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Chemical composition and biological activity of the essential oil from *Helichrysum microphyllum* Cambess. *ssp. tyrrhenicum* Bacch., Brullo e Giusso growing in La Maddalena Archipelago, Sardinia.

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Abstract: *Helichrysum microphyllum* Cambess. subsp. *tyrrhenicum* Bacch., Brullo e Giusso (Asteraceae), previously known as *Helichrysum italicum ssp. microphyllum* (Willd.) Nyman, is one of the many endemic species growing in Sardinia, Corsica and Balearic Islands. In the present work the composition of the essential oil obtained from a population of *H. microphyllum ssp. thyrrenicum* growing in a littoral location of La Maddalena Archipelago was investigated by GC-FID and CG-MS. The major compounds of the oil were the monoterpene ester neryl acetate (18.2%), the oxygenated sesquiterpene 5-eudesmen-11-ol (rosifoliol, 11.3%), the sequiterpene hydrocarbons δ-cadinene (8.4%) and γ-cadinene (6.7%), showing a peculiar composition in comparison with other Sardinian populations. The oil was tested for cytotoxicity on three human tumor cell lines (MDA-MB 231, HCT116 and A375) by MTT assay showing a strong inhibitory activity on human malignant melanoma cells A375 (IC_{50} of 16 μg/ml). In addition the oil was assessed for antioxidant activity by DPPH and ABTS assay.

Key words: *Helichrysum*, neryl acetate, rosifoliol, δ-cadinene, γ-cadinene

1 Introduction

Helichrysum microphyllum subsp. *tyrrhenicum* (Asteraceae), is one of the many endemic species growing in Sardinia, Corsica and Balearic Islands.

This plant is a shrub, 25-40 cm in height, strongly aromatic, with linear leaves, the lower not longer than 12 mm; the flowers are grouped in yellow heads and fruits are brown achenes[1)]. It is used in Sardinian traditional medicine to nurse cough, burns and as antirheumatic and analgesic[2)].

There are several studies on the essential oils of *Helychrysum* species and their activity, such as antispasmodic, antioxidant, antibacterial and antiallergic[3−6)]. Alike, many are the phytoproducts isolated from the genus *Helichrysum* (s.l.), although few oils and extracts are used in the food, cosmetics and pharmaceutical industries. *H. microphyllum* subsp. *tyrrhenicum* is less widespread with respect to the other subspecies, and its chemical composition is peculiar with respect to plants growing in the peninsula. In particular from this subspecies a heterodimeric phloroglucinol pyrone arzanol endowed with interesting biological activities as well as an unusual class of lipids named santinols have been recently characterized[7, 8)].

H. italicum was proven to exhibit both morphological and genetic variation among and within the Mediterranean and Sardinian populations[9, 10)]. This variability influenced the volatile composition with quali-quantitative differences in essential oils among populations coming from different regions[11−15)]. Therefore, our aim was to verify if geographical isolation in La Maddalena Archipelago lead to differenc-

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Table 1 Essential oil composition of *Helichrysum microphyllum subsp. tyrrhenicum*.

