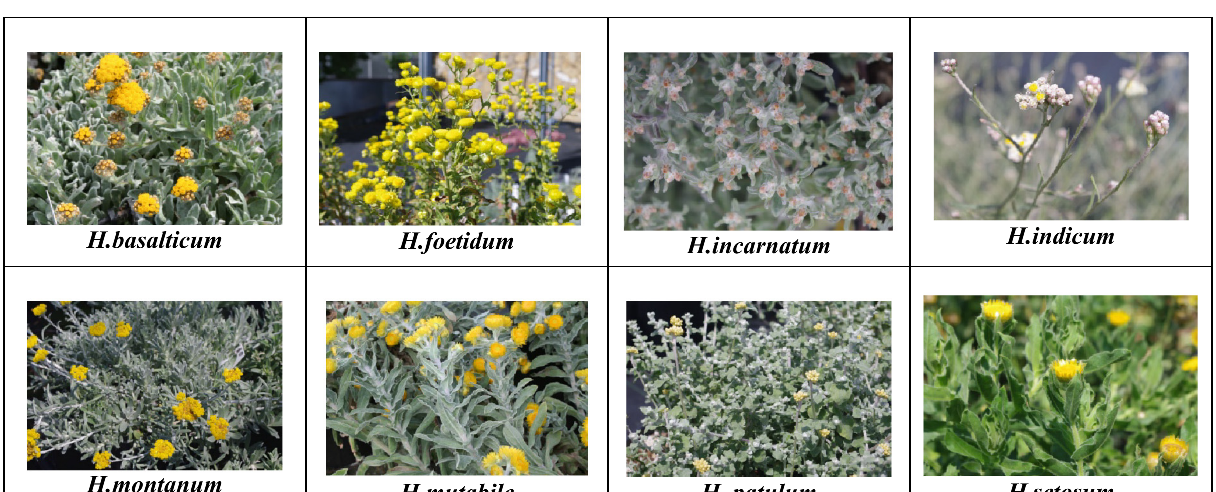

Fig. 1. Images of the eight *Helichrysum* spp. studied

Table 1
Main information of the eight *Helicrysum* spp. studied herein.

Helichrysum species	Botanical characteristics and habitat
Helichrysum basalticum Hilliard *(H. bas.)*	*Habitat*: Small mats on basalt sheets and bare stony ground. *Life form and plant size*: Perennial herb *Diagnostic characters*: Velvety leaves, large and broad Basal leaf rosette present Medium-sized heads, often felted together at the base Compact inflorescence *Blossoming*: February–April *Voucher No*: HMGBH.e/9006.2017.001
Helichrysum foetidum (L.) Cass. *(H. foe.)*	*Habitat*: Often a constituent of shrubby growth in valleys, on hill and mountain slopes, particularly in damp places along streams or on forest margins. *Life form and plant size*: Stout biennial herb, up to c. 1 m tall *Diagnostic characters*: Leaves with glandular upper suface, lower surface white tomentose Large heads in open branched inflorescences Bright yellow bracts *Blossoming*: October–May *Voucher No*: HMGBH.e/9006.2017.003
Helichrysum incarnatum DC. *(H. inc.)*	*Habitat*: Sandy flats in arid biomes *Life form and plant size*: Bushy half-shrub c. 100–200 mm tall *Diagnostic characters*: Bracts rosy pink, lanceolate. opaque Large solitary flower heads *Blossoming*: September–November *Voucher No*: HMGBH.e/9006.2018.002
Helichrysum indicum (L.) Grierson *(H. ind.)*	*Habitat*: Sandy flats and slopes. *Life form and plant size*: Annual herb, stems 70–300 mm long *Diagnostic characters*: Branched flower heads White-cream bracts Medium-sized heads *Blossoming*: October–February *Voucher No*: HMGBH.e/9006.2018.003
Helichrysum montanum DC. *(H. mon.)*	*Habitat*: Sthick mats on cliff faces, in rocky gullies, and on rock outcrops on steep mountain slopes. *Life form and plant size*: Mat-forming dwarf shrub ca. 100–450 mm tall, often 1 m or more across *Diagnostic characters*: Bright yellow bracts Large heads Compact inflorescences *Blossoming*: January–April *Voucher No*: HMGBH.e/9006.2017.005
Helichrysum mutabile Hilliard *(H. mut.)*	*Habitat*: Rocky sites in woodland or in grassland near forest patches. *Life form and plant size*: Perennial herb up to ca. 1 m tall *Diagnostic characters*: Large heads in compact branched inflorescences Bright yellow (brownish) bracts Leaves woolly to glabrous *Blossoming*: March–May *Voucher No*: HMGBH.e/9006.2017.006
Helichrysum patulum (L.) D. Don *(H. pat.)*	*Habitat*: Sshrub communities on coastal dunes and inland on S-facing mountain slopes up to c. 600 m above sea level. *Life form and plant size*: Well-branched subshrub up to 1 m tall *Diagnostic characters*: Medium-sized heads Compact inflorescence Cream bracts Small hairy leaves *Blossoming*: September–February, mainly in December–January *Voucher No*: HMGBH.e/9006.2018.005
Helichrysum setosum Harv. *(H. set.)*	*Habitat*: *Life form and plant size*: Herbaceous perennial or subshrub, stems up to 1 m tall *Diagnostic characters*: Bright yellow bracts Large solitary heads *Blossoming*: May–August *Voucher No*: HMGBH.e/9006.2017.007

hydrocarbons (58.5%) in its spontaneous emission, which make it distinctive from the other spp. where the amount of this class of compounds did not exceed 18.7% (*H. foetidum*) of the total composition. The analysis of the single compounds showed that only two or three compounds were responsible of at least 34% of the total volatile composition. *Helichrysum mutabile* evidenced limonene (10.3%) as the major compound, followed by sabinene (8.9%), 1-decene (7.6%) and 1-hexanol (7.0%). Alpha and β-pinene together reached 72.5% of the total composition of *H. patulum*, while 51.9% of the *H. setosum* VOCs was represented by α-pinene and α-fenchene (36.3 and 15.6 %, respectively). Myrcene (29.3%), tricyclene (21.5%) and limonene (20.7%) were the main constituents of *H. indicum*, whereas (Z)-β-ocimene (34.6%), tricyclene (16.2%) and myrcene (10.0%) characterised the volatiles emitted by *H. incarnatum*. *Helichrysum foetidum* showed the highest amount of sabinene which alone was the 57.7% of the total composition. However, sabinene (16%) together with β-caryophyllene (13.7%) and α-guaiene (11.9%) were the most abundant constituents of *H. basalticum*. *Helichrysum montanum* was characterised by tricyclene (32.1%) and (E)-3-hexanol acetate (20.1%).

