Basma Najar*, Valeria Nardi, Claudio Cervelli, Francesca Mancianti, Simona Nardoni, Valentina Virginia Ebani and Luisa Pistelli

Helichrysum araxinum Takht. ex Kirp. grown in Italy: volatiloma composition and in vitro antimicrobial activity

https://doi.org/10.1515/znc-2020-0025
Received February 7, 2020; revised March 19, 2020; accepted March 28, 2020

Abstract: In the present work the composition of biogenic volatile organic compounds (BVOCs) and the essential oil (EO) of *Helichrysum araxinum* Takht. ex Kirp. aerial parts, together with the antimicrobial activity, were investigated. The results showed the prevalence of sesquiterpene hydrocarbons in both spontaneous emissions as well as in the EO. The main compounds of BVOCs were γ-curcumene (10.7%), γ-muurolene (9.2%), and β-selinene (8.5%). This latter constituent also showed a similar amount in the EO and represented the most abundant compounds together with α-selinene (8.0%). It is Interesting to note the same percentage of monoterpene hydrocarbons (MHs) in both the aroma profile and the EO (18.0%) with the same most abundant compounds: β-pinene (6.3% in BVOCs vs. 5.1% in EO, respectively) and limonene (4.5% in VOCs vs. 4.9% in EO, respectively). With regard to the antimycotic activity, the EO showed to be inactive against the tested strains, while a moderate antibacterial activity was shown against *Staphylococcus* isolates.

***Corresponding author: Basma Najar,** Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy, E-mail: basmanajar@hotmail.fr
Valeria Nardi: Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy
Claudio Cervelli: CREA-Centro di Ricerca Orticoltura e Florovivaismo, Corso Inglesi 508, 18038 Sanremo, Italy
Francesca Mancianti and Valentina Virginia Ebani: Dipartimento di Scienze Veterinarie, Università di Pisa, Viale delle Piagge 2, 56124 Pisa, Italy; and Centro Interdipartimentale di Ricerca Nutraceutica e Alimentazione per la Salute "Nutrafood", Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy
Simona Nardoni: Dipartimento di Scienze Veterinarie, Università di Pisa, Viale delle Piagge 2, 56124 Pisa, Italy
Luisa Pistelli: Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy; and Centro Interdipartimentale di Ricerca Nutraceutica e Alimentazione per la Salute "Nutrafood", Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy.
https://orcid.org/0000-0002-6898-6510

Keywords: bacterial strains; BVOC; essential oil; fungi strains.

1 Introduction

Helichrysum genus, *Gol-e-Bimarg* as it is called in the Persian traditional language, belongs to the Asteraceae family with 600 species mainly distributed in Africa and Madagascar [1]. Plants of this genus are known for their richness in secondary metabolites, including flavonoids, acetophenones, phloroglucinols, pyrones, triterpenoids, and sesquiterpenes [2]. These secondary metabolites, used as a biochemical defense mechanism against bacteria and fungi, have recently been of great interest [3–6]. *Helichrysum araxinum* Takht. ex Kirp. is a native species of Turkey where it grows in rocky limestone slopes, forest clearings and steppes at 900–2500 m above sea level. It is a strongly suffruticose plant, 18–5-cm high, from subglaborous to thin lanate, with numerous erect twiggy stems and median cauline leaves. It shows very close and neat corymb with turbinate-cylindrical straw-colored capitula, each 5–6 mm long, from three to 10 in each corymb. All flowers are hermaphrodite [7].

The literature reports many medicinal benefits of the different *Helichrysum* species. The most recent report dates back to more than 10 years ago (2008) when Lourens et al. [8], in his review on the traditional use of the South African *Helichrysum* species, cited their use in treating gall bladder disorders, due to its bile regulation and diuretic effects. The *H. araxinum* herbal products were also used as relief for stomach ache, for their anti-infective, hepaprotective, cholagogic and choleretic effects; to stimulate the secretion of gastric juices, and for the treatment of coughs and diabetes mellitus. Furthermore, its antimicrobial [9] and cytotoxic properties [10] are well known.