N.	Component[a]	RI[b]	RI lit[c]		%[d]	ID[e]
			ADAMS	NIST08		
1	nerol	1230	1229	1230	1.3	RI,MS
2	2-undecanone	1296	1294	1296	0.1	RI,MS
3	silphinene	1334	1347		0.1	RI,MS
4	α-ylangene	1366	1375	1366	0.0	RI,MS
5	neryl acetate	1369	1361	1369	19.2	RI,MS
6	α-isocomene	1377	1388		0.7	RI,MS
7	italicene	1394	1405		0.7	RI,MS
8	α-gurjunene	1400	1407		0.1	RI,MS
9	(*E*)-caryophyllene	1409	1418	1410	0.8	Std
10	γ-elemene	1430	1436		0.2	RI,MS
11	aromadendrene	1430	1441	1429	0.2	RI,MS
12	6,9-guaiadiene	1438	1444		0.1	RI,MS
13	α-humulene	1445	1454	1446	0.3	Std
14	*allo*-aromadendrene	1452	1460	1452	2.0	RI,MS
15	neryl propanoate	1458	1454		3.6	RI,MS
16	*trans*-cadina-1(6),4-diene	1468	1476		0.3	RI,MS
17	γ-muurolene	1471	1479	1473	1.6	RI,MS
18	α-amorphene	1474	1484	1474	1.2	RI,MS
19	α-neocallitropsene	1477	1476		0.7	RI,MS
20	*ar*-curcumene	1480	1480	1480	0.7	RI,MS
21	δ-selinene	1485	1492	1485	0.5	RI,MS
22	*cis*-cadina,1-4-diene	1487	1495		1.2	RI,MS
23	α-muurolene	1494	1500	1494	2.5	RI,MS
24	δ-amorphene	1500	1512		0.2	RI,MS
25	γ-cadinene	1507	1513	1507	6.7	RI,MS
26	*trans*-calamenene	1518	1522	1518	tr[f]	RI,MS
27	δ-cadinene	1519	1523		8.4	RI,MS
28	*trans*-cadina,1-4-diene	1526	1534	1525	1.0	RI,MS
29	α-cadinene	1531	1538	1532	1.2	RI,MS
30	α-calacorene	1536	1545		0.8	RI,MS
31	geranyl butanoate	1541	1564	1540	0.2	RI,MS
32	germacrene B	1547	1561	1547	0.4	RI,MS
33	gleenol	1579	1587		0.3	RI,MS
34	neryl isovalerate	1586	1583		1.3	RI,MS
35	guaiol	1592	1600	1592	1.9	RI,MS
36	rosifoliol	1598	1600		11.3	RI,MS
37	1,10-di-*epi*-cubenol	1606	1619		0.6	RI,MS
38	tetradecanal	1613	1612	1613	0.3	RI,MS
39	1-*epi*-cubenol	1621	1628		1.5	RI,MS
40	muurola-4,10(14)-dien-1-β-ol	1621	1631		1.5	RI,MS

Table 1 Continued.

N.	Component[a]	RI[b]	RI lit[c]		%[d]	ID[e]
			ADAMS	NIST08		
41	β-eudesmol	1627	1650	1630	1.3	RI,MS
42	*epi*-α-cadinol	1634	1640		2.0	RI,MS
43	*epi*-α-muurolol	1634	1642		2.0	RI,MS
44	α-muurolol	1641	1646		1.5	RI,MS
45	α-eudesmol	1644	1653	1644	0.5	RI,MS
46	α-cadinol	1648	1654	1650	1.5	RI,MS
47	bulnesol	1660	1671	1662	0.8	RI,MS
48	cadalene	1667	1676	1668	0.3	RI,MS
49	geranyl tiglate	1674	1696	1675	0.4	RI,MS
50	pentadecanal	1714		1717	1.2	RI,MS
51	drimenol	1769	1767		0.2	RI,MS
52	2-pentadecanone, 6,10,14-trimethyl-	1846		1846	1.3	RI,MS
53	pimaradiene	1943	1949	1947	0.2	RI,MS
54	*n*-pentacosane	2500	2500	2500	0.1	Std
55	*n*-heptacosane	2700	2700	2700	0.1	Std
56	*n*-nonacosane	2900	2900	2900	0.1	Std
57	*n*-untriacontane	3100	3100	3100	0.1	Std
	Total identified				89.7	
	Grouped compounds					
	Monoterpene esters				24.7	
	Oxygenated monoterpenes				1.3	
	Sesquiterpene hydrocarbons				33.1	
	Oxygenated sesquiterpenes				28.3	
	Others				2.2	

[a] Compounds are listed in order of their elution from a HP-5MS column. [b] Linear retention index on HP-5MS column, experimentally determined using homologous series of C_8-C_{30} alkanes. [c] Linear retention index taken from Adams (2007) and NIST 08 (2008). [d] Percentage values are means of three determinations with a RSD% in all cases below 10%. [e] Identification methods: std, based on comparison with authentic compounds; MS, based on comparison with WILEY, ADAMS and NIST 08 MS databases; RI, based on comparison of LRI with those reported in ADAMS, FFNSC 2 and NIST 08. [f] tr, % below 0.1%.

es in essential oil composition of *H. microphyllum* subsp. *tyrrhenicum* with respect to other areas of Sardinia, as resulted for *A. arborescens* in our previous paper[16]. To complete the work, we assayed the essential oil for cytotoxicity against some tumor cell lines by the MTT method, and for antioxidant activity by DPPH, ABTS and FRAP methods.