The dendrogram obtained with the HCA (Fig. 2) showed the specificity of *H. basalticum* which alone constitutes a sub-group apart (Sup-group II.a) and, as previously reported, was characterised by its high percentage of sesquiterpene hydrocarbons. Sup-group II.b represented by both *H. mutabile* and *H. montanum* exhibited an important amount of non-terpene derivatives if compared to all the other species analysed. Group I included *H. indicum*, *H. patulum*, *H. setosum*, *H. incarnatum* and *H. foetidum* where the predominance of monoterpene hydrocarbons was evidenced.

Only a few studies detailed the volatiles composition of some South African species of *Helichrysum*. Bandeira Ridel et al. (2017) highlighted a high percentage of sesquiterpene hydrocarbons in flower EO of *H. nudifolium* (92% of identified fraction) with β-caryophyllene (79,4%) and bicyclogermacrene (5.5%) as the main constituents. 93.7% of identified compounds in leaf EO of the same species were represented by non-terpene derivatives with (Z)-3-hexenol acetate (70.5%) and 3-octanone (6.6%) as the most abundant compounds. Another four South African species were also investigated for their aroma profile in the work of Giovanelli et al. (2018): *H. cymosum*, *H. odoratissimum*, *H. petiolare* and *H. tenax*. All these oils were characterised by their relevant percentage of monoterpene hydrocarbons, which accounted for at least 51% of the identified VOCs. Three *Helichrysum* spp. (*H. odoratissimum*, *H. petiolare* and *H. tenax*) showed α- and β-pinene as major compounds even though their percentage varied from one species to the other. These results were in agreement with all *Helicrysum* spp. studied herein, except for *H. basalticum*, especially for their main class of compounds even though the composition wasn't the same and α- and β-pinene constitute the major compounds only for *H. setosum* and *H. patulum*. On the contrary *H. cymosum* was noted for its large amount of both *(Z)* and *(E)*-β-ocimene (44% and 6.6%, respectively). These latter compounds were present among the identified fraction of *H. incarnatum* with a good amount. β-caryophyllene was also one of the main constituents of *H. cymosum* and *H. odoratissimum* (8.5% and 12.7%, respectively), present herein in similar percentage only in *H. basalticum* and *H. foetidum*.

3.2. Essential oil profile

The composition of *Helichrysum* essential oils is reported in Table 3. 234 different compounds were reported accounting for at least 91% of the total composition. The extraction yield was very low for half of the studied species (*H. mutabile*, *H. montanum*, *H. basalticum* and *H. indicum*) and approx. 0.1% (*v*/*w*) for the other species.

All the studied oils shared a common unique compound, β-selinene, a sesquiterpene hydrocarbon, whose percentage ranged from 0.1% (*H. incarnatum* and *H. foetidum*) to 0.9% (*H. basalticum*).

Oxygenated sesquiterpenes dominated in all the EOs, ranging from 31.2% (*H. indicum*) to 74.4% in *H. foetidum*, except for *H. incarnatum* and

Table 2
Volatile emission profiles of the analysed *Helichrysum* spp.

	Compounds			*H.bas*	*H.foe*	*H.inc*	*H.ind*	*H.mon*	*H.mut*	*H.pat*	*H.set*
1	1-octene	nt	790						5.9		
2	*(E)*-2-hexenal	nt	860							0.8	
3	*(E)*-3-hexan-1-ol	nt	868			0.1		3.7			0.2
4	1-hexanol	nt	875		0.1			2.1	7.0	0.1	
5	Bicyclo[2.2.1]hept-2-ene. 1.7.7-trimethyl-	mh	909					0.3			4.8
6	santolina triene	mh	910				0.1		0.7		
7	5.5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	mh	920				1.3				
8	α-thujene	mh	932		0.7			0.1	4.6	0.3	0.5
9	tricyclene	mh	938	5.5	6.2	16.2	21.5	32.1	3.5		
10	α-pinene	mh	940							27.6	36.3
11	α-fenchene	mh	951								15.6
12	camphene	mh	955	0.6	0.1	0.3	2.8	2.5	1.4	0.3	
13	1-heptanol	nt	973					2.3	0.6		
14	sabinene	mh	978	16.0	57.7	2.0	4.5	0.3	8.9	0.9	1.9
15	β-pinene	mh	981			6.7		3.3	4.0	44.9	
16	3-octanone	nt	987						0.3		
17	1-decene	nt	991						7.6		
18	myrcene	mh	993	0.5	0.8	10.0	29.3	2.1		2.6	1.0
19	6-methyl-5-hepten-3-one	nt	995					0.1			
20	2-octanol	nt	998						0.1		
21	*(E)*-3-hexanol cetate	nt	1004			0.1		20.1			
22	α-phellandrene	mh	1006	0.1							0.3
23	*(Z)*-3-hexanol acetate	nt	1008	0.6	0.4			3.0	0.9		0.4
24	δ-3-carene	mh	1012			0.2					
25	α-terpinene	mh	1019	0.5	1.4		0.6		1.9	0.2	8.8
26	4-Hexen-1-ol. acetate	nt	1024					1.6			
27	*p*-cymene	mh	1028	0.3	0.7		0.6	0.9	2.4		1.8
28	limonene	mh	1032	2.0	2.4	4.0	20.7	3.3	10.3	2.9	2.6
29	β-phellandrene	mh	1033		1.0						
30	1,8-cineole	om	1036	4.4		2.1		1.9	2.6	6.8	
31	*(Z)*-β-ocimene	mh	1042			34.6	2.5			5.2	0.1
32	*(E)*-β-ocimene	mh	1053		0.2	7.1	2.5		0.3	0.3	0.6
33	γ-terpinene	mh	1062	1.4	1.1	0.1	1.7	0.6	5.5	0.3	0.7
34	*cis*-sabinene hydrate	om	1072		0.8			1.2	3.5	0.2	0.1
35	1-octanol	nt	1075					0.4			
36	terpinolene	mh	1090	0.4	0.5	0.3	1.7		1.9	0.1	0.6
37	2-nonanone	nt	1095						5.8		
38	*trans*-sabinene hydrate	om	1101		3.0				2.1		
39	linalool	om	1102					0.3	1.3		0.2
40	isopentyl isovalerate	nt	1106					1.5			
41	*trans*-thujone	om	1108						0.4		
42	*exo*-fenchol	om	1116				0.2				
43	*(E)*-4.8-Dimethylnona-1.3.7-triene	nt	1117					1.1			
44	*cis-p*-menth-2-en-1-ol	om	1126					1.2	0.1		4.7
45	*allo*-ocimene	om	1133			2.0				0.2	
46	terpinen-1-ol	om	1138								0.1
47	*trans*-pinocarveol	om	1142		0.2			0.2			
48	camphor	om	1148			0.1					
49	hexyl isobutyrate	nt	1153					0.1			
50	pinocarvone	om	1166					0.1			
51	borneol	om	1169				0.1				
52	pinocampheol	om	1170					0.1			
53	artemisyl acetate	om	1173						0.1		
54	*cis*-pinocamphone	om	1177		0.1						
55	4-terpineol	om	1180	0.2	0.5			0.1	0.9		0.3
56	α-terpineol	om	1192	0.2							
57	myrtenol	om	1195		0.3						3.6
58	1-octanol acetate	nt	1214					0.7			
59	nerol	om	1228								1.9
60	butanoic acid. 3-methyl-3-hexen-1-yl ester (Z)	nt	1236	0.3							
61	heptyl isobutyrate	nt	1250					0.5			
62	*trans*-ethyl chrysanthemumate	nt	1277		0.9						
63	*trans*-pinocarvyl acetate	om	1300					0.1			
64	Methyl 6.6-dimethylbicyclo[3.1.1]hept-2-ene-2-carboxylate	om	1301		0.1						0.4
65	*iso*-ascaridole	om	1305		0.1						0.1
66	myrtenyl acetate	om	1327		0.2						0.2
67	δ-elemene	sh	1340			0.5			0.1		
68	cyclosativene	sh	1371					0.2	3.1		0.2
69	α-ylangene	sh	1375						0.1		
70	*iso*-ledene	sh	1375	1.1		0.1					
71	α-copaene	sh	1376	0.6	0.4		0.1	0.6	0.9	0.6	2.0
72	β-bourbonene	sh	1383		0.8				1.0		
73	β-elemene	sh	1392		0.6						
74	sativene	sh	1395						0.2		
75	italicene	sh	1406		0.2						

