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Short Communication: Chemical composition and antioxidant activity of essential oil from *Salvia hypoleuca* at different growth stages

KIANI-DEHKIAN HABIB[1], BARZIN GITI[1,♥], MAZOOJI ALI[2]

[1] Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Tehran, Iran. ♥email: gitibarzin@iiau.ac.ir
[2] Department of Biology, Roudehen Branch, Islamic Azad University, Roudehen, Tehran, Iran

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Abstract. *Habib KD, Giti B, Mazooji Ali M. 2016. Chemical composition and antioxidant activity of essential oil from* Salvia hypoleuca *at different growth stages. Nusantara Bioscience 8: 145-149*. The objective of this study was to investigate the chemical composition of essential oils extracted from *Salvia hypoleuca* aerial parts, wild type from Iran at various growth stages. Samples of the plant were taken during its vegetative, flowering and fruit stages. The different plant part samples were crushed and subjected separately to hydro-distillation. The obtained essential oil components were then analyzed by a combination of capillary GC and GC-MS. The DPPH method was used to evaluate antioxidant activity of the essential oils. Fifty nine components were identified in essential oils. The major components of essential oils from the aerial parts were caryophyllene oxide (21.3-37.8%), the *E*-caryophyllene (13-18.1%), the bicyclogermacrene (6.7-13.1%), and germacrene D (3.8-10.3%). Antioxidant evaluations of the oils showed a moderate antioxidant activity with IC_{50} values of 32.6, 25.0, and 41.2 mg/mL of samples taken at the vegetative, flowering and fruit stages, respectively.

Keywords: Antioxidant activity, Essential oil, GC, Iran, Lamiaceae, *Salvia hypoleuca*

INTRODUCTION

It is well-known that free radicals cause oxidation of unpreserved aliments rich in unsaturated fatty acids (Li et al. 2008). Nowadays, there is much interest world-wide in finding new and safe antioxidants from natural sources. Many essential oils of plants have therapeutic effect. Also, they are used as antioxidant agents in food preservation and drug industries (Stefanakis et al. 2013).

The genus *Salvia*, which belongs to the Lamiaceae family, contains 1000 species distributed all over the world (Walker et al. 2004). Various *Salvia* species are used in traditional medicine worldwide (Ulubelen 2003). The extracts of these species exhibit antibacterial, antioxidant, antidiabetic, antitumor, antiplasmodial, antifungal, antiprotozoal, cytotoxic, and HIV inhibitory properties (Howes et al. 2003; Topcu 2006; Kabouche et al. 2007; Chan et al. 2011; Farimani et al. 2012; Ebrahimi et al. 2013). Sixty one species of *Salvia* have been reported in Flora of Iran, of which 17 are endemic (Jamzad 2012). *Salvia hypoleuca* is an endemic and range-restricted species that occurs naturally in the southern slopes of the Elburz Mountains North of Tehran. The species has been investigated for it′s terpenoid contents (Rustaiyan et al. 1982; Saeidnia et al. 2012), and flavonoids (Wollenweber et al. 1992).

Many authors have studied the essential oil content of *S. hypoleuca* natural populations (Rustaiyan et al. 1999; Bigdeli et al. 2005; Nickavar et al. 2005), but there have been no reports to date on secondary metabolite changes in essential oil composition of this genus at different growth stages.

According to literature survey, *S. hypoleuca* is a source of various extracts containing biologically active constituents that possess antioxidant activity (Firuzi et al. 2013; Javdan and Estakhr 2011), but there are no reports on antioxidant activity of the essential oils. Therefore, this study was performed to assess the chemical composition and antioxidant activity of oil of *S. hypoleuca* at different growth stages.

MATERIALS AND METHODS

Plant material

The aerial flowering parts of *Salvia hypoleuca* were collected from early May to mid-June, from Mazandaran of Iran. A voucher specimen of plant were deposited at the IAUN Herbarium (14029).

Isolation of the essential oil

The dried aerial parts of the plant (100 g) were subjected to hydro distillation for 3 h using a Clevenger-type apparatus according to the European Pharmacopoeia (1975). The oil was dried over anhydrous sodium sulfate and kept at 4℃ in a sealed brown vial until required.