2 Results and discussion

2.1 Essential oil Analysis

The composition of the essential oil from aerial parts of *Helichrysum microphyllum* subsp. *tyrrhenicum* growing in the Isle of La Maddalena (Sardinia, Italy), is reported in Table 1. A total of fifty-seven volatile components were identified, accounting for 89.7% of the total composition. The major compounds were neryl acetate (19.2%), δ-cadinene (8.4%) and rosifoliol (11.3%), which are representative of monoterpene esters (24.7%), sesquiterpene hydrocarbons (33.1%) and oxygenated sesquiterpenes (28.3%), respectively. Instead, completely missing resulted the monoterpene hydrocarbons. Other components occurring in significant amounts (≥ 2%) were neryl propanoate (3.6%) for monoterpene esters, γ-cadinene (6.7%),

Table 2 Major volatile constituents (>5%) of Sardinian samples of *H. microphyllum ssp. thyrrenicum*.

Origin	Major constituents	Reference
Sardinia (location not specified)	Group 1: linalool (9.1%), nerol (10.7%), neryl acetate (28.9%), neryl propionate (11.4%), γ-curcumene (11.4%). Group 2: linalool (14.9%), γ-curcumene (18.2%), δ-cadinene (5.6%), 2-naphthalenmethanol,2,3,4, 4a,5,6,7,8-octahydro-α,α,4a,8-tetramethyl-[2R-(2α,4aβ,8β9] (20.2%)	11)
Northwestern Sardinia (Vignola, Porto Ferro, Calangianus)	linalool (3.7-5.6%), nerol (5.3-8.1%), neryl acetate (44.7-52.5%), eudesm-5-en-11-ol (4.3-11.5%)	12)
Central Sardinia (Esterzili)	limonene (2.5-9.0%), linalool (7.4-26.5%), nerol (5.3-13.5%), neryl acetate (20.0-35.7%), neryl propionate (1.9-15.6%), γ-curcumene (7.3-17.1%), ar-curcumene (4.0-6.8%), rosifoliol (5.8-15.1%)	13)
Northwestern Sardinia (Costa Paradiso, Trinità d' Agultu, Calangianus)	April samples: α-terpineol (1.5-6.7%), nerol (3.4-6.3%), neryl acetate (28.1-55.9%), neryl propionate (2.3-5.8%), eudesm-5-en-11-ol (4.5-21.5%)	15)
Northeastern Sardinia (La Maddalena)	neryl acetate (19.2%), γ-cadinene (6.7%), δ-cadinene (8.4%), rosifoliol (11.3%)	

Table 3 *In vitro* cytotoxic activity of *Helichrysum microphyllum subsp. tyrrhenicum* essential oil.

Essential oil	Cell line (IC_{50} μg/ml)		
	MDA-MB 231[a]	HCT116[b]	A375[c]
Helichrysum microphyllum subsp. tyrrhenicum	44.29	29.38	16.03
95% C.I.	40.58-49.9	24.92-34.64	12.50-20.54
Reference			
Cisplatin	2.96	2.45	0.42
95% C.I.	2.23-3.15	2.05-2.89	0.29-0.49

IC_{50} = The concentration of compound that affords a 50% reduction in cell growth (after 72 h of incubation). C.I. = Confidence Interval. [a] Human breast adenocarcinoma cell line. [b] Human colon carcinoma cell line. [c] Human malignant melanoma cell line.

α-muurolene (2.5%) and *allo*-aromadendrene (2.0%) for sesquiterpene hydrocarbons, *epi*-α-cadinol (2.0%) and *epi*-α-muurolol (2.0%) for oxygenated sesquiterpenes.

Comparing our results with those reported in literature[11−15, 17], we can notice similarities in the composition taking into account such an inter-populations variability linked to genetic, geographic and climatic factors. Notably, the geographical isolation in La Maddalena Archipelago, could have led to the detected differences in the essential oil composition with respect to populations from different Sardinian locations (Table 2). The main features of the investigated population seem to be a lower content of neryl acetate and more in general of the oxygenated monoterpenes, the absence of monoterpene hydrocarbons together with that of linalool and γ-curcumene which are reported as two of the major components in other populations[18], and the high content (about 61%) of sesquiterpenes among which γ-cadinene, δ-cadinene and rosifoliol were the most representatives. In addition it has not been observed substantial decreasing of the principal compounds between oils derived from fresh or dried plants[15].