(continued on next page)

Table 2 (*continued*)

	Compounds			H.bas	H.foe	H.inc	H.ind	H.mon	H.mut	H.pat	H.set
76	β-isocomene	sh	1407								0.1
77	α-gurjunene	sh	1410	3.4	0.9	0.1					
78	*cis*-α-bergamotene	sh	1417	8.7							
79	β-cedrene	sh	1418								1.0
80	β-caryophyllene	sh	1418	13.7	9.0	0.8	5.6	0.7	2.8	2.7	0.1
81	β-copaene	sh	1429		0.3				0.7		0.1
82	β-gurjunene	sh	1434	0.1							
83	*trans*-α-bergamotene	sh	1437	3.6		0.1					
84	α-guaiene	sh	1440	11.9	0.9	0.3		0.7	1.3		0.2
85	aromadendrene	sh	1445	0.3	0.2						
86	selina-5.11-diene	sh	1446	1.3							
87	*epi*-β-santalene	sh	1449	0.3							
88	α-*neo*-clovene	sh	1454		0.2						
80	α-humulene	sh	1456	0.7	0.4	6.3	1.5		0.2	0.7	
90	*(E)*-β-farnesene	sh	1460		0.1						
91	*allo*-aromadendrene	sh	1461	1.8	0.2			0.2		0.1	
92	dehydro-aromadendrene	sh	1463						0.1		
93	α-acoradienne	sh	1464	0.2							
94	9-*epi*-*(E)*-caryophyllene	sh	1467	0.2							
95	γ-patchoulene	sh	1474								0.1
96	β-chamigrene	sh	1475	0.1				0.3			
97	γ-gurjunene	sh	1476	0.4							
98	γ-muurolene	sh	1477				0.2		0.5		0.2
99	γ-curcucumene	sh	1481	0.2						0.1	
100	germacrene D	sh	1481	4.0	2.8				1.7		
101	2-Isopropenyl-4a.8-dimethyl-1.2.3.4.4a.5.6.7-octahydronaphthalene	sh	1485					0.5			2.8
102	β-selinene	sh	1485	1.3	0.3			0.4			0.3
103	*cis*-β-guaiene	sh	1489	0.6							
104	α-selinene	sh	1495		0.1						
105	bicyclogermacrene	sh	1495		0.2	6.0		1.2	1.3		
106	valencene	sh	1496		0.1		0.1				0.5
107	epizonarene	sh	1498				0.2				
108	β-dihydrogarofuran	os	1499					1.5			
109	*trans*-β-guaiene	sh	1500		0.1				0.2		
110	α-bulnesene	sh	1505		0.4						0.2
111	β-bisabolene	sh	1509	1.0	0.2						
112	lavandulyl isovalerate	om	1510				0.8	0.2			
113	β-curcumene	sh	1513	0.6							
114	*trans*-γ-cadinene	sh	1513		0.3			0.1	0.2		0.1
115	*(Z)*-γ-bisabolene	sh	1515	0.6							
116	7-epi-α-selinene	sh	1519		0.1			0.1			0.5
117	δ-cadinene	sh	1523	0.1	0.2		0.4	0.2	0.2	1.4	0.2
118	*(E)*-γ-bisabolene	sh	1535	1.6							
119	*cis*-sesquisabinene hydrate	os	1545	0.4							
120	germacrene D-4-ol	os	1576		0.1						
121	spathulenol	os	1577					0.2			
122	caryophyllene oxide	os	1582	0.5	0.2			0.5			
123	globulol	os	1584		0.1					0.1	
124	viridiflorene	os	1593	4.1	0.6						0.8
125	myrtenyl angelate	os	1611								0.7
126	humulane1-6-dien-3-ol	os	1615								0.4
127	agaruspirol	os	1643	0.6							
128	trans-Guai-11-en-10-ol	os	1655		0.1						
129	intermedeol	os	1667					0.1			0.1
130	valeranone	os	1674		0.8						
131	3-heptanone 4-methyl	nt					0.8				
132	heptanol acetate	nt						1.1			
	Unknown			2.8	0.3	0.2	0.2	3.7	1.2	0.7	1.7
	Total identified			97.2	99.7	99.8	99.8	96.3	98.8	99.3	98.3
	Class of compound			H.bas	H.foe	H.inc	H.ind	H.mon	H.mut	H.pat	H.set
	Monoterpene hydrocarbons (mh)			27.3	72.6	81.4	89.8	45.3	45.5	85.7	75.8
	Oxygenated monoterpenes (om)			4.8	5.2	4.1	1.1	5.4	11.0	7.1	11.5
	Sesquiterpene hydrocarbons (sh)			58.5	18.7	14.1	8.1	5.1	14.2	5.6	8.6

