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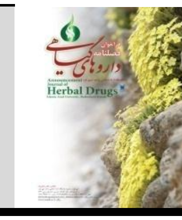

Chemical composition of the volatile oils of three Lamiaceae species growing wild in North of Iran

Salar Hafez Ghoran[1]*, Boshra Azadi[2], Hidayat Hussain[3]

[1]*Department of Chemistry, Faculty of Sciences, Golestan University, Gorgan 4913815739, Iran;*
Email:** ***S.Hafezghoran@gu.ac.ir and S_Hafezghoran@yahoo.com

[2]*Phytochemistry Department, Faculty of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran;*

[3]*UoN Chair of Oman's Medicinal plants and Marine Natural products, University of Nizwa, Nizwa, Sultanate of Oman;*

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ABSTRACT

Background & Aim: The Lamiaceae or Labiatae (commonly referred to as the mint or deadnettle family) are an exceedingly large and widespread of flowering plants (Angiospermae). Most member of Lamiaceae plants are used in Iranian folk medicine as stimulant, antiseptic, aphrodisiac, stomachic, expectorant, carminative, anti-diarrheic and anti-bacterial. The aim of the present work was to obtain a better insight into the nature of essential oil.

Experimental: The essences of three Lamiaceae species viz., *Satureja macrantha* C.A. Mey., *Ziziphora persica* Bunge, and *Lavandula stoechas* L. obtained by hydro-distillation method of flowers growing wild in the north of Iran. The study led to the identification of 67 components by a combination HP-5 GC-FID and GC-MS analytical techniques and the oil yields were 1.3 %, 1.64 %, and 1.16 % on fresh flowers, respectively.

Results & Discussion: The chemical components of *S. macrantha* were found to contain mainly: Carvacrol (43.3 %), γ-Terpinene (24.1 %) and *p*-Cymene (21.6 %). On the other hand the major oil components for *Z. persica* were Isopulegone (46.6 %), followed by *p*-mentha-1(7),8-diene (16.4 %), 1,8-Cineole (7.0 %), Isomenthol (5.5 %) and Isoledene (3.3 %), accounting for 98.0 % of the total components. Camphor (61.5 %), 1,8-Cineole (10.1 %), Fenchone (4.2 %) and Linalool (3.8 %) were found to be the major constituents of the oil of *L. stoechas*. The essential oils of *S. macrantha*, *Z. persica*, and *L. stoechas* were identified by much larger amount of monoterpenes (96.52 %, 89.31 %, and 93.49 %, respectively) and sesquiterpenes (1.87 %, 8.10 %, and 5.04 %, respectively).

Industrial and practical recommendations: Based on the results of this study, the chemical variations of three volatile oils might be correlated with geographical regions and environmental conditions (temperature, humidity, interaction with other species and etc.) play a significant role in biosynthesis of the components of the oil

1. Introduction

Volatile oils, essential oils or ethereal oils are usually obtained from the appropriate plant material by steam distillation. If certain components are unstable at these temperatures, however, then other, less harsh techniques, such as expression or solvent extraction, may be employed. These oils, which typically contain a complex mixture of low boiling-point components, are widely used in flavoring, perfumery, and aromatherapy (Isman, 2000). *Satureja macrantha* C. A. Mey., *Ziziphora persica* Bunge, and *Lavandula stoechas* L. are three species of aromatic herbs of the family Lamiaceae (=Labiatae) and are widely distributed in the northern part of Iran.

Satureja species grow in different geographical areas of Iran. There are 12 annual and perennial species (eight species are endemic of Iran), which have been used as a foodstuff and traditional medicine. *S. macrantha* C. A. Mey.; is a small shrub divided in western, northwestern and northern parts of Iran (Mozaffarian, 2013).