Analysis of the essential oils

The analyses of the oils were performed by using Agilent gas chromatograph (FID) with a DB-5 fused silica column (30 m × 0.32 mm; 0.25 µm film thickness).

Nitrogen was used as gas carrier at a constant flow of 1.1 mL/min. The oven temperature was programmed from 60 to 250 ºC at 4 ºC/min, and then isothermal for 15 min. The injector and FID temperatures were set at 240 ºC and 250 ºC, respectively. The injection volume was 0.1 mL. GC-MS analysis was carried out on a Thermoquest Finnigan Trace GC-MS instrument equipped with a DB-5 column (60 m × 0.25 mm; 0.25 µm film thickness) programmed as above with helium as the carrier gas with flow rate 1.1 mL/min and a split ratio of 1:100. The MS operating parameters were: ionization voltage, 70 ev; ion source temperature, 200 ºC. Identification of the compounds was performed by comparison of the retention indexes (relative to a homologue C_6-C_{24} *n*-alkane series) obtained in the same column, with those of reference compounds. Additionally, each mass spectra obtained was compared with those from usual electronic libraries (Adams 2007).

Antioxidant activity

DPPH assay

The antioxidant capacity of the essential oils were measured from the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH assay was performed as described before (Mensor et al. 2001). Butylated hydroxytoluene (BHT) was used as reference materials. Briefly, the various volumes of the samples were mixed with 1 mL of 0.004% DPPH solution and filled up with 95% methanol to a final volume of 4 mL. The samples were first kept in a dark place at room temperature and their absorbance was read at 517 nm after 30 min. Inhibition of free radical by DPPH in percent was calculated as follows:

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

Where, A_{blank} is the absorbance of the control reaction (containing all reagents except the oil), A_{sample} is the absorbance of the sample and *RSC* is the free radical scavenging capacity. The oil concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentages against oil concentrations.

β-Carotene-linoleic acid assay

In this assay antioxidant capacity is determined by indirectly measuring the inhibition of the volatile organic compounds and the conjugated diene hydro peroxides arising from linoleic acid oxidation (Dapkevicius et al. 2002). A stock solution of β-carotene-linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 mL of chloroform (HPLC grade), 25 µL linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL distilled water saturated with oxygen (30 min 100 mL/min.) was added with a vigorous shaking. 2.5 mL of this reaction mixture was dispersed to test tubes and 350 µL portions of the oils prepared at 2 g/L concentrations were added and emulsion system was incubated up to 48 hours at room temperature. After this incubation period absorbance of the mixtures were measured at 470 nm.

RESULTS AND DISCUSSION

The yields of essential oils, obtained by hydro distillation from *S. hypoleuca* aerial parts were 0.17, 0.25 and 0.22% at vegetative, flowering and fruit stages, respectively (Table 1). These yields were calculated on the basis of dry weight, by using the following equation: Y (%) = WEO/Wdp × 100; where WEO is the weight of essential oils and Wdp is the weight of dry plant. Results showed significantly different essential oil contents of aerial parts at different plant growth stages. Indeed, low yield (0.17%) was recorded in plants harvested at the vegetative stage compared to the higher yield of 0.25% at the flowering stage. Chemical composition of the oil samples was mainly investigated using GC-FID and GC-MS techniques. Fifty-nine components were identified in the essential oil of aerial parts (including vegetative, flowering and fruit stages) representing 98.9, 97.6, and 97.2% of the total oil, respectively. Evaluations of qualitative and quantitative essential oil composition are presented in Table 2, which compounds are listed in order of elution on the DB-5 column. Figure 1 shows the GC-MS chromatogram of essential oil of plant at the flowering stage.

The most abundant class of compounds at the vegetative stage was sesquiterpene hydrocarbons with 47.8% of constituents. This high percentage of sesquiterpene hydrocarbons was mainly due to high contents of *E*-caryophyllene (18.1%), bicyclogermacrene (13.7%) and germacrene D (10.3%). The second most abundant class was oxygenated sesquiterpenes (30.4%), largely represented by caryophyllene oxide (22.2%). Other important constituents were monoterpene hydrocarbons (15.5%). Oxygenated monoterpenes and diterpenes accounted for only 4.2 and 1.0%, respectively.