Phytochemical investigation on *H. araxinum* reported in the literature has shown the presence of some flavonoids such as apigenin, luteolin, naringenin, astragalin, helichrysins A and B, isosalipurposide, apigenin 4′- and 7-glucosides, and quercetin 3-glucoside in the capitula,

while kaempferol, quercetin, astragalin, and quercetin-3-glucoside were found in the leaves [11].

The present work investigates the chemical characterization by gas chromatography-mass spectrometry (GC-MS) of the spontaneous volatile emission of *H. araxinum* aerial parts and the essential oil (EO) composition for the first time. Furthermore, the in vitro antimicrobial activity of its EO against fungi and bacteria has also been reported.

2 Experimental

2.1 Plant material

Helichrysum araxinum Takht. ex Kirp., grown in pots under uniform environmental condition at the Centro di Ricerca Orticoltura e Florovivaismo (CREA), Sanremo, Italy, was collected in 2018 and identified by one of us (C. Cervelli). A voucher specimen was deposited at the Herbarium of Giardini Botanici Hanbury (La Mortola, Ventimiglia, Italy) (HMGBH.e/9006.2019.001).

2.2 EO extraction

Helichrysum araxinum air-dried aerial parts (50 g) were subjected to hydro-distillation for 2 h, using a Clevenger apparatus. The obtained oil was dehydrated over anhydrous magnesium sulfate, then diluted to 0.5% in *n*-hexane high-performance liquid chromatography (HPLC) grade prior to GC-MS injection. The injections were performed immediately after extraction.

2.3 Headspace solid phase microextractions (HS-SPME)

The headspace from fresh aerial parts of *H. araxinum* (about 2 g) was sampled by solid phase microextraction (SPME). The adsorption of volatiles was performed with a Supelco polydimethylsiloxane fiber assembly (100-µm coating thickness, Supelco, St. Louis, MO, USA) preconditioned according to the manufacturer's instructions. After the equilibration time, the septum of each vial was perforated by the holder (syringe), then the fiber was exposed to the headspace of the sample for 30 min at room temperature. Once the sampling was complete, the fiber was retracted into the holder and directly injected in the GC-MS apparatus for separation and analysis.

2.4 GC-MS analyses

The GC-MS analysis was carried out with an Agilent 7890D gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an Agilent HP-5MS (Agilent Technologies Inc.) capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) and an Agilent 5977B single quadrupole mass detector (Agilent Technologies Inc.).

The GC oven temperature was set to rise from 60 to 240 °C at a rate of 3 °C/min. The split ratio was adjusted at 1:25. The carrier gas helium was at 1 mL/min; an injection of 1 µL (0.5% HPLC grade *n*-hexane solution). The acquisition parameters were as follows: full scan; scan range: 30–300 m/z; scan time: 1.0 s.

2.5 Peak identification

Identification of the EO components was carried out either through the comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) with a series of *n*-hydrocarbons, and by a computer matching against commercial (NIST 14 and ADAMS 2007) and laboratory-developed mass spectra library built up from pure substances and components of known oils and MS literature data [12–17].

2.6 Antimycotic activity

The antimycotic activity of the *H. araxinum* EO was checked against different fungal species. In detail, two feline clinical isolates of *Microsporum canis* and *Trichophyton mentagrophytes*, a clinical isolate from turtles of *Fusarium solani* and two environmental fungi, *Aspergillus niger* and *Aspergillus flavus*. All the microorganisms were used for in vitro sensitivity assays and were maintained on malt extract agar (MEA) at room temperature until use.

The minimum inhibitory concentration (MIC) value was calculated by a microdilution test, performed as recommended by the Clinical and Laboratory Standards Institute (CLSI M38-A2) for molds (2008) [18], starting from a 5% EO dilution. Five percent, 2.5%, 1.5%, and 1% dilutions were carried out. All assays were performed in triplicate.