There are four species of *Ziziphora* genus which grow in Iran and other countries such as Afghanistan, Armenia, Pakistan, Turkey, and Turkmenistan (Mozaffarian, 2013). Moreover *Ziziphora* species are present in high altitude areas in Iran, especially northern and eastern parts. *Z. persica* Bunge: is an edible medicinal plant, which the aerial parts used in foods to offer flavor and aroma (Ozturk *et al*., 1995). Furthermore the volatile oils of *Z. persica* have antibacterial activity, which can be used as conservative in foods, to protect them from pathogens (Ozturk & Ercisli, 2006).

Lavandula species are important members of family Lamiaceae, which are mainly cultivated for their essential oils. There are three species of *Lavandula* genus, which are wildly distributed in north of Iran (Golestan province) (Mozaffarian, 2013). The dried flowers of *L. stoechas* have been used in perfumery, cosmetics, food processing and traditional medicine because of their various biological activities such as antifungal (Angioni *et al*., 2006), antibacterial (Goren *et al*., 2002; Bouzouita *et al*., 2005). Moreover the volatile oils of *L. stoechas* were shown effective cytotoxic activity on COL-2 (human colon cancer) (Goren *et al*., 2002).

Interestingly the extracts of three investigated plants are used in folk medicine as stimulant, antiseptic, aphrodisiac, stomachic, expectorant, and carminative, anti-diarrheic, anti-bacterial and also have anti-inflammatory activity (Ozturk *et al*., 1995; Goren *et al*., 2002; Ozturk & Ercisli, 2006; Beikmohammadi, 2011). The aim of the present work was to obtain a better insight into the nature of essential oil of three Lamiaceae herbs from north of Iran (Golestan province) by using GC-FID and GC/MS.

2. Materials and Methods

2.1. Plant material

The aerial parts of *S. macrantha*, *Z. persica*, and *L. stoechas* were collected in full flowering stages in August 2013 from Golestan province, Iran. Voucher specimens (No. 6240, No. 6241, and No. 6238, respectively) have been deposited in the Herbarium of the faculty of biology (Golestan University, Gorgan, Iran). Plant specimens were identified by Dr. Mohammad Fatemi from that institute. The dried flowering aerial parts for three herbs were individually extracted by hydro-distillation method using Clevenger-type apparatus for 4-5 hours to give the following yield (w/w): 1.3%, 1.04%, and 1.16%, respectively. Anhydrous sodium sulfate was used to remove water after extraction and the volatile oils stored in refrigerator at 4°C.

2.1. Isolation procedure of S. macrantha, Z. persica, and L. stoechas essential oil

The determination of retention data and the area percentage of the identified oil components are mentioned in Table 1. The experiments were analyzed by an Agilent 7890A gas chromatograph (manufactured by America) equipped with a 30 m × 0.32 *i.d*., 0.25 µm film thicknesses with silica HP-5 column (capillary). In addition, GC-MS was measured in Hewlett Packard 5975C GC-MS system with a HP-5 fused silica column using the aforementioned chromatographic conditions (Table 1).

2.3. Identification and characterization of the volatile oil constituents

The retention indices (RI) for three herbs oil were individually determined by conjunction of the sample with containing the homologous series of C_8 to C_{26} *n*-alkanes (Van del Dool & Kratz, 1963). The constituents of *S. macrantha*, *Z. persica*, and *L. stoechas* oils were identified by their retention indices compared with data published in the literature (Adams, 2001), computer library (NIST/NIH libraries) and Wiley library (Massada, 1976). Finally, the percentage of each oil component was carried out by peak area normalization measurement.

3. Results and discussion

The essential oil compounds of *S. macrantha*, *Z. persica*, and *L. stoechas* were analyzed by using GC-MS analysis. The yield oils (yellow liquids) were 1.3 %, 1.04 %, and 1.16 % (w/w), based on dry weights, respectively. Finally, 67 components were identified in three Lamiaceae species (see Tables 2).