H. patulum, where the main class of constituents was represented by monoterpene hydrocarbons (60.7%) and sesquiterpene hydrocarbons (36.4%), respectively. It is worth noting that the percentages of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated diterpenes in *H. mutabile* EO was approximately the same, around 12% (11.1% of mh, 13.1% of om, 14.1% of sh and 12.6% of od). *Helichrysum patulum* showed a similar amount of monoterpene hydrocarbons as well as oxygenated sesquiterpenes (26.7% and 29.2%, respectively). On the contrary *H. indicum* and *H. mutabile* evidenced a good percentage of non-terpene derivatives (6.7% and 5,2%, respectively). However, they did not share the same constituents, with the exception of 2-nonanone and *n*-heneicosane, even though with different amounts (Table 3). This class of compounds (nt) showed less relevance in the composition of the other *Helychrisum* EOs (ranging from 0.4% in *H. setosum* to 3.0% *H. basalticum*), while *H. foetidum* was free from non-terpenes. Moreover, the highest percentage of non-terpenes (10.8%),

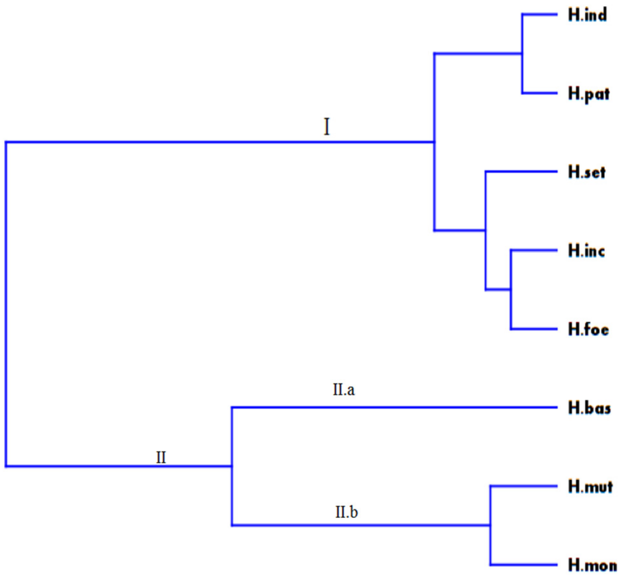

Fig. 2. Hierarchical Cluster Analysis (HCA) of volatile emissions of the eight *Helichrysum* spp. studied.

represented mainly by isopropyl hexadecanoate (3.5%), was identified in *H. montanum*

The HCA analysis, based on the main class of compounds, specify *H. incarnatum*, which is distant from the other since it was the only oil with the highest percentage of monoterpene hydrocarbons (60.6 %) (Group II.a) (Fig. 3). Groups I, which includes *H. basalticum*, *H. setosum*, H. *foetidum* and *H. montanum*, differs from group II.b (consisting of *H. patulum*, *H. mutabile* and *H. indicum*) with its high amount of oxygenated sesquiterpenes (at least 49% of the total identified).

The total number of compounds varied among the species, with *H. foetidum* EO characterised by the lowest number of constituents (42) and *H. mutabile* being the richest (74). Despite this abundance of compounds, only six constituents were responsible for almost 43% of the total identified in *H.mutabile*: sandarocopimarinol (12.3%, od), abieta,8 (14),13(15)-diene (4.6%, dh), spathulenol (6.3%, os), caryophyllene oxide (5.6%, os), and 1,8-cineole (3.2%, om). The first two compounds were found only in this EO.

Ledol (16.3%, os), viridiflorol (11.8%, os), valerianol (11.2%, os), valeranone (8.4%, os), caryophyllene oxide (7.4%, os) and himachalol (6.3%, os) were predominant in *H. foetidum* EO and constitute 62% of the total identified fraction. Himachalol typified this latter oil since it was present only here.

Table 3
Major constituents of the *Helichrysum* EOs

	Compounds	Class	LRI	*H.bas*	*H.foe*	*H.inc*	*H.ind*	*H. mon*	*H. mut*	*H.pat*	*H.set*
1	santolina triene	mh	910				0.1		0.3		
2	α-thujene	mh	932		0.3	0.2			0.3		
3	4-heptanone 3-methyl	nt	932				0.6				
4	tricyclene	mh	938	0.2					1.1		
5	α-pinene	mh	940	0.2	0.3	11.6	3.3	2.1		9.1	1.9
6	camphene	mh	955			0.1	0.7		0.8		0.9
7	1-heptanol	nt	973					0.2			
8	sabinene	mh	978		0.9	0.8		0.3	0.6		
9	β-pinene	mh	981	0.5	3.1	9.5	0.6		0.7	13.5	
10	1-octen-3-ol	nt	982					0.1			
11	6-methyl-5-hepten-2-one	nt	990	0.1							
12	myrcene	mh	993			5.4	6.2	0.4	1.9	0.7	
13	Furan. 2-pentyl-	nt	995	0.3							0.2
14	*n*-octanal	nt	1005	0.1				0.5			
15	α-phellandrene	mh	1006			0.2					0.1
16	α-terpinene	mh	1019		0.8	0.2	0.2	0.1	1.0	0.1	2.0
17	p-cymene	mh	1028		0.2		0.2		1.1		1.5
18	limonene	mh	1032	0.1	0.7		2.8	0.6	1.2	1.7	0.7
19	β-phellandrene	mh	1033			12.0					
20	1.8-cineol	om	1036	0.3			2.5		3.2	1.7	
21	*(Z)*-β-ocimene	mh	1042			17.0	1.7			1.2	
22	*(E)*-β-ocimene	mh	1053			3.0	1.8				
23	γ-terpinene	mh	1062		1.4	0.4	0.5	0.4	1.6	0.3	0.3
24	*trans*-arbusculene	nt	1071							0.1	
25	cis-sabinene hydrate	om	1072		1.2			0.5	1.3		
26	terpinolene	mh	1090		0.3	0.3	0.7		0.5	0.1	0.2
27	p-cymenene	mh	1090								0.2
28	2-nonanone	nt	1095				0.1		2.0		
29	*trans*-sabinene hydrate	om	1101		1.8				1.6		
30	linalool	om	1102			0.2		0.1	0.7		1.3
31	*n*-nonanal	nt	1104	1.0				1.0	1.0	0.1	
32	(-)-myrtenol	om	1112								5.7
33	1.3.8-p-menthatriene	mh	1114								0.1
34	exo-fenchol	om	1116				1.1				
35	1-octen-3-yl acetate	nt	1117					0.4			
36	*trans*-thujone	om	1120	0.1							
37	*cis-p*-menth-2-en-1-ol	om	1126		0.2			0.1	1.0		4.5
38	*allo*-ocimene	om	1133			0.3					
39	*trans*-verbenol	om	1139						0.3		
40	*trans*-pinocarveol	om	1142					0.6			0.2
41	*trans-p*-menth-2-en-1-ol	om	1145		0.2	0.1			0.6		
42	camphor	om	1148	0.1							