2.2 Cytotoxic activity

Cytotoxic activity of the essential oil from *Helichrysum microphyllum* subsp. *tyrrhenicum* was evaluated on a selection of human tumour cell lines. MDA-MB 231 human breast adenocarcinoma, A375 human malignant melanoma, and HCT116 human colon carcinoma cell lines were tested by MTT assay. All cell lines were submitted to increasing concentrations of essential oil for 72 h. The results show that essential oil exhibited a significant cytotoxicity against the human tumour cells examined (Table 3). The essential oil induced a concentration-dependent inhibitory effect on all cell lines tested in the dilutions ranging from 1.56 to 400 μg/ml. The IC_{50} values of the oil were 16.03, 29.38, and 44.29 μg/ml on the A375, HCT116 and MDA-MB 231 cell lines, respectively. The essential oil resulted active mainly on human melanoma cell line.

Table 4 *In vitro* radical scavenging activities of the essential oil from *Helichrysum microphyllum subsp. tyrrhenicum*.

Essential oil	DPPH		ABTS		FRAP
	TEAC μmol TE/g	IC_{50} μg/ml	TEAC μmol TE/g	IC_{50} μg/ml	TEAC μmol TE/g
Helichrysum microphyllum subsp. tyrrhenicum	20.3 (±1.7)	2060.0 (±20)	37.8 (±1.1)	370.0 (±4.2)	2.4 (±0.6)
Reference					
Trolox		10.7 (±0.2)		3.6 (±0.2)	

IC_{50} = The concentration of compound that affords a 50% reduction in the assay. TEAC=trolox equivalent (TE) antioxidant concentration.

The chemical composition of the essential oil does not put in evidence the presence of a main compound that could be responsible for the cytotoxic activity. The main compounds present in the essential oil are neryl acetate (19.2%), rosifoliol (11.3%), δ-cadinene (8.4%), and γ-cadinene (6.7%). There are few data in literature related to cytotoxic activity of these compounds. Only δ-cadinene has been reported to exert high cytotoxic activity on human breast carcinoma cell line BT-20 and human epithelial adenocarcinoma cell line HeLa[19]. All the other compounds are present in the oil at concentrations below 3.6%, the minor components could contribute to cytotoxicity or may be involved in some type of synergism with the other compounds.

2.3 Antioxidant activity

H. italicum is a medicinal plant with promising pharmacological activities that continues to play an important role in the traditional medicine of Mediterranean countries[20]. The plant is widely used due to different properties as anti-inflammatory[20], antibacterial[8, 13, 20] and antioxidant[4, 6]. In the cosmetic field, products based on extracts and essential oil, have also anti-aging property, because they help to regenerate skin tissue and relieve inflammation through antioxidant properties which protect the skin from damaging free radicals[21]. *H. italicum* solvent extracts showed high antioxidant activity[6, 21, 22] while less activity is normally showed by essential oils owing to the lack of phenols.

Due to the interest on the use of this oil, in our work we characterized the antioxidant activity of the essential oil from *H. microphyllum* subsp. *tyrrhenicum* by DPPH and ABTS assays. All data are presented in Table 4. According to the results obtained by DPPH assay, the essential oil exhibited scavenging activity with an IC_{50} value about 187-fold higher than that reported for Trolox, while the total antioxidant power determined towards $ABTS^+$ assay showed an IC_{50} value of about 100-fold higher than that of Trolox. The different activity observed against the two radicals is probably due to the more specificity of the oil components towards $ABTS^+$ radical cation. The antioxidant activity of *H. microphyllum* subsp. *tyrrhenicum* oil was also determined by ferric reducing antioxidant power (FRAP) method. As reported in Table 4 the essential oil did not show important reducing ability. As regards the contribution of the oil components to the antioxidant activity, the major oil constituent (neryl acetate) is not known as a strong antioxidant, while the sesquiterpene alcohol rosifoliol (11.3%) exhibited a significant, protective effect against linoleic acid oxidation[6]. Nonetheless, other minor compounds may act as electron donors, contributing to the total activity of the oil.