Twenty-eight compounds were identified form the essential oils of *S. macrantha*. The major components of the essential oils of *S. macrantha* were carvacrol (43.02%), γ-terpinene (24.1%) and *p*-cymene (21.6%). The high percentages of carvacrol and p-cymene proved that this essential oil clearly belongs to the aromatic chemo-type (see Table 2). According to the previous studies, Sefidkon and Jamzad studied the oil of *S. macrantha*, which collected in northeast of Iran (Khorasan), consists of p-cymene (25.8%), limonene (32.3%) and thymol (8.1%) (Sefidkon & Jamzad, 2005). The relative quantities of γ-terpinene and p-cymene are noticeably smaller in the *S. macrantha* from Turkey but carvacrol (64.4%) was higher (Azaz *at al*., 2005). Golestanian *S. macrantha* oil has carvacrol as the most abundant component, while volatile oil of *S. macrantha* obtained from the northwest of Iran (Urmia); contains spathulenol (14.0%), vanillin (13.4%) and *p*-cymene (12.3%), as the main constituents (Gohari *et al*., 2006).

Moreover, 32 components, representing 98.09 % of the oil content of Golestanian *Z. persica* were characterized, which are mentioned in Table 2. It is interesting to note that the most abundant compounds were isopulegone (46.6%), followed by *p*-mentha-1(7),8-diene (16.4%), 1,8-Cineole (7.0%), isomenthol (5.5%) and isoledene (3.3%).

The *Z. persica* oil consisted of 31.6% monoterpene hydrocarbons, 7.3% monoterpene alcohols, 0.7% monoterpene esters, 7.0% monoterpene ethers, 49.3% monoterpene ketones and 8.1% sesquiterpenes. It has been reported by Dembitskii *et al* that major component of the essential oils of *Z. persica* was pulegone and that species was collected from Kazakh and Azerbaijanian (Dembitskii *et al*., 1994). Moreover, the major components of the essential oils of Turkey origin *Z. persica* were (+)-pulegone (79.3%), limonene (6.8%) and piperitenone (4.2%). On the other hand, Golestanian *Z. persica* oils were identified by high percentage of monoterpene ketone, such as isopulegone. These data indicated that monoterpene ketones (isopulegone and pulegone) are mainly chemo-type for *Z. persica* in Iran, Turkey, Kazakhstan and Azerbaijan (Dembitskii *et al*., 1994; Ozturk & Ercisli, 2006), while *Z. persica* from the eastern parts of Iran is mostly monoterpene alcohol chemo-type, with high contents of neomenthol (22.5%) and *p*-menth-3-en-8-ol (18.1%) (Rustaiyan *et al*., 2006).

Essential oil compounds of *Z. persica* from Golestan constitute the first report on savory oil of the isopulegone chemo-type.

Table 2 reports the chemical constituents of *L. stoechas*. Thirty-seven components were identified, which accounted for 98.55% of the total volatile fraction in the *L. stoechas* aerial parts. In a research on *Lavandula* essential oils, Angioni *et al* (2006) noted the enormous variability in the chemical compositions of *L. stoechas*. In spite of the variability, there are some apparent chemical constituents. Not only are there fenchone-rich, camphor-rich, myrtenyl acetate-rich, α-pinene-rich and camphene-rich chemotypes as previously noted by Angioni *et al*, but also in Golestanian *L. stoechas*, Camphor was the main compound, accounting 61.32%, followed by 1,8-Cineole, fenchone, linalool and borneol (10.08%, 4.21%, 3.77% and 3.19%, respectively).

β-Selinene, β-eudesmol, camphene and α-pinene were the most representative components. The Grecian *L. stoechas* essential oil obtained from aerial parts were identified by fenchone (30.9%), camphor (9.6%) and 1,8-cineole (8.1%), and it was rich in pinocarvyl acetate (10.2%) (Kokkalou, 1988). The Tunisian essential oil from aerial parts of *L. stoechas* was characterized by the low amount of 1, 8-cineole (4.9%), but it was rich in fenchone (68.2%) (Bouzouita *et al*., 2005).

Table 1. Temperature programming for determination of retention data and the area percentage of *Satureja macrantha* C. A. Mey; *Ziziphora persica* Bunge and *Lavandula stoechas* L.