2.7 Antibacterial activity

The EO was tested against three field bacterial strains: *Escherichia coli* (Gram-negative), *Staphylococcus aureus* and *Staphylococcus pseudointermedius* (Gram-positive). The strains have been previously isolated from canine clinical specimens, typed and stored at −80 °C in glycerol broth.

The antibacterial activity of the EO, diluted at 10% in dimethyl sulfoxide (DMSO, Oxoid Ltd., Basingstoke, Hampshire, UK) was tested using the Kirby-Bauer agar disc diffusion method [19]. A commercial disk impregnated with chloramphenicol (30 μg) (Oxoid) and a paper disk impregnated with 10 μL of DMSO were included as positive and negative controls, respectively. All tests were performed in triplicate. Successively, the MIC was determined with the broth microdilution method following the guidelines of the CLSI [20] with some modifications as previously reported [21].

3 Results and discussion

The complete identification of the spontaneous emission as well as the EO composition is reported in Table 1. Sesquiterpene hydrocarbons represented the most abundant chemical class of compounds in both biogenic volatile organic compounds (BVOCs) and EO, accounting for 79.5% and 53.1%, respectively. The identified compounds belonging to this class are very different in the SPME and EO. γ-Curcumene (10.7%), γ-muurolene (9.2%), β- and α-selinene (8.5 and 7.3%, respectively) as well as guaia-6,9-diene (7.3%) were the most abundant sesquiterpene hydrocarbons in the VOC emission of *H. araxinum*. These compounds were also the most represented in the EO, where β- and α-selinene together with γ-curcumene accounted for over 23%. Conversely, γ-muurolene was significantly less represented in the EO (0.4%).

Oxygenated sesquiterpenes showed a more relevant relative abundance in the EO composition. Among them, selina-6-en-4α-ol, cubenol, α- and β-eudesmol showed the highest contents (5.8%, 1.7%, 1.6% and 1.4%, respectively), whereas their presence was not detected in the spontaneous emission.

Monoterpene hydrocarbons were present in the same relative abundance both in BVOCs and in the EO (about 18.0%), with β-pinene (6.3% vs. 5.1%, respectively), limonene (4.5% vs. 4.9%, respectively), and α-pinene (3.2% vs. 3.7%, respectively) as the main constituents.

To the best of our knowledge there are no reports in the literature on the spontaneous emission of the studied *Helichrysum* species. The only study on the EOs from leaves, stems and flowers of Iranian *H. araxinum* [11] showed limonene as the most abundant compound in all the plant parts (29.2, 23.6, and 21.2%, respectively), together with α-pinene, a monoterpene hydrocarbon, present in leaves and stems (14.4% and 13.4%, respectively). On the contrary, the EO analyzed in the current study, showed a very low amount of these two constituents (only 4.9% of the total composition of limonene followed by 4% of α-pinene).

The published EO composition of *H. araxinum* flowers [11] was, instead, dominated by α-cadinol which represented over 18%, followed by borneol (11.9%), δ-cadinene (9.0%), bornyl acetate (8.0%), and α-humulene (7.3%). Except for borneol and bornyl acetate, which were present in very low amounts (1.2% and 0.5%, respectively), the other three compounds were not detected in the EO studied herein.

The EO did not show any antimycotic activity at the tested dilution and the MIC value was fixed at >5%. The EO resulted not active against *E. coli* with both the methods employed. It showed moderate antibacterial activity when tested against the staphylococcal strains. In fact, the agar disk diffusion method showed a growth inhibitory zone of 8 mm with both *S. aureus* and *S. pseudointermedius* isolates, and 5% (v/v) MIC values were determined with them (Table 2).

