SHORT REPORT

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Comparison of Chemical Composition, Antifungal and Antibacterial Activities of Two Populations of *Salvia macilenta* Boiss. Essential Oil

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Abstract: *Salvia macilenta* Boiss. is a fragrant subshrub which grows wild in some regions of Iran. In this work, we contrasted essential oil contents and components of two wild populations under two different ecological situations (Kerman and Baluchistan Provinces, Center and South East of Iran, respectively). For the first time the antibacterial and the antimycotic properties of these essential oils were also evaluated against seven bacterial and fungal strains. The essential oils were isolated by hydrodistillation method and the chemical compositions of the samples were examined by GC and GC-MS. Kerman (K) specimen was found to be rich in α-pinene (29.0%), *p*-cymene (10.7%), veridiflorol (9.1%), α-eudesmol (8.7%), bornyl acetate (7.3%) with lesser concentrations of borneol (4.9%). Principal components were identified as α-eudesmol (35.6%), α-pinene (7.7%), bornyl acetate (7.6%), (*E*)-nerolidol (6.5%) and veridiflorol (5.9%) from Baluchistan (B) sample. Oxygenated sesquiterpenes were found to be the principal class of components in the oil of Baluchistan sample (53.0%) whereas monoterpene hydrocarbons were the main class in Kerman specimen (46.6%). The results demonstrated that the variety in the volatile compounds could be considered as chemotaxonomic importance and it may be ascribed to their different ecological and geographical source.

Keywords: *Salvia macilenta* Boiss.; essential oil; antibacterial activity; antifungal properties; GC/MS.

1. Plant Source

Salvia macilenta is a fragrant subshrub which grows wild in Iran (Hormozgan, Baluchistan, Kerman and Yazd provinces). The aerial segments of *S. macilenta* of different origins were collected at the full flowering stage in April 2015 from Khash area, Zahedan, Baluchistan Province, Iran, and from Orzouyeh, Jiroft, Kerman Province, Iran. Voucher specimens have been deposited in the Medicinal Plants and Drugs Research Institute Herbarium (MPH-56 and MPH-2442 for Baluchistan and Kerman samples respectively), Shahid Beheshti University, Tehran, Iran.

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2. Previous Studies

The genus *Salvia* is one of the greatest and substantial fragrants of the Labiateae family. There are many reports on the chemical composition of the essential oils extracted from the plants belonging to the genus *Salvia*. In many *Salvia* species, *α*-pinene, *β*-pinene, 1,8-cineole, borneol and camphene were observed as major components [1,2].

Salvia macilenta, an endemic species from Iran, a member of this great genus is a fragrant subshrub