In the oil of plant harvested during the flowering period, sesquiterpene hydrocarbons represented the most abundant compound class (32.9%). Other abundant classes were oxygenated sesquiterpenes (28.8%), and monoterpene hydrocarbons (25.1%). However, contents of sesquiterpene hydrocarbons and oxygenated sesquiterpenes were lower (32.9 vs. 47.8% and 30.4 vs 28.8% respectively; Table 2) while monoterpene hydrocarbons exhibited a slight increase (15.5 vs 25.1%; Table 2). A slight increase was also observed for oxygenated monoterpenes and oxygenated diterpenes. *E*-Caryophyllene (13.0%), and bicyclogermacrene (10.3%) were the principal sesquiterpene hydrocarbons and caryophyllene oxide (21.3%) was the main component of oxygenated sesquiterpenes. Also, the major monoterpene hydrocarbons detected in this oil were *α*-pinene (9.7%) and *β*-pinene (9.8%). The essential oil of fruit phase, were dominated by oxygenated sesquiterpenes (51.0%), and sesquiterpene hydrocarbons (32.6%), and largely represented by caryophyllene oxide (37.8%) and salvial-4 (14) en-1-one (7.3%). The second most abundant class was composed of sesquiterpene hydrocarbons (32.6%). The content of monoterpene hydrocarbons of oil was extremely reduced (5.4%). However, the other chemical classes of volatiles were detected at comparable levels in oil taken of the flowering stage i.e., oxygenated monoterpenes (5.1 vs

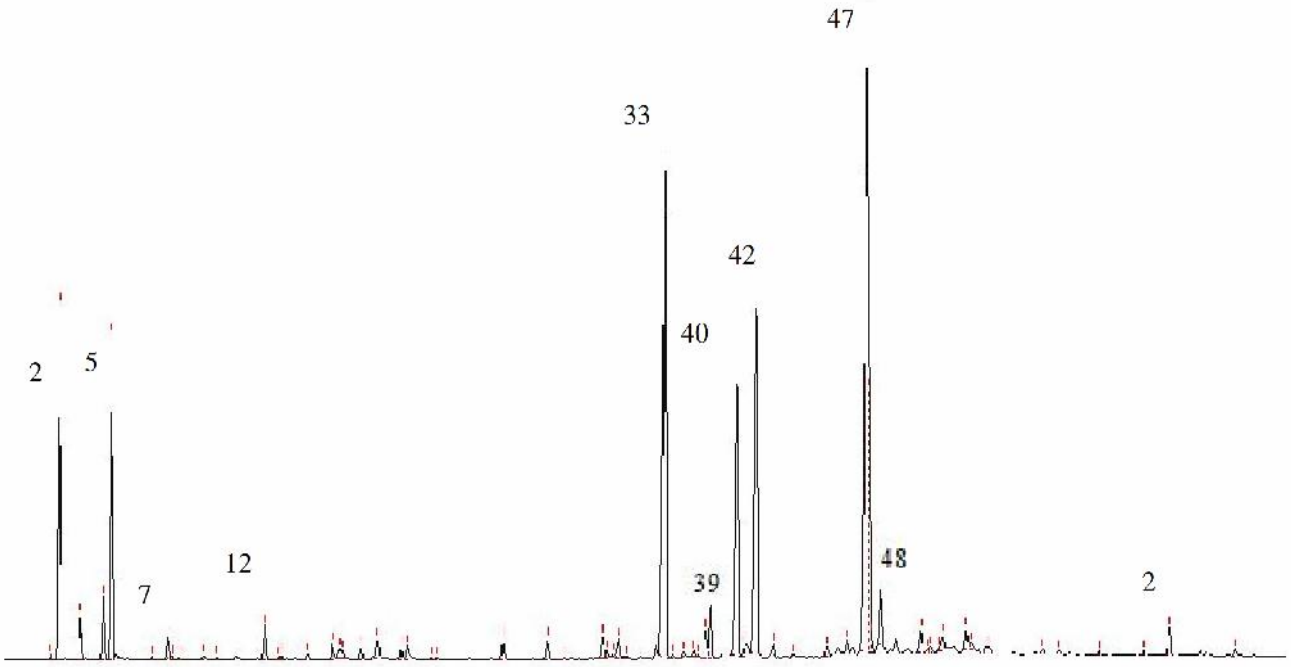

Figure 1. GC-MS Chromatogram of *Salvia hypoleuca* essential oil from aerial parts in flowering stage