(continued on next page)

Table 3 (*continued*)

	Compounds	Class	LRI	*H.bas*	*H.foe*	*H.inc*	*H.ind*	*H. mon*	*H. mut*	*H.pat*	*H.set*
43	camphene hydrate	om	1153				0.4				
44	photocitral A	om	1154				0.5				
45	nerol oxide	om	1158								0.6
46	*(E)*-2-nonen-1-al	nt	1166	0.1				0.2	0.3		
47	pinocarvone	om	1166					0.2		0.1	
48	borneol	om	1169	0.1			2.0	0.2			
49	lavandulol	om	1170				1.5				
50	1-nonanol	nt	1174				0.1	0.6			
51	*neo*-menthol	om	1176	0.1							
52	*cis*-pinocamphone	om	1177				0.1				
53	4-terpineol	om	1180		3.1	0.4	0.6	0.7	3.1	0.2	1.3
54	α-terpineol	om	1192				1.5		0.2		1.6
55	myrtenol	om	1195		0.3			0.2			
56	dihydrocarveol	om	1196			0.1		0.3			
57	*cis*-piperitol	om	1197						0.5		
58	*n*-decanal	nt	1206	0.2				0.4	0.3		
5	*trans*-piperitol	om	1210						0.2		
60	1-octyl acetate	nt	1214					1.1			
61	*cis*-geraniol	om	1235								6.2
62	heptyl isobutyrate	nt	1250					0.3			
63	geraniol	om	1259								1.5
64	*(E)*-2-decenal	nt	1266						0.2		
65	*n*-decanol	nt	1275					0.2			
66	isobornyl acetate	om	1287						0.2		
67	carvacrol	om	1301								0.3
68	1-nonanol acetate	nt	1312					0.8			
69	*(E,E)*-2.4-decadienal	nt	1317					0.2			
70	myrtenyl acetate	om	1327								0.3
71	Butanoic acid. 3-methyl-. heptyl ester	nt	1340					0.8			
72	δ-elemene	sh	1340			0.3	0.1				
73	7-epi-silphiperfol-5-ene	sh	1345			0.4					
74	α-longipinene	sh	1351		0.2						
75	2.3.4-trimethyl benzaldehyde	om	1352							0.3	
76	siphiperfol-4.7(14)-diene	sh	1361			0.2					
77	neryl acetate	om	1368	0.1							0.2
78	*n*-undecanol	nt	1370					0.2			
79	longicyclene	sh	1370						0.3		0.3
80	cyclosativene	sh	1371						0.9		
81	*iso*-ledene	sh	1375	0.3							
82	α-copaene	sh	1376	0.4			0.2			0.7	0.7
83	silphiperfol-6-ene	sh	1377						0.3		
84	β-maaliene	sh	1379			1.1					
85	β-patchoulene	sh	1380	0.2							
86	β-bourbonene	sh	1383		0.3				0.7		
87	*trans*-myrtanol acetate	om	1384						0.2		
88	*(E)*-β-damascenone	ac	1386					0.2			0.3
80	α-iscomene	sh	1386	0.1		3.9					
90	β-elemene	sh	1392							0.2	
91	sativene	sh	1395	0.2							
92	β-iscomene	sh	1405			0.6					
93	α-gurjunene	sh	1410	2.3		0.1				0.7	
94	β-caryophyllene	sh	1418	3.5	2.5	0.6	11,0	0.6	1.9	13.4	
95	lavandulyl isobutyrate	om	1425				0.2				
96	1H-Cyclopropa[a]naphthalene. decahydro-1.1.3a-trimethyl-7-methylene-. [1aS-(1aα.3aα.7aβ.7bα)]-	sh	1427	0.3							
97	β-copaene	sh	1429								0.1
98	β-gurjunene	sh	1432	0.6							
99	γ-maaliene	sh	1435	0.3							
100	*trans*-α-bergamotene	sh	1437			1.0	0.4				
101	α-guaiene	sh	1440	1.6	0.1	0.2		1.1	2.4		
102	aromadendrene	sh	1445	11.7		0.6					
103	α-himachalene	sh	1450				0.1				
104	*(E)*-geranylacetone	ac	1455			0.4	1.6	0.2	0.2		
105	α-humulene	sh	1456	0.3	0.2	4.2	3.5			2.3	
106	*(E)*-β-farnesene	sh	1460				0.7		0.5		0.1
107	*allo*-aromadendrene	sh	1461	1.6	0.2			0.5		0.6	
108	β-chamigrene	sh	1475					0.8		0.7	
109	γ-gurjunene	sh	1476	0.3						1.2	2.0
110	γ-muurolene	sh	1477	0.3	0.5		1.0		0.5	0.5	0.6
111	α-amorphene	sh	1480				0.3				
112	germacrene D	sh	1481			0.1			0.2		
113	*(E)*-β-ionone	ac	1485					0.3			
114	2-Isopropenyl-4a.8-dimethyl-1.2.3.4.4a.5.6.7-octahydronaphthalene	sh	1485					1.1			
115	β-selinene	sh	1485	0.9	0.1	0.1	0.3	0.7	0.9	0.7	0.3

Table 3 (*continued*)