The *H. araxinum* EO showed a moderate activity against mold. Moreover, our results are in agreement with those found by other authors who analyzed the antibacterial activity of *Helichrysum italicum* EO and found that Gram-negative were more resistant than Gram-positive bacteria [24–26].

Cui et al. [27] found *H. italicum* oil active against *E. coli* and *S. aureus*. The antibacterial mechanism of this oil, which had an amount of γ-curcumene (11.64%) similar to our oil (10.7%), was related to the disruption of cell membranes with consequent losses of intracellular constituents.

From the literature, the EO of *Piper reticulatum* studied by Santana and his collaborators [28], showed a similar chemical composition of *H. araxinum* EO investigated herein; in fact, sesquiterpene hydrocarbons were the main class of compounds especially represented by β-selinene (19.0%), β-elemene (16.1%), and α-selinene (15.5%). The antifungal activity of that oil was tested on *A. flavus* and *T. mentagrophytes* and the obtained results confirmed the inactivity of these oils on these fungi, in agreement with our results.

Table 1: Chemical composition of VOCs and EO of *H. araxinum* aerial parts.

	Compounds	L.R.I.[a]	L.R.I.[b]	Class	SPME	EO
					Relative percentage (%)	
1	Bornylene	908	908[c]	MH	0.1±0.01	0.1±0.02
2	α-Pinene	939	939	MH	3.2±0.24	3.7±0.16
3	α-Fenchene	950	953	MH	–	1.0±0.08
4	Camphene	954	954	MH	1.3±0.88	0.6±0.03
5	β-Pinene	979	979	MH	6.3±1.15	5.1±0.16
6	Myrcene	991	991	MH	0.2±0.10	0.2±0.01
7	(E)-3-Hexen-1-ol acetate	1005	1002	NT	0.3±0.07	–
8	α-Terpinene	1017	1017	MH	0.6±0.21	0.5±0.02
9	*p*-Cymene	1025	1025	MH	0.1±0.01	0.2±0.02
10	Limonene	1029	1029	MH	4.5±0.15	4.9±0.21
11	γ-Terpinene	1060	1060	MH	1.0±0.37	0.9±0.03
12	Terpinolene	1089	1089	MH	0.7±0.25	0.8±0.03
13	Exo-fenchol	1122	1122	OM	0.1±0.09	0.5±0.02
14	3-Caraneol	1143	1125[d]	OM	–	0.2±0.02
15	Camphene hydrate	1148	1150	OM	–	0.2±0.01
16	Borneol	1169	1169	OM	0.3±0.04	1.2±0.03
17	4-Terpineol	1177	1177	OM	0.4±0.07	0.7±0.01
18	α-Terpineol	1189	1189	OM	0.6±0.08	1.9±0.01
19	Methyl 8-methyl-nonanoate	1277	1265	NT	0.1±0.01	0.2±0.01
20	Bornyl acetate	1289	1289	OM	0.3±0.06	0.5±0.02
21	Perilla alcohol	1297	1295	OM	–	0.4±0.03
22	*p*-Mentha-1,4-dien-7-ol	1330	1333	OM	–	0.1±0.00
23	7-Epi-silphiperfol-5-ene	1348	1348	SH	0.3±0.01	0.1±0.01
24	Neryl acetate	1362	1362	OM	0.2±0.03	0.3±0.01
25	α-ylangene	1375	1375	SH	1.3±0.03	0.4±0.03
26	Di-epi-α-cedrene-(I)	1382	1385	SH	0.7±0.11	0.3±0.01
27	Modephene	1385	1384	SH	3.6±0.11	1.4±0.07
28	α-Isocomene	1388	1388	SH	1.9±0.13	0.9±0.03
29	(+)-Sativene	1396	1392	SH	0.1±0.08	–
30	Italicene	1406	1406	SH	2.4±0.23	1,2±0.01
31	(±)-β-Isocomene	1412	1412	SH	0.3±0.03	–
32	β-Isocomene	1412	1407	SH	–	0.2±0.02
33	*Cis*-α-bergamotene	1413	1413	SH	1.1±0.22	0.3±0.01
34	β-Caryophyllene	1420	1419	SH	1.3±0.24	0.2±0.01
35	*Trans*-α-bergamotene	1435	1435	SH	1.2±0.31	0.5±0.01
36	Aromadendrene	1440	1441	SH	3.3±0.29	–
37	γ-Patchoulene	1441	1441	SH	–	2.0±0.06
38	Guaia-6,9-diene	1443	1447	SH	7.3±0.50	4.5±0.18
39	β-Gurjunene	1447	1434	SH	3.1±0.22	1.6±0.06
40	(*E*)-β-Farnesene	1457	1457	SH	0.6±0.03	–
41	α-Elemene	1462	1469	SH	–	0.1±0.01
42	9-Epi-(*E*)-caryophyllene	1466	1466	SH	–	0.1±0.01
43	α-Acoradiene	1466	1466	SH	0.1±0.09	–
44	γ-Gurjunene	1473	1477	SH	0.8±0.08	–
45	4-Epi-α-acoradiene	1475	1475	SH	–	0.1±0.01
46	Aristolochene	1476	1479	SH	0.3±0.05	0.2±0.02
47	γ-Muurolene	1480	1480	SH	9.2±1.22	0.4±0.02
48	γ-Curcumene	1483	1483	SH	10.7±1.40	6.7±0.38
49	4,11-Selinadiene	1485	1485	SH	–	5.6±0.18
50	β-Selinene	1486	1490	SH	8.5±0.72	8.5±0.25
51	Valencene	1496	1496	SH	4.5±0.83	–
52	δ-Selinene	1497	1493	SH	4.7±0.35	6.0±0.25
53	α-Selinene	1498	1498	SH	7.3±0.76	8.0±0.38
54	α-Muurolene	1500	1500	SH	0.3±0.02	0.1±0.05
55	Epizonarene	1501	1502	SH	0.1±0.08	–
56	γ-Cadinene	1513	1514	SH	–	0.2±0.01