8.5%), and oxygenated diterpenes (3.1 vs 2.6%). Our results are in accordance with previous studies of *S. hypoleuca* essential oils which showed the predominance of sesquiterpenes compounds. Indeed, Nickavar et al. (2005) described the chemical constituents of the *S. hypoleuca* Benth essential oils and displayed that the main classes of compounds were sesquiterpenes. Another study was conducted by Rustaiyan et al. (1999), dealing the essential oils of the Iran *S. hypoleuca* aerial parts, showed that the major compounds identified in this essential oil were *β*-caryophyllene (22.0%), ^{TM}-elemene (15.5%), and bicyclogermacrene (15.1%). The essential oil of *S. hypoleuca* root has been investigated and hexadecanoic acid (24.7%), viridiflorol (14.9%), spathulenol (7.7%), and caryophyllene oxide (5.8%) were determined as the most abundant constituents (Bigdeli et al. 2005). The comparison of the chemical composition of the *S. hypoleuca* essential oils with some other species of *Salvia* showed that the compounds are qualitatively similar. In fact, according to a report of Gursoy et al. (2012), GC and GC-MS analysis of *S. palaestina* essential oil identified 70 components, which the main compounds were caryophyllene oxide (16.1%) and (*E*)-caryophyllene (4.5%). Furthermore, the major constituents of *S. hydrangea* essential oil collected from Iran were *β*-caryophyllene (25.1%), 1,8-cineole (15.2%), and caryophyllene oxide (11.5%) (Sonboli et al. 2006). Mirza and Sefidkon (1999) investigated the chemical composition of *S. nemorosa* essential oil collected from Tehran, which *β*-caryophyllene (41.6%) and germacrene B (21.3%) were determined as major components. Bornyl acetate (25.6 and 43.3%), camphor (18.4 and 12.0%), and camphene (15.1 and 9.9%) were identified as major components of *S. pinnata* essential oils at flowering and fruiting stages, respectively (Somer et al. 2015). Caryophyllene oxide (15.97%), and β-caryophyllene (12.74%) were also reported the most abundant component of *S. ballotiflora* with 15.9, and 12.7% of total oil, respectively (Cardenas-Ortega et al. 2015).

Antioxidant activities of the oils have been determined by two different test systems DPPH and β-carotene-linoleic acid. All of the data are presented in Table 3. In the DPPH assay, the reduction ability of DPPH radicals' formation was determined by the decrease in its absorbance at 517 nm induced by antioxidants. In general, the volatile oils exhibited moderate levels of free-radical-scavenging properties with IC_{50} values of 32.6, 25.0, and 41.2 mg/mL at vegetative, flowering and fruit stages of growth, respectively. In β-carotene-linoleic acid assay, antioxidant capacity of oils is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Barriere et al. 2001). Table 3 depicts the inhibition of β-carotene bleaching by the essential oils of *S. hypoleuca*. In this system, we could conclude that results were consistent with the data obtained from the DPPH test. As can be seen from Table 3, the most active sample was the oil of the plant in flowering stage with IC_{50} value of 9.5 mg/mL. The IC_{50} value of oil of the plant in vegetative stage was 13.8 mg/mL, while the oil of fruit stage showed low antioxidant activity with an IC_{50} of 19.2 mg/mL.

Table 1. Yields of essential oils at different growth stages

Essential oil	Vegetative stage	Flowering stage	Fruit stage
Yield (%)	0.17	0.25	0.22

Table 2. Chemical composition of the essential oils of aerial parts in vegetative, flowering and fruit stages of *S. hypoleuca*