	Compounds	Class	LRI	*H.bas*	*H.foe*	*H.inc*	*H.ind*	*H. mon*	*H. mut*	*H.pat*	*H.set*
116	*cis*-β-guaiene	sh	1489	0.3							
117	*trans*-muurola-4(14).5-diene	sh	1492		0.2						
118	δ-selinene	sh	1493	0.3							
119	α-selinene	sh	1495	0.7							
120	viridiflorene	sh	1495	2.9							0.4
121	bicyclogermacrene	sh	1495			13.1					
122	valencene	sh	1496				0.8			0.6	0.5
123	epizonarene	sh	1498				1.3				
124	β-Dihydroagarofurane	os	1499					15.2			
125	β-himachelene	sh	1499		0.4						
126	α-muurolene	sh	1499							0.4	0.2
127	γ-patchoulene	sh	1502						2.8	1.1	0.3
128	α-bulnesene	sh	1505		0.4				0.6	0.1	0.4
129	lavandulyl 2-methyl butyrate	os	1512				10.9				
130	*trans*-γ-cadinene	sh	1513		0.2				0.3	0.4	0.2
131	cubebol	os	1518		1.3				0.3		0.4
132	(+)-Cycloisolongifol-5-ol	os	1518	0.3							
133	7-*epi*-α-selinene	sh	1519							0.6	0.7
134	δ-cadinene	sh	1523	0.1	1.3	0.2	3,0		1.6	11.5	0.7
135	*trans*-cadina-1(2)-4-diene	sh	1533				0.2				
136	α-cadinene	sh	1537				0.1				
137	α-calacorene	sh	1542		0.3		0.2			0.7	
138	α-agarofuran	os	1548	0.3				1.0			
139	elemol	os	1553	0.6						0.2	
140	β-vetivenene	sh	1553	0.3							
141	germacrene B	sh	1556	2.3							
142	*epi*-longipinanol	os	1560						0.2		
143	ledol	os	1565	1.1	16.3	0.3				7.1	
144	*trans*-nerolidol	os	1566				0.2				5.4
145	caryophyllene alcohol	os	1571				3.2				
146	germacrene D-ol	os	1576	0.4							
147	spathulenol	os	1577	0.4		2.1		0.3	6.3		
148	caryophyllene oxide	os	1582	1.0	7.4		1.3	12.1	5.6	1.2	
149	globulol	os	1584	10.3		1.5		3.8			
150	α-cedrene epoxide	sh	1585						0.2		
151	gleenol	os	1586			0.3					
152	thujopsan-2-α-ol	os	1588				0.3				
153	viridiflorol	os	1591	3.6	11.8	0.5		18.3	2.3	5.8	3.9
154	isoaromadendrene espoxide	os	1595	0.6							
155	longiborneol	os	1596				2.6				
156	guaiol	os	1597				0.1				
157	cedrol	os	1599						0.4		
158	5-*epi*-7-*epi*-α-eudesmol	os	1606	2.0						2.0	
159	β-oplopenone	os	1606		0.5						
160	humulene epoxide II	os	1607			0.2	0.6	0.2	0.2		
161	*epi*-cedrol	os	1611							0.6	
162	rosifoliol	os	1613	2.7		0.4		1.3			
163	1.10-di-*epi*-cubenol	os	1614				4.8			0.2	
14	α-*epi*-7-*epi*-5-Eudesmol	os	1616	1.4							
165	*(E)*-β-farnesene epoxide	os	1624								6.7
166	10-*epi*-γ-eudesmol	os	1627					4.3			
167	1-*epi*-cubenol	os	1630		3.7		0.2		0.4	0.7	1.0
168	eremoligenol	os	1630	0.3				0.7			
169	2-Butenoic acid. 2-methyl-. 3.7-dimethyl-2.6-octadienyl ester. (E.Z)-	os	1631			0.3					
170	α-acorenol	os	1633			0.5				0.8	
171	γ-eudesmol	os	1634	0.7			1.8	1.5	0.2	0.3	
172	caryophylla-4(14).8(15)-dien-5-ol	os	1636		2.1		0.2		0.7		
173	hinesol	os	1638	0.2						0.2	1.0
174	τ-cadinol	os	1642		3.1		1.0				
175	cedr-8(15)-en-9-α-ol	os	1644					1.7			
176	agarospirol	os	1646			0.2					
177	himachalol	os	1646		6.3						
178	τ-muurolol	os	1649							1.4	
179	β-eudesmol	os	1649	1.3			0.5	0.5	2.7	0.3	
180	α-muurolol	os	1651				0.5		0.6		1.3
181	α-eudesmol	os	1654	2.6							
182	α-cadinol	os	1655				2.3				
183	valerianol	os	1655	16.3	11.2			1.5		1.1	30.1
184	curcumen-15-al	os	1660			0.1					
185	*neo*-intermediol	os	1660							0.8	
186	patchouli alcohol	os	1660					0.4			
187	intermediol	os	1666			0.4		2.0	9.4		
188	ledene oxide-(II)	os	1666	1.1							

(continued on next page)

Table 3 (*continued*)

	Compounds	Class	LRI	*H.bas*	*H.foe*	*H.inc*	*H.ind*	*H. mon*	*H. mut*	*H.pat*	*H.set*
189	bulnesol	os	1666							0.1	
190	cis-9-tetradecen-1-ol	nt	1667			0.5					
191	guaia-3.10(10)-dien-11-ol	os	1672					0.7			
192	β-bisabolol	os	1672				0.2				
193	valeranone	os	1673		8.4					1.9	
194	*(Z)*-α-santalol	os	1675						2.5	1.9	
195	*cis*-α-santalool	os	1675		2.3						
196	*cis*-11-tetradecen-1-ol	nt	1678			0.2					
197	bicyclovetivenol	os	1680			0.1		2.2			
198	α-bisabolol	os	1684							1.3	
199	*epi*-α-bisabolol	os	1685				0.4			0.3	
200	acorenone	os	1689					0.4			
201	*(Z)-trans*-α-bergamotol	os	1697							1.0	
202	mayurone		1711						0.2		
203	6-Isopropenyl-4.8a-dimethyl-1.2.3.5.6.7.8.8a-octahydro-naphthalen-2-ol	os	1714	3,0							
204	pentadecanal	nt	1718	0.1					0.6		
205	khusimol	os	1735	6.4				0.7			
206	7R.8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	os	1746	0.2							
207	*(Z)*-lanceol	os	1761					0.8			
208	tertradecanoic acid	nt	1766	0.6		0.2	1.8				0.2
209	*(Z,E)*-farnesyl acetate	ac	1805							0.7	
210	hexahydrofarnesylacetone	ac	1845	1.4			0.6	0.8	1.0		0.2
211	flourensadiol	os	1864						0.3		
212	farnacyl acetone	ac	1920			0.6	1.5				
213	phytol	od	1950						0.1		
214	beyerene	dh	1962						0.5		
215	*n*-hexadecanoic acid	nt	1972	0.5		0.2	3.2			0.7	
216	bifloratriene	dh	1977							0.9	
217	isopropyl hexadecanoate	nt	1981					3.5			
218	manoyl oxide	od	1987				0.7			0.1	0.9
219	*n*-eicosane	nt	2000						0.2		
220	*epi*-13-manoyl oxide	od	2010								1.2
221	*n*-pentacosane	nt	2017			0.2	0.7	0.1			
222	kaurene	dh	2034			0.2	1.0				
223	epi-manool	od	2056								1.7
224	manool	od	2056		1.5						1,0
225	abietadiene	dh	2079						0.7		
226	*n*-heneicosane	nt	2100				0.2		0.6		
227	abieta-8(14).13(15)-diene	dh	2154						4.6		
228	*n*-docosane	nt	2200								
229	phyllocladanol	od	2210						0.2		
230	sclareol	od	2239							1.8	
231	paradol	os	2245				0.1				
232	sandaracopimarinol	od	2271						12.3		
233	*n*-tricosane	nt	2300					0.2			
	unknown			5.2	2.4	2.1	4.3	6.5	4.6	1,0	5.4
	Total identified			94.8	97.6	97.9	95.7	93.5	95.4	99.0	94.6
	Yields			0.001	0.102	0.109	0.001	0.001	0.001	0.104	0.093
	Compounds			H.bas	H.foe	H.inc	H.ind	H. mon	H. mut	H.pat	H.set
	Monoterpene hydrocarbons (mh)			1.0	8.0	60.7	18.8	3.9	11.1	26.7	7.9
	Oxygenated monoterpenes (om)			0.8	6.8	1.1	10.4	2.9	13.1	2.3	23.7
	Sesquiterpene hydrocarbons (sh)			31.8	6.9	26.7	23.2	4.8	14.1	36.4	7.5
	Oxygenated sesquiterpenes (os)			56.8	74.4	6.9	31.2	69.6	32.1	29.2	49.8
	Diterpene hydrocarbons (dh)			-	-	0.2	1.0	-	5.8	0.9	-
	Oxygenated diterpenes (od)			-	1.5	-	0.7	-	12.6	1.9	4.8
	Non-terpene derivatives (nt)			3.0	-	1.3	6.7	10.8	5.2	0.8	0.4
	Others			-	-	-	-	-	0.2	0.1	-

