RESEARCH ARTICLE

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Chemical composition of the essential oil from *Scutellaria orientalis* L. subsp. *virens* (Boiss. & Kotschy) J. R. Edm., grown in Iran: Gas Chromatography/Mass Spectrometry Data

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ABSTRACT

Scutellaria orientalis L. subsp. *virens* (Boiss. & Kotschy) J. R. Edm. which is a perennial species of *Scutellaria* genus and *Lamiaceae* family is widely used as one of the most popular traditional Iranian herbal remedies against various diseases. This study was aimed to identify the essential oil components of *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. by Gas Chromatography/Mass Spectrometry (GC/MS) following hydro-distillation using a Clevenger-type apparatus. Essential oil analysis revealed 61 components including: monoterpenes (51.9%), sesquiterpenes (8.8%), hydrocarbons (20.4%), ketones (0.8%), aldehydes (0.8%), oxygenated diterpene (1.6%), acetate (1.3%) and isoprenoid (1.4%). The main constituents were oxygenated monoterpenes (51.6%), with trans-anethole (48.7%) as the major one. In conclusion, the results revealed the presence of biologically active compounds that can be used for a variety of herbal formulations.

Key words: GC/MS, Monoterpene, *Scutellaria*, trans-anethole

Introduction

Scutellaria L., a member of *Lamiaceae* family, has been distributed worldwide with over 350 species (Evans, 1989). 20 species and two hybrids of this genus already exist in Iranian flora, with 10 species and two hybrids being present as the native ones (Ghahreman & Attar, 1999). *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. is a perennial herb and medicinal plant which has been spread in Tabriz, Iran. It grows on soils with little clay, at the height of 1650 meters above sea level, and an annual rainfall of 250 to 300 mm (Figure 1).

All plant parts including leaves, fruits, roots as well as seeds are widely used in traditional Iranian medicine to treat constipation, wounds, stress, neurological disorders, dermatitis, bronchitis, and inflammation (Zargari, 1997). Pharmacological activities of *Scutellaria* genus are due to the presence of 4′-deoxyflavones such as chrysin, baicalein, wogonin, and their glycosides (baicalin and wogonoside) (Samarghandian et al., 2011). Baicalein, wogonin, baicalin, wogonoside, and chrysin are the main flavonoids of *S. orientalis* species (Gharari et al., 2020a; Gharari et al., 2020b). About essential oil components, already there are different reports on the phytochemical composition of different subspecies of *S. orientalis* essential oil: subsp. *virens* from Iran (Delnavazi et al., 2014), subsp. *virens* (Boiss. & Kotschy) J. R. Edm., subsp. *carica*, subsp. *haussknechtii*, subsp. *macrostegia*, subsp. *orientalis* and subsp. *santolinoides* from Turkey (İçen et al., 2016; Yilmaz et al., 2020). According to essential oil analysis in these subspecies, the main component of the essential oil was determined as germacrene D (16.5%), terpinolene (15.6%), and β-caryophyllene (13.4%) in subsp. *virens* (Delnavazi et al., 2014); β-Caryophyllene (22.08%), γ-Cadinene (19.92%) and Camphene (6%) in subsp. *virens* (Boiss. & Kotschy) J. R. Edm. (İçen et al., 2016); germacrene D (57.2%), bicyclogermacrene (7.4%) and β-caryophyllene (5.2%) in subsp. *carica*; germacrene D (23.5%), β-caryophyllene (14.2%), bicyclogermacrene (9.0%) and caryophyllene oxide (8.1%) in subsp. *haussknechtii*; germacrene D (47.8%), β-caryophyllene (16.3%) and bicyclogermacrene (12.0%) in subsp. *macrostegia*; caryophyllene oxide (23.5%), germacrene D (20.1%) and β-caryophyllene (14.5%) in subsp. *orientalis*; germacrene D (42.1%), β-caryophyllene (13.7%) and bicyclogermacrene (11.3%) in subsp. *santolinoides* (Yilmaz et al., 2020). Differences in the essential oil content of these sub-species prompted us to re-analyze the essential oil content of this species collected from its habitat in Marand, Iran and identify and quantify its essential oil phytochemical profiles by GC/MS analysis.