Table 1 (continued)

	Compounds	L.R.I.[a]	L.R.I.[b]	Class	SPME	EO
					Relative percentage (%)	
57	β-Curcumene	1516	1516	SH	0.3±0.09	0.2±0.02
58	7-Epi-α-selinene	1517	1522	SH	0.2±0.07	0.3±0.01
59	β-Cadinene	1518	1517	SH	2.2±0.43	1.3±0.05
60	δ-Cadinene	1523	1523	SH	0.3±0.01	0.2±0.06
61	Selina-3,7(11)-diene	1542	1547[c]	SH	0.9±0.23	0.40.01
62	(4aR,8aS)-4a-Methyl-1-methylene-7-(propan-2-ylidene) decahydronaphthalene	1544	1544[c]	SH	0.6±0.22	0.7±0.00
63	α-Calacorene	1546	1546	SH	–	0.4±0.01
64	Epi-globulol	1585	1585	OS	–	0.2±0.04
65	Viridiflorol	1591	1593	OS	–	1.0±0.02
66	Guaiol	1596	1601	OS	–	0.6±0.04
67	Humulene-1,6-dien-3-ol	1619	1619[c]	OS	–	1.1±0.03
68	Selina-6-en-4-ol	1624	1624[c]	OS	–	0.7±0.11
69	Selina-6-en-4α-ol	1636	1636[c]	OS	–	5.8±0.26
70	Cubenol	1642	1647	OS	–	1.7±0.13
71	Hinesol	1642	1642	OS	–	0.3±0.13
72	Agarospirol	1645	1648	OS	–	0.4±0.04
73	β-Eudesmol	1649	1651	OS	–	1.4±0.10
74	α-Eudesmol	1654	1654	OS	–	1.6±0.14
75	Neointermedeol	1660	1660	OS	–	3.5±0.11
76	Intermedeol	1667	1667	OS	–	0.1±0.07
77	β-Bisabolol	1671	1675	OS	–	0.4±0.05
78	Ylangenal	1675	1674[c]	OS	–	0.1±0.11
79	α-Bisabolol	1686	1685	OS	–	0.3±0.07
80	Juniper camphor	1693	1700[c]	OS	–	0.3±0.02
81	*Trans*-α-bergamotol	1700	1691	OS	–	0.9±0.02
82	β-Santalol	1715	1716	OS	–	0.1±0.08
83	*Cis*-lanceol	1763	1761	OS	–	0.1±0.01
84	3,5,6,7,8,8a-Hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-2(1H)naphthalenone	1781	1773[c]	OS	–	0.1±0.02
	Yield of EO (% w/w)					1.4±0.01
	Chemical classes				**SPME**	**EO**
	Monoterpene hydrocarbons (MH)				18.0±7.57	18.0±0.74
	Oxygenated monoterpenes (OM)				1.9±0.31	6.0±0.10
	Sesquiterpene hydrocarbons (SH)				79.5±7.90	53.1±1.95
	Oxygenated sesquiterpenes (OS)				–	20.7±1.30
	Non-terpene derivatives (NT)				0.4±0.06	0.2±0.01
	Total identified (%)				99.8±0.10	98.0±0.50