Treatment	Rate (ºC/min)	Column temperature (ºC)	Holding time (min)	Injector temperature (ºC)	Detector temperature (ºC)	Flow rate (mL/min)	Carrier gas
		50	3	250	260	1	Helium
S. macrantha	4	250	0				
	5	280	3				
		50	10	250	260	1	Nitrogen
Z. persica	4	200	0				
	10	270	5				
		60	4	250	260	1	Nitrogen
L. stoechas	3	100	2				
	4	260	0				

Table 2. Chemical components of the essential oils of *Satureja macrantha*, *Ziziphora persica*, and *Lavandula stoechas* from north of Iran (Golestan province).

Volatile Compounds [a]	LRI [b]	*S. macrantha*	*Z. persica*	*L. stoechas*	Method of identification [c]
Tricyclene [d]	923	0.43	-	-	MS, RI
α-Thujene [d]	930	1.19	0.14	-	MS, RI
α-Pinene	939	0.58	0.85	1.07	MS, RI, Std
Camphene [d]	951	0.24	0.33	1.21	MS, RI
Verbenene [d]	967	-	1.76	0.38	MS, RI
β-pinene	979	0.21	2.35	0.96	MS, RI, Std
Myrcene [d]	991	-	0.25	-	MS, RI
p-Mentha-1(7),8-diene [d]	1002	-	16.4	-	MS, RI
α-Phellandrene [d]	1005	0.23	-	-	MS, RI
δ-3-Carene [d]	1011	0.09	0.79	-	MS, RI
α-Terpinene [d]	1017	2.94	-	0.34	MS, RI
o-Cymene [d]	1022	-	-	0.32	MS, RI
p-Cymene [d]	1025	21.6	0.82	0.21	MS, RI
β-Phellandrene [d]	1026	0.17	0.17	0.25	MS, RI
Limonene [d]	1029	0.28	0.84	-	MS, RI
1,8-Cineole	1031	0.11	6.96	10.08	MS, RI, Std
β-Trans-ocimene [d]	1054	-	-	0.32	MS, RI
γ-Terpinene	1062	24.09	-	-	MS, RI, Std
Trans-4-thujanol [d]	1064	0.11	-	-	MS, RI
β-Terpinene [d]	1071	-	0.81	-	MS, RI
trans-Linalool oxide [d]	1084	-	-	0.75	MS, RI
Fenchone	1087	-	-	4.21	MS, RI, Std
α-Terpinolene [d]	1088	0.07	0.21	-	MS, RI
Linalool	1098	-	-	3.77	MS, RI, Std
α-Thujone [d]	1105	-	0.47	0.49	MS, RI
Chrysanthenone [d]	1123	-	0.53	-	MS, RI
α-Campholene aldehyde [d]	1132	-	-	0.15	MS, RI
Camphor	1143	-	-	61.32	MS, RI, Std
p-Menthone [d]	1147	-	1.54	-	MS, RI
Isopulegone	1153	-	46.58	-	MS, RI, Std
Pinocarvone [d]	1160	-	-	0.24	MS, RI
Borneol	1165	0.3	0.53	3.19	MS, RI, Std
Terpinen-4-ol [d]	1177	0.29	0.74	0.33	MS, RI
Isomenthol [d]	1182	-	5.54	-	MS, RI
Myrtenol [d]	1194	-	-	0.16	MS, RI