3 Experimental

3.1 Plant Materials

Plant material was collected in La Maddalena Archipelago, located a short distance from the rugged north-east coast called Baia Trinita, at the beginning of the flowering stage (April 2013) The botanical identification was performed by Dr. Cinzia Sanna "Consorzio Interuniversitario Co.S.Me.Se." (Università di Cagliari). A sample of the studied plant is stored in our laboratory under the accession number: Herbarium CAG 729.

3.2 Extraction of essential oil

The fresh plant material (546 g) was subjected to hydro-distillation using a Clevenger type apparatus for 3 h according to the European Pharmacopoeia[23] (European Pharmacopoeia, 2002). The obtained hydro-distillate was extracted with hexane (3 times) and the organic layers was collected and dried on sodium sulfate. The organic solvent was eliminated at room temperature, under nitrogen flux. The obtained oil was stored in sealed vials under nitrogen atmosphere at −20℃ in the dark, until analysis. The essential oil was obtained in yield of 0.11% (w/w).

3.3 GC-FID and GC/MS analysis

For gas chromatographic separations, an *Agilent* 4890D instrument coupled to an ionization flame detector (FID)

was used. Volatile components were separated on a *HP-5* capillary column (5% phenylmethylpolysiloxane, 25 m, 0.32 mm i.d.; 0.17 μm film thickness) (*J and W Scientific*, Folsom, CA), with the following temperature program: 5 min at 60℃, subsequently 4℃/min up to 220℃, then 11℃/min up to 280℃, held for 15 min, for a total run of 65 min. Injector and transfer line temperatures were 280℃. Helium was used as the carrier gas, at a flow rate of 1.4 ml/min; injection volume: 1 μl; split ratio, 1:34. A mixture of aliphatic hydrocarbons (C8-C30) (*Sigma*, Milan, Italy) in *n*-hexane, was directly injected into the GC injector under the above temperature program, in order to calculate the linear retention index of each compound. Oil samples were diluted 1:100 in *n*-hexane and injected at a volume of 1 μl. Analysis was repeated three times. Data were collected by using *HP3398A* GC Chemstation software (*Hewlett Packard*, Rev. A.01.01). Quantification of essential oil components was obtained by FID peak-area internal normalization by using correction factors[24].

GC-MS analysis was performed on an *Agilent 6890N* gas chromatograph coupled to a *5973N* mass spectrometer using a *HP-5MS* (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness) (*J & W Scientific*, Folsom) capillary column. The temperature program was the same as above. Injector and transfer line temperatures were 280℃. Helium was used as the carrier gas, at a flow rate of 1 ml/min. Split ratio: 1:50; acquisition mass range: 29-400 *m/z*. All mass spectra were acquired in electron-impact (EI) mode with an ionization voltage of 70 eV. Oil samples were diluted 1:100 in *n*-hexane and the volume injected was 2 μl. Data were analyzed by using MSD ChemStation software (*Agilent*, Version G1701DA D.01.00). Whenever possible volatile components were identified by co-injection with authentic standards purchased from Sigma-Aldrich (Milan, Italy). Otherwise, the peak assignment was carried out according to the recommendations of the *International Organization of the Flavor Industry* (IOFI, http://www.iofi.org/)[25], i.e., by the interactive combination of chromatographic linear retention indices that were consistent with those reported in MS libraries[26, 27] for apolar stationary phases, and MS data consisting in the computer matching with the following libraries: *WILEY275*, *NIST 08* and *ADAMS*. In addition, a home-made library, based on the analyses of reference oils and commercially available standards was used as well.