Helichrysum montanum EO was rich in viridiflorol (18.3%) and caryophyllene oxide (12,1%). However, this oil could be distinguished by the presence of β-dihydroagofuran (15.2%) 10-epi- γ-eudesmol (4.3%) and bicyclovetivenol (2.2%).

The EOs of *H. basalticum* and *H. setosum*, both rich in oxygenated sesquiterpenes, showed a high amount of valerianol (16.3% and 30.1%, respectively), even though the *H. basalticum* species displayed good percentages of globulol (10,3%) and aromadendrene (11.7%) together with khusimol (6.4%).

Epizonarene was evidenced only in the EO of *H. indicum*, but its percentage was very low (1.3%). *Helichrysum incarnatum*, with the highest percentage of monoterpene hydrocarbons with (Z)-β-ocimene (17.0%), α-pinene (11.6%) and β-pinene (9,5%) as major compounds. This EO was also characterised by an appreciable amount of bicyclogermacrene (13.1%) followed by β-phellandrene (12.0%), identified for the first time in all the oils studied herein.

This work evidenced that the essential oil composition of all the *Helichrysum* species studied differed one from the other even though the plants belong to the same genus, although the diversity among the species of this genus is well documented (Lourens et al., 2008). Previous studies on the EO composition of *H. krausii* and *H. rugulosum* from South Africa showed a good content of β-caryophyllene (30.7% and 12.6%,

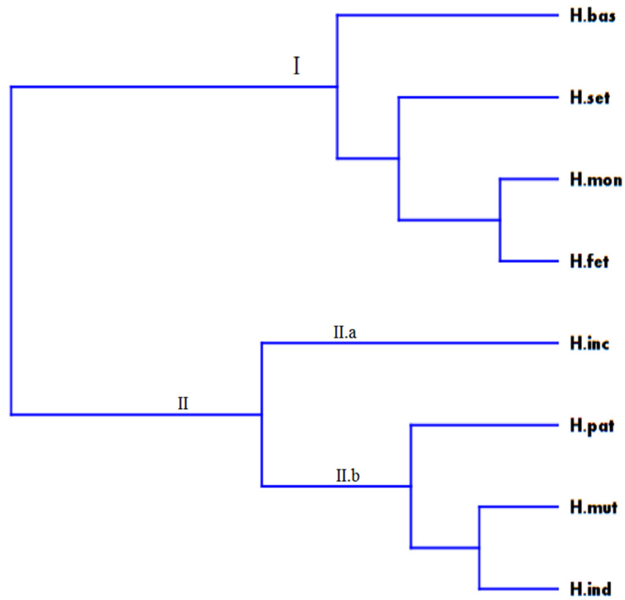

Fig. 3. Hierarchical Cluster Analysis (HCA) of essential oils from the eight *Helichrysum* spp. studied.

respectively) (Bougatsos et al., 2003) similar to that reported here for *H. indicum* and *H. basalticum*. β-caryophyllene, known as an anti-inflammatory agent, was one of the major components in the EOs of other *Helichrysum* species from Greece (*H. heldreichii*, 38.5% and *H. stoechas* subsp. *barrelieri*, 15.6%) (Roussis et al., 2002; Chinou et al., 1997), from Cameroon (*H. odoratissimum*, 13.8%) (Kuiate et al., 1999) and from Madagascar (*H. gymnocephalum,* 1.6%; *H. bracteiferum*, 7.1%; *H. selagnifolium*, 7.5%; *H. cordifolium*, 55.6%; *H. faradifani*, 34.6%; and *H. hypnoides*, 34.0%) (Cavalli et al., 2001).

Bagci et al. (2013) pointed out a high percentage of caryophyllene oxide (8%) in Turkish *H. graveolens*, also present in *H. montanum* (12.1%), *H. foetidum* (7.4%) and *H. mutabile* (5.6%) analysed here. The Turkish species showed also the presence of epizonarene (1%), a typical compound found in *H. indicum* with the same percentage (1.3%).

Other EOs from three indigenous South African species (*H. dasyanthum*, *H. excisum* and *H. petiolare*) were characterised by the presence of 1,8-cineole (20–34 %), α-pinene (3–17 %) and *p*-cymene (6–10%). These compounds were present in most *Helichrysum* studied but in low percentages. Another point of interest was the presence of large amount of viridiflorol in *H. foetidum* (11.8%) and *H. montanum* (18.3%) from this work, in agreement with that reported in *H. excisum* (18.2 %) (Lourens et al., 2004). A different trend was reported for *H. hypnoides* and *H. bracteriferum* EOs from Madagascar with 1,8-cineol as main compound (51.5% and 24.8%, respectively) (Baser et al., 2002).