Myrtenal [d]	1195	-	-	0.37	MS, RI
Eucarvone (Verbenone) [d]	1204	-	-	0.18	MS, RI
cis-Carveol [d]	1226	-	-	0.34	MS, RI
Carvone [d]	1239	-	-	0.39	MS, RI
Cuminaldehyde [e]	1242	-	-	056	MS, RI
Geraniol	1249	-	-	0.57	MS, RI, Std
2′,4′-Dimethylacetophenone [d]	1255	-	0.21	-	MS, RI
Phellandral [d]	1273	-	-	0.4	MS, RI
Anethole [d]	1282	-	-	0.19	MS, RI
Thymol [d]	1290	0.09	-	-	MS, RI
p-cymen-7-ol [d]	1295	-	-	0.37	MS, RI
Carvacrol	1298	43.02	0.49	-	MS, RI, Std
Terpinen-4-ol acetate [d]	1340	0.25	-	-	MS, RI
α-Cubebene [d]	1348	-	0.31	-	MS, RI
Isoledene [d]	1373	-	3.33	-	MS, RI
β-Bourbonene [d]	1384	-	0.7	-	MS, RI
β-Cubebene [d]	1388	0.1	-	-	MS, RI
α-Cedrene [d]	1411	1.04	0.97	-	MS, RI
β-Caryophyllene [d]	1419	0.11	-	-	MS, RI
Iridomyrmecin [d]	1436	-	0.68	-	MS, RI
β-Farnesene [d]	1440	-	-	0.3	MS, RI
α-Humulene	1455	0.15	-	-	MS, RI, Std
Selina-4,11-diene [d]	1475	-	0.24	-	MS, RI
γ-Gurjunene [d]	1477	0.19	2.16	-	MS, RI
β-Selinene [d]	1489	-	-	1.25	MS, RI
β-Bisabolene	1505	-	-	0.45	MS, RI, Std
δ-Cadinene [d]	1523	0.15	-	0.16	MS, RI
α-Cadinene [d]	1538	-	0.23	-	MS, RI
Germacrene B	1561	0.2	0.16	-	MS, RI, Std
Caryophyllene oxide [d]	1582	-	-	1.05	MS, RI
β-Eudesmol [d]	1649	-	-	1.3	MS, RI
α-Bisabolol [d]	1685	0.36	-	0.36	MS, RI
Farnesol	1732	-	-	0.17	MS, RI, Std
Monoterpene hydrocarbons		52.12	25.72	5.06	
Oxygenated Monoterpenes		44.17	63.59	88.45	
Sesquiterpene hydrocarbons		2.94	8.10	2.16	
Oxygenated Sesquiterpene		0.27	-	2.88	
Other		-	0.68	-	
Total identified		98.5 %	98.09 %	98.55 %	

[a] Compounds are listed in order of their elution from a HP-5MS column using homologous series of C_8 to C_{26} *n*-alkanes. [b] LRI, literature retention indices on HP-5MS column. [c] Method of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; Std, by injection of an authentic sample. [d] Tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (LRI).

However, the essential oil obtained from *L. stoechas*, harvested in a different Turkish region, appeared to be of a peculiar chemotype characterized by the presence of pulegone (40.4%), menthol (18.1%), menthone (12.6%) and 1,8-cineole (3.9%) (Goren *et al*., 2002). Overall, the Golestanian *L. stoechas* essential oil analyzed in this study showed a lower content of fenchone and the presence of borneol in quite high with respect to literature data.

4. Conclusions

The incompatibility between the results mentioned in different literature on *S. macrantha*, *Z. persica* and *L. stoechas* could still be logical due to the influence of several factors mentioned such as experimental conditions, the climate conditions, harvest conditions, and geographical source (Kokkalou, 1988; Goren *et al.*, 2002; Bouzouita *et al*., 2005; Sefidkon & Jamzad, 2005; Angioni *et al*., 2006; Gohari *et al*., 2006; Rustaiyan *et al*., 2006). Interestingly α-pinene, camphene, β-pinene, p-cymene, β-phellandrene, 1,8-cineole, borneol and terpinen-4-ol were present in three investigated species viz., *S. macrantha*, *Z. persica*, and *L. stoechas*.

According to many studies, the chemical variations of *S. macrantha*, *Z. persica*, and *L. stoechas* volatile oils - given the same species with similar part of plant and method of distillation - might be correlated with geographical regions and environmental conditions (temperature, humidity, interaction with other species and etc.) play a significant role in biosynthesis of the components of the oil. It should also be noted that the chemical composition of an essential oil prepared from the same organ of one species varies to same degree according to the ecological conditions under which the herb has grown.

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