3.4 Cytotoxicity assay

MDA-MB 231 (human breast adenocarcinoma) and A375 cells (human malignant melanoma) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS). HCT116 (human colon carcinoma) cells were cultured in RPMI1640 medium with 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and supplemented with 10% HI-FBS. Cells were cultured in a humidified atmosphere at 37℃ in presence of 5% CO_2. The MTT assay was used as a relative measure of cell viability. Cell-viability assays were carried out as described by Quassinti[28] *et al.*. Briefly, cells were seeded at the density of 2×10^4 cells/ml. After 24 h, samples were exposed to different concentrations of essential oil. Ten experimental groups were tested with the following concentrations of essential oil: 0, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 μg/ml. Each group was performed in quadruplicate. Cells were incubated for 72 h in a humidified atmosphere of 5% CO_2 at 37℃. At the end of incubation, each well received 10 μl of 3-(4,5-dimethylthiozol-2-yl) 2-5 diphenyltetrazolium bromide (MTT) (5 mg/ml in phosphate-buffered saline, PBS) and the plates were incubated for 4 h at 37℃. The extent of MTT reduction was measured spectrophotometrically at 540 nm using a Titertek MultiscanmicroElisa (Labsystems, FI-Helsinki). Experiments were conducted in triplicate. Cytotoxicity was expressed as the concentration of compound inhibiting cell growth by 50% (IC_{50}). The IC_{50} values were determined with GraphPad Prism 4 computer program (GraphPad Software, S. Diego, CA, USA).

3.5 Antioxidant activity

DPPH free radical scavenging activity was evaluated on a microplate analytical assay as previously described[29]. Stock solution of DPPH was prepared methanol and stored at −20℃ until needed. The working solution was obtained by mixing stock solution with methanol to obtain an absorbance of 1 unit at 517 nm. The absorbance of tested and blank control solutions (the same chemicals, except for the sample) was recorded spectrophotometrically at 517 nm after incubation for 30 min in the dark. Free Radical Scavenging Capacity (RSC) of solution was then calculated as percent inhibition according to the following equation:

$$RSC(\%\ inhibition) = 100(A_{(blank)} - A_{(sample)})/A_{(blank)}$$

Antioxidant activity of the essential oil was expressed as IC_{50} values, which represented the concentrations of the essential oil that caused 50% inhibition; it was determined by linear regression analysis from the obtained RSC values. Trolox was used as reference. Results were expressed in μmoles of Trolox equivalents (TE)/g of essential oil.

ABTS assay, was performed following the procedure previously described by Re and coworkers[30], applied to a 96-well microplate assay[31]. The $ABTS^+$ stock solution was prepared by mixing the two solutions of ABTS (7.4 mM) and potassium persulfate (2.6 mM) in equal quantities and allowing them to react for 12 h in the dark at room temperature. The working solution was then obtained by mixing $ABTS^+$ solution with methanol to obtain a final solution with absorbance of 1 unit at 734 nm measured with a Varian Cary 1 spectrophotometer. Trolox was used as refer-

ence. Results were expressed in μmoles Trolox equivalents (TE)/g of essential oil. The capacity of free radical scavenging (IC_{50}) was determined using the previously reported equation. All data were expressed as means ± standard deviations (SD) of triplicate measurements. The confidence limits were set at $p<0.05$. SD did not exceed 5% for the majority of the values obtained.

The FRAP assay was carried out as reported previously[32]. The stock solutions contained 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s -triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5mL $FeCl_3$ $6H_2O$ solution and then warmed at 37℃ before used. Aliquots of diluted oil solution in methanol allowed to react with 500 μL of the FRAP solution in the dark for 30 min. Samples were centrifuged at 10000 rpm and aliquots (280 μL), then withdrawn and read in 96-well microplates. Readings of the coloured product [ferrous tripyridyltriazine complex] were then carried out at 593 nm. The standard curve was linear between 25 and 800 μM Trolox (TE). Results were expressed in μmoles Trolox equivalents (TE)/g of essential oil.

4 Conclusions

The composition detected in *Helichrysum microphyllum subsp. tyrrhenicum* growing in La Maddalena Archipelago confirmed the existence of a worthy chemical polymorphism among populations coming from different areas. In comparison to the composition of the oils from other populations our sample showed a lower content of neryl acetate (19.2%) and a high content of sesquiterpenes (about 61%). Moreover, other marker components detected in other Sardinian populations such as linalool and γ-curcumene are completely missing in our sample. We assume that geographical isolation affected the essential oil composition of the investigated population with respect to those from other regions of the Isle. The essential oil showed a significant cytotoxicity against the human tumor cell examined, notably on melanoma cells, deserving further studies for a possible use as anticancer agent. Finally, the antioxidant activity of the essential oil resulted weak as a consequence of the poorness of components endowed with hydrogen- or electron-donor functional groups.

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