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Figure 1. *Scutellaria orientalis L. subsp. virens (Kotcshy & Boiss.) J. R. Edm. during flowering.*

Materials and Methods

Plant material collection

S. orientalis L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. shoots were collected from Marand city, Iran during the summer season (July, 2018). The plant was identified by Dr. Talebpour (plant taxonomy section, University of Tabriz, Tabriz, Iran) and a voucher specimen was deposited in the herbarium of the East-Azarbaijan Agricultural and Natural Resources Research and Education Center, Tabriz, Iran (No. 2488).

Essential oil extraction

Aerial parts of the plant during its foliation–flowering stage were used for essential oil extraction. Plant leaves, flowers, and shoots were kept in the shade to get dried, powdered by a grinder, and used as the crude source. The essential oil was extracted by hydro-distillation in a Clevenger-type apparatus for three hours using around 100 g fresh weight of total plant material. Following extraction, anhydrous sodium sulfate was utilized to absorb the little water content of the obtained essential oil. Subsequently, each sample was tagged and stored at 4°C.

Gas Chromatography/Mass Spectrometry (GC/MS)

A qualitative assessment of essential oil was carried out by GC/MS. GC analysis was conducted in a Hewlett-Packard (HP, Palo Alto, CA) HP 7890A gas chromatograph system. Helium (99.999%) was utilized as the carrier gas, at the flow rate of 1 ml/min. The injection port temperature was set at 240 °C; column temperature was firstly held at 40 °C for 1 min, and next slowly risen to 240 °C at the rate of 3 °C/min.

Identification and quantification of the compounds

The chemical components of the oil were determined by comparing their mass spectra with those of GC/MS library (WILEY 7n D. 04.00 and NIST) and by calculating their retention indices (RI) compared with n-alkanes (C6-C24) and the essential oil on HP-5 MS capillary column, under the same chromatographic conditions (Adams, 2007). The relative percentage of oil components was quantified based on GC peak areas.

Results and Discussion

The extraction and analysis of plant compounds is crucial for development, improvement as well as quality control of herbal formulization. Therefore this study was directed to find out the bioactive compounds of essential oil of *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. by GC/MS analysis. The bioactive constituents along with their abundance percentage (%) and Retention Index (RI) are presented in Table 1. GC-MS chromatogram of *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. essential oil is presented in Figure 2a. *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. dried aerial parts provided a yellowish essential oil of 0.62% (v/w) yield. Sixty-one compounds were identified in *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. essential oil, representing 87% of the total sample. Monoterpenes were the major constituents (51.9%). The extracted oil was rich in oxygenated monoterpenes (51.6%), hydrocarbons (20.4%), and oxygenated sesquiterpene (5%). The major component of oxygenated monoterpenes was trans-anethole (48.7%) (Figure 2b, c). Notably, monoterpene hydrocarbons and sesquiterpene hydrocarbons represented only 0.3% and 3.8% of the oil, respectively. Aldehydes (0.8%), oxygenated diterpenes (1.6%), acetates (1.3%), ketones (0.8%), and isoprenoids (1.4%) were other main compounds obtained from *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. essential oil (Table 1). The essential oils composition from the aerial parts of *S. orientalis* subspecies was previously investigated by GC/MS analysis (Delnavazi et al., 2014; İçen et al., 2016; Yilmaz et al., 2020). The study by Delnavazi et al. (2014) showed that the major components of *S. orientalis* subsp. *virens* essential oil are hydrocarbon sesquiterpenes followed by hydrocarbone monoterpenes (Delnavazi et al., 2014); on the other hand, the study by other groups revealed the presence of hydrocarbon sesquiterpenes and oxygenated sesquiterpenes in high amounts (İçen et al., 2016; Yilmaz et al., 2020). However, the present study results showed the monoterpenes as the main components of *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. essential oil (Table 1). The values of oxygenated monoterpenes were significantly high (51.6%), with *trans*-anethole (48.7%) as the major one. The differences in the essential oil constituents of *S. orientalis* grown in different locations are probably due to the influence of environmental factors, soil, and climatic conditions. GC/MS analysis of essential oils in other species and subspecies of *Scutellaria* have been conducted in a number of studies (Skaltsa et al., 2005; Skaltsa et al., 2000; Valarezo et al., 2012; Yu et al., 2004). According to these reports, oxygenated monoterpenes were identified as the major essential oil component of *S. albida* ssp. albida, *S. sieberi* Benth. and *S. rupestris* Boiss. from Greece (Skaltsa et al., 2005; Skaltsa et al., 2000), *S. barbata* from China (Yu et al., 2004), *S. volubilis* from Ecuador (Valarezo et al., 2012) and *S. araxensis* from Iran (Gharari et al., 2020c). In agreement with these studies, the present research was revealed the *trans*-anethole (48.7%) as the main part of oxygenated monoterpenes.