Helichrysum cymosum EO, on the contrary, was characterised by the presence of (Z)-β-ocimene (50.4%), *trans*-caryophyllene (15.0%), 1,8-cineole (9.4%), α-humulene (5.3%), (E)-β-ocimene (7.9%) and caryophyllene oxide (1.7%) (Giovanelli et al., 2018). It is noteworthy the presence of all these constituent in *H. indicum* studied here, although with lower amount.

4. Conclusion

Since no data have previously been reported on these indigenous plants this work represents the first contribution on the volatile and EO composition of eight *Helichrysum* spp., typical of the South African area, but grown in the Italian environment. Each *Helichrysum* studied differed one from the other both in their aroma profile and in the EO composition. This genus continues to be a good resource of natural bioactive compounds to be used for medical or cosmetic purposes but also as ornamental plants.

References

Al-Rehaily, A.J., Albishi, O.A., El Olemy, M.M., Mossa, J.S., 2008. Flavonoids and terpenoids from *Helichrysum forskahlii*. Phytochemistry 69, 1910–1914.
Antunes Viegas, D., Palmeira-de-Oliveira, A., Salgueiro, L., Matninez-de-Oliveira, J., Palmeira-de-Oliveira, R., 2014. *Helichrysum italicum*: From traditional use to scientific data. J. Ethnopharmacol. 151, 54–65.
Bagci, E., Elkiran, O., Evren, H., 2013. Constituents of the essential oils of *Helichrysum graveolens* (Bieb.) sweet from Turkey. Asian J. Chem. 13, 7254–7256.
Bandeira Reidel, R.V., Cioni, P.L., Ruffoni, B., Cervelli, C., Pistelli, L., 2017. Aroma Profile and Essential Oil Composition of *Helichrysum* species. Nat. Prod. Commun. 12, 977–982.
Baser, K.H.C., Demirci, B., Kirimer, N., 2002. Composition of the essential oils of four *Helichrysum* species from Madagascar. J. Essen. Oil Res. 14, 53–55.
Bigovic, D., Savikin, K., Jankovic, T., Menkovic, N., Zdunic, G., Stanokovic, T., Djuric, Z., 2011. Antiradical and cytotoxic activity of different *Helichrysum plicatum* flower extracts. Nat. Prod. Commun. 6, 819–822.
Bougatsos, C., Meyer, J.J.M., Magiatis, P., Vagias, C., Chinou, I.B., 2003. Composition and antimicrobial activity of the essential oils of *Helychrysum kraussii* Sch. Bip. and *H. rugulosum* Less. from South Afric. Flavour Frag. J. 18, 48–51.
Cavalli, J.F., Ranarivelo, L., Rtasimbason, M., Bernardini, A.F., Casanova, J., 2001. Constituents of the essential oil of six *Helichrysum* species from Madagascar. Flavour Frag. J. 16, 253–256.
Chinou, I.B., Roussis, V., Perdetzoglou, D., Tzakou, A., Loukis, A., 1997. Chemical and antibacterial studies of two *Helichrysum* species of Greek origin. Planta Med. 63, 181–183.
Czinner, E., Hagymasi, K., Blazovics, A.K., Kery, A., Szoke, E., Lemberkovics, E., 2000. In vitro antioxidant properties of *Helichrysum arenarium* (L.) Moench. J. Ethnopharmacol. 73, 437–443.
Demasi, S., Caser, M., Lonati, M., Cioni, P.L., Pistelli, L., Najar, B., Scariot, V., 2018. Latitude and altitude influence secondary metabolite production in peripheral Alpine population of the Mediterranean species *Lavandula angustifolia* Mill. Front. Plant Sci. 9, 983. https://doi.org/10.3389/fpls.2018.00983.
Ghassemi-Dehkordi, N., Sadeghi, M., Kaviani, M.R., Zolfaghri, B., 2015. Analysisof *Helichrysum oligocephalum* DC. essential oil. Res. J. Pharmacog. 2, 47–52.
Giovanelli, S., De Leo, M., Cervelli, C., Ruffoni, B., Ciccarelli, D., Pistelli, L., 2018. Essential oil composition and volatile profile of seven *Helichrysum* species grown in Italy. Chem. Biodiv., 15 https://doi.org/10.1002/cbdv.201700545.
Kuiate, J.R., Zollo, P.H.A., Nguefa, E.H., Bessiere, J.M., Lamaty, G., Menut, C., 1999. Composition of the essential oils from the leaves of *Microglossa pyrifolia* (Lam.) O. Kuntze and *Helichrysum odoratissimum* (L.) Less. growing wild in Cameroon. Flav. Frag. 14, 82–84.
Legoalea, P.B., Mashimbyeb, M.J., Van Rec, T., 2013. Antiinflammatory and antioxidant flavonoides from *Helichrysum kraussii* and *H. odoratissimum* flower. Nat. Prod. Commun. 8, 1403–1404.
Leonardi, M., Giovanelli, S., Ambryszewska, K.E., Ruffoni, B., Cervelli, C., Pistelli, La, Flamini, G., Pistelli, L., 2018. Essential oil composition of six Helichrysum species grown in Italy. Biochem. Systemat. Ecol. 79, 15–20.
Lourens, A.C., Reddy, D., Başer, K.H., Viljoen, A.M., Van Vuuren, S.F., 2004. In vitro biological activity and essential oil composition of four indigenous South African *Helichrysum* species. J. Ethnopharmacol. 95, 253–258.
Lourens, A.C.U., Viljoen, A.M., Van Heerden, F.R., 2008. South African *Helichrysm* species: a review of the traditional uses. biological activity and phytochemistry. J. Ethnopharmacol. 119, 630–652.
Pooley, E., 2003. Mountain Flowers: A Field Guide to the Flora of the Drakensberg and Lesotho. 1st ed. 44. The Flora Publications Trust, Durban, pp. 102–110 146–157. 222–225.
Roussis, V., Tsoukatou, M., Chinou, I.B., Harvala, C., 2002. Composition and antibacterial activity of the essential oils of two *Helichrysum stoechas* varieties growing in the island of Crete. J. Essen. Oil Res. 14, 459–461.
Satta, M., Tuberoso, C.I.G., Angioni, A., Pirisi, F.M., Cabras, P., 1999. Analysis of the essential oil of *Helichrysum italicum* G. Don. ssp. *microphyllum* (Willd) Nym. J. Essen. Oil Res. 11, 711–715.