Data are reported as mean values (n = 3; ±standard deviation [SD]); L.R.I. [a]Linear retention time experimentally determined; L.R.I. [b]Linear retention time reported by Adams [22]; [c]Linear retention time reported by NIST [23]; [d]Linear retention index in chemspider (www.chemspider.com). VOCs, volatile organic compounds.

Table 2: Antimycotic and antibacterial activity of the *H. araxinum* EO.

Fungi strains					Bacterial strains					
M. canis	***T. mentagrophytes***	***A. flavus***	***A. niger***	***T. solani***	***S. aureus***		***S. intermedius***		***E. coli***	
MIC (%)					**MIC (%)**	**Disk (mm)**	**MIC (%)**	**Disk (mm)**	**MIC (%)**	**Disk (mm)**
>5	>5	>5	>5	>5	5	8	5	8	Non-effective	

MIC, mean inhibitory concentration.

References

1. Najar B, Cervelli C, Ferri B, Cioni PL, Pistelli L. Essential oils and volatile emission of eight South African species of *Helichrysum* grown in uniform environmental conditions. S Afr J Bot 2019;124:178–87.
2. Maffei Facino R, Carini M, Franzoi L, Pirola O, Bosisio E. Phytochemical characterization and radical scavenger activity of flavonoids from *Helichrysum italicum* G. Don (Compositae). Pharmacol Res 1990;22:709–21.
3. Sharifi-Rad M, Roberts TH, Matthews KR, Bezerra CF, Morais-Braga MF, Coutinho HD, et al. Ethnobotany of the genus *Taraxacum* – phytochemicals and antimicrobial activity. Phytother Res 2018;32:2131–45.
4. Stojanović-Radić Z, Pejcić M, Stojanović N, Sharifi-Rad J, Stanković N. Potentail of *Ocimum basilicum* L. and *Salvia officinalis* L. essential oils against biofilms of *P. aeruginosa* clinical isolates. Cell Mol Biol (Noisy-le grand) 2016;62:27–33.
5. Salehi B, Sharopov F, Martorell M, Rajkovic J, Ademiluyi AO, Sharifi-Rad M, et al. Phytochemicals in *Helicobacter pylori* infections: what are we doing now? Int J Mol Sci 2018;19:Pii:E2361.
6. Sharifi-Rad J, Ayatollahi SA, Varoni E, Salehi B, Kobarfard F, Sharifi-Rad M, et al. Chemical composition and functional properties of essential oils from *Nepeta schiraziana* Boiss. Farmacia 2017;65:802–12.
7. Davis PH. Flora of Turkey and the East Aegean Island. Vol. 5. Edinburgh 1975:80–97.
8. Lourens AC, Viljoen AM, van Heerden FR. South African *Helichrysum* species: a review of the traditional uses, biological activity and phytochemistry. J Ethnopharmacol 2008;119:630–52.
9. Albayrak S, Aksoy A, Sagdic O, Hamzaoglu E. Compositions, antioxidant and antimicrobial activities of *Helichrysum* (Asteraceae) species collected from Turkey. Food Chem 2009;119:114–22.
10. Bigović D, Šavikin K, Janković T, Menković N, Zdunić G, Stanojković T, et al. Antiradical and cytotoxic activity of different *Helichrysum plicatum* flower extracts. Nat Prod Commun 2011;6:819–22.