No.	Compounds	RI[a]	Vegetative (%)	Flowering (%)	Fruit (%)
1	Tricyclene	926	tr	0.2	Tr
2	**α-Pinene**	**934**	**6.4**	**9.7**	**1.7**
3	Camphene	949	0.8	1.2	0.3
4	Sabinene	973	1.2	1.4	0.4
5	**β-Pinene**	**979**	**6.4**	**9.8**	**2.5**
6	α-Terpinene	1017	tr	0.1	Tr
7	*p*-Cymene	1024	0.6	1.2	0.4
8	Limonene	1029	0.1	1.5	0.1
9	1,8-Cineole	1031	0.1	0.1	0.2
10	γ-Terpinene	1050	0.1	0.2	0.1
11	*Z*-Sabinene hydrate	1063	0.1	tr	Tr
12	Terpinolene	1091	0.7	1.1	0.7
13	Linalool	1101	tr	tr	Tr
14	*E*-Sabinene hydrate	1103	0.1	0.2	Tr
15	α-Campholenal	1127	0.2	0.3	0.3
16	*E*- Pinocarveol	1143	0.4	1.0	0.7
17	*E*-Verbenol	1147	0.2	0.6	0.2
18	Camphor	1151	0.2	0.3	0.5
19	Pinocarvone	1165	0.3	0.7	0.6
20	Borneol	1170	0.6	1.8	0.1
21	4-Terpineol	1180	0.3	0.63	0.3
22	α-Terpineol	1194	0.4	0.97	0.8
23	Myrtenal	1200	tr	0.1	0.1
24	Myrtenol	1202	tr	0.1	0.1
25	Bornyl acetate	1289	0.5	0.4	0.4
26	Bicycloelemene	1341	0.5	0.4	0.2
27	Eugenol	1352	tr	tr	Tr
28	α-Copaene	1380	0.5	0.3	0.4
29	β-Bourbonene	1389	0.1	0.1	Tr
30	β-Elemene	1396	0.1	tr	0.2
31	*Z*-Caryophyllene	1406	0.6	0.2	0.3
32	α-Gurjunene	1411	0.1	0.1	0.1
33	***E*-Caryophyllene**	**1431**	**18.1**	**13.0**	**14.3**
34	β-Copaene	1434	0.1	0.1	0.1
35	Aromadendrene	1444	0.1	0.7	0.1
36	*E*-Geranyl acetone	1454	0.1	0.1	0.1
37	α-Humulene	1458	0.1	tr	0.2
38	α-Gurjunene	1463	0.7	0.4	0.6
39	*9-epi*-(*E*)-Caryophyllene	1467	1.6	2.0	2.9
40	**Germacrene D**	**1488**	**10.3**	**3.8**	**5.3**
41	δ-Selinene	1492	0.5	1.0	0.7
42	**Bicyclogermacrene**	**1504**	**13.7**	**10.3**	**6.1**
43	*E*,*E*-α-Farnesene	1508	0.2	0.2	0.2
44	δ-Cadinene	1526	0.1	tr	0.1
45	Isopatchoulane	1558	0.3	0.2	0.7
46	Spathulenol	1572	0.3	0.3	0.6
47	**Caryophyllene oxide**	**1591**	**22.2**	**21.3**	**37.8**
48	**Salvial-4(14)en-1-one**	**1607**	**3.6**	**2.4**	**7.3**
49	Viridiflorol	1621	1.5	0.9	0.2
50	*Z*-Cadin-4-en-7-ol	1649	0.6	0.9	0.7
51	Isospathulenol	1656	0.1	0.1	0.2
52	Caryophyll-5-en-12-al	1665	0.2	0.2	0.4
53	α-Cadinol	1670	0.1	0.5	0.2
54	neo-Intermedeol	1674	0.6	0.7	0.7
55	Aromadendrene oxide II	1686	0.7	0.8	2.0
56	8-Cedren-13-ol	1697	0.4	0.3	0.7
57	*Z*-Lanceol	1750	0.1	0.4	0.2
58	Sclareoloxide	1896	0.8	2.0	2.8
59	Manool oxide	2023	0.2	0.3	0.3
Total			98.9	97.6	97.2

Note: [a]RI, Retention indices relative to C_8 -C_{28} *n*-alkanes on DB-5 column. The components are listed in order of elution from the DB-5 column. tr, trace (<0.1%)

Table 3. Antioxidant activity of the essential oils of *S. hypoleuca* and positive control (BHT)

Test sample	IC_{50} (mg/mL)	
	DPPH	β-Carotene / linoleic acid
Oil in vegetative stage	32.6	13.8
Oil in flowering stage	25.0	9.5
Oil in fruit stage	41.2	19.2
BHT	0.4	0.1

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