Table 1. *Compounds identified in the essential oil of Scutellaria orientalis L. subsp. virens (Kotcshy & Boiss.) J. R. Edm. using GC-MS.*

No	Compound	%	RI*	RI**	No	Compound	%	RI*	RI**
1	Heptane	0.1	699	700	32	*γ*-Selinene	0.5	1480	-
2	Octane	0.1	800	800	33	*α*-Selinene	0.1	1496	1496
3	Decane	0.6	1000	1000	34	*α* -muurolene	0.2	1500	1500
4	Acetophenone	0.7	1074	-	35	Pentadecane	1.7	1500	1500
5	Fenchone	0.7	1094	1094	36	*β*-Bisabolene	0.1	1508	1509
6	Undecan	1	1100	1100	37	*γ*–cadinene	0.3	1517	1517
7	Camphor	0.1	1126	-	38	Phenol, 2,4-di-tert-butyl-	1	1519	-
8	Pinocarvone	t	1164	1165	39	Germacrene D-4-ol	0.2	1574	-
9	*cis*-verbenol	0.1	1188	1188	40	Caryophyllene oxide	1.4	1576	-
10	Myrtenal	t	1193	-	41	Octadecane	1.3	1600	1600
11	Dodecane	1	1201	1200	42	Dillapiole	0.1	1621	-
12	Verbenone	0.1	1205	-	43	Spathulenol	1.7	1640	1640
13	Decanal	0.1	1206	1206	44	*α*-cadinol	0.4	1640	1640
14	Fenchyl acetate	t	1220	-	45	Vulgarone	0.4	1645	1647
15	Pulegone	0.1	1235	1237	46	*β*–eudesmol	0.2	1650	-
16	Carvone	0.4	1253	1253	47	Vulgarol B	0.7	1688	-
17	*cis*-anethole	1.4	1262	1262	48	Heptadecane	1.5	1699	1700
18	Anisaldehyde	0.7	1274	1275	49	Methyl myristate	0.2	1728	1726
19	Tridecane	0.3	1299	1300	50	Hexadecane	2.2	1799	1800
20	*trans*-anethole	48.7	1302	1303	51	Phytane	1.4	1812	1811
21	*α*-longipinene	0.1	1346	1347	52	Methyl isohexadecanoate	1.1	1887	-
22	*α* -cubebene	0.4	1352	1352	53	Nonadecane	2.8	1900	1900
23	*α*-copaene	0.1	1377	1377	54	Eicosane	0.2	2000	2000
24	*β*- bourbonene	t	1384	1385	55	Heneicosane	3.5	2099	2100
25	*β*-cubebene	0.1	1387	1387	56	Phytol	1.6	2114	2114
26	Tetradecane	1.3	1400	1400	57	Docosane	2.4	2200	2200
27	*β*-Gurjunene	0.1	1432	1432	58	Heptacosane	0.1	2700	2700
28	Aromadendrene	0.2	1440	1440	59	Octacosane	0.1	2800	2800
29	*α*-himachalene	0.1	1447	1447	60	Oxirane, hexadecyl-	0.1	2912	-
30	*β*-farnesene	0.2	1452	1454	61	Pentatriacontane	0.2	3500	3500
31	Germacrene D	1.5	1480	1481					