11. Oji KA, Shafaghat A. Constituents and antimicrobial activity of the essential oils from flower, leaf and stem of *Helichrysum armeneium*. Nat Prod Commun 2012;7:671–4.
12. Adams RP. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Carol Stream, IL: Allured Publishing Corporation, 1995.
13. Davies NW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and carbowax 20M phases. J Chromatogr A 1990;503:1–24.
14. Jennings W, Shibamoto T. Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography. Food/Nahrung. New York: Academic Press, 1980.
15. Masada Y. Analysis of essential oils by gas chromatography and mass spectrometry. New York, NY: John Wiley & Sons, Inc., 1976.
16. Stenhagen E, Abrahamsson S, McLafferty FW. Registry of mass spectral data. New York, NY: Wiley & Sons, 1974.
17. Swigar AA, Silverstein RM. Monoterpenes. Milwaukee, WI: Aldrich Chemical Company, 1981.
18. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved 541 standard, 2nd ed. CLSI document M38-A2. Wayne, PA: CLSI, 2008.
19. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; approved standard – 11th ed. CLSI document. Wayne, PA: Clinical and Laboratory Standards Institute, 2012. M02-11.
20. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A2. Villanova, PA: CLSI, 1990.
21. Ebani VV, Nardoni S, Bertelloni F, Giovanelli S, Rocchigiani G, Pistelli L, et al. Antibacterial and antifungal activity of essential oils against some pathogenic bacteria and yeasts shed from poultry. Flavour Frag J 2016;31:302–9.
22. Adams RP. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy, 4th ed. Carol Stream, IL: Allured Publishing Corporation, 2007.
23. NIST 14/EPA/NIH Mass Spectra Library; I. Hoboken, NJ: Wiley and Sons, Inc., 2014.
24. Nardoni S, Giovanelli S, Pistelli L, Mugnaini L, Profili G, Pisseri F, et al. In vitro activity of twenty commercially available, plant-derived essential oils against selected dermatophyte species. Nat Prod Commun 2015;10:1473–8.
25. Stupar M, Gbric ML, Dzamic A Unkovic N, Ristic M, Vukojevic J. Antifungal activity of *Helichrysum italicum* (Roth) G. Don (Asteraceae) essential oil against fungi isolated from cultural heritage objects. Arch Biol Sci 2014;66:1539–45.
26. Bouzid D, Nouioua W, Soltani E, De Haro JP, Angeles Esteban M, Zerroug M. Chemical constituents of *Helichrysum italicum* (Roth) G. Don essential oil and their antimicrobial activity against Gram-positive and Gram-negative bacteria, filamentous fungi and *Candida albicans*. Saudi Pharm J 2017;25:780–7.
27. Cui H, Zhao C, Lin L. Antibacterial activity of *Helichrysum italicum* oil on vegetables and its mechanism of action. J Food Process Pres 2015;39:2663–72.
28. Santana AI, Vila R, Cañigueral S, Gupta MP. Chemical composition and biological activity of essential oils of different species of piper from Panama. Planta Med 2017;82:986–91.