RI: The Kovats retention indices determined on HP-5 capillary

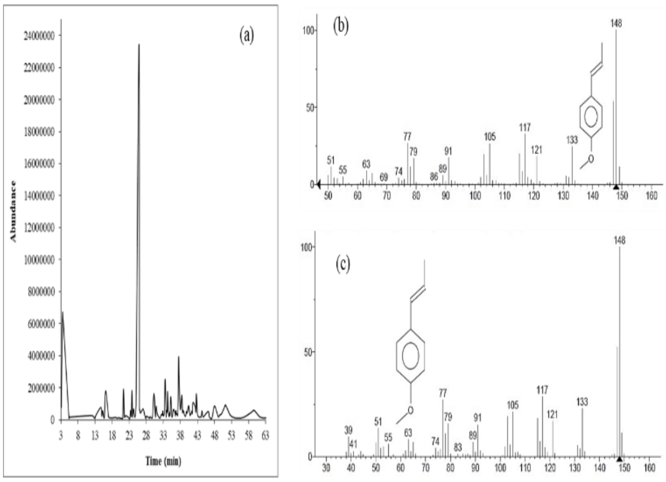

Figure 2. *(a) GC-MS chromatogram of Scutellaria orientalis L. subsp. virens (Kotcshy & Boiss.) J. R. Edm. essential oil, (b, c) mass spectrum of trans-anethol.*

Trans-anethole has shown a number of biological activities such as antibacterial, anti-oxidative (Freire et al., 2005), antiviral (Astani et al., 2011) and anti-inflammatory properties (Domiciano et al., 2013). Dillapiole, a derivative of phenylpropene and a member of monoterpene ketones, also was identified from *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. essential oil. This compound is an organic chemical compound commonly extracted from dill weed with some medicinal activities including inhibition of DNA polymerase beta lyase activity (Prakash Chaturvedula et al., 2004), antifungal activity against wild type *Candida albicans* (Treyvaud Amiguet et al., 2006) and antimitotic activity against Paracentrotus lividus embryo (Semenov et al., 2010). Our recent report on the essential oil composition of two Iranian *Scutellaria* species revealed the presence of different groups of chemical compounds (Gharari et al., 2019). A comparison of the main groups of compounds obtained from these species is listed in Table 2. According to these results, the chemical composition of *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. essential oil is very similar to that of *S. araxensis* (Gharari et al., 2020c). Oxygenated monoterpenes were the major components of *S. araxensis* essential oil (40%), with cis-anethole (28.5%) and p-Menthan-3-one (7.5%) as the main ones (Gharari et al., 2020c). However, the main active odor compounds of *S. bornmuelleri* and *S. multicaulis* essential oil were sesquiterpene hydrocarbons and steroids, respectively (Gharari et al., 2019). In conclusion the presence of different biologically active components in *S. orientalis* L. subsp. *virens* (Kotcshy & Boiss.) J. R. Edm. essential oil indicates its great potential for the future pharmaceutical industry.

Table 2. *Comparisons of main group of compounds obtained from essential oil of 4 species of Scutellaria from Iran*.

Compounds			* *S. bornmuelleri*	* *S. multicaulis*	** *S. araxensis*	✓*S. orientalis*
Terpenes	Monoterpenes	Oxygenated	0.9%	4%	40%	51.6%
		Hydrocarbon	3.7%	7.1%	0.9%	0.3%
	Sesquiterpenes	Oxygenated	4.3%	5.8%	1.3%	5%
		Hydrocarbon	7.1%	24%	2.5%	3.8%
	Diterpenes	Oxygenated	-	-	-	1.6%
Hydrocarbons			22.5%	24.2%	18.1%	20.4%
Acids			5.4%	12.5%	0.6%	-
Ketones			3.5%	3%	21.2%	0.8%
Aldehydes			32.9%	0.1%	0.5%	0.8%
Steroids			0.5%	1.7%	-	-
Acetates			0.2%	6.8%	0.1%	1.3%
Alcohols			0.1%	0.5%	2.2%	-
Amides			-	0.4%	-	-
Isoprenoids			-	-	-	1.4%
Metal-linked compounds			2.1%	2.3%	-	-

** The data for S. bornmuelleri and S. multicaulis essential oil components was obtained from Gharari et al (2019) study.*
*** The data for S. araxensis essential oil components was obtained from Gharari et al. (2020c) study.*
✓Data reported for S. orientalis essential oil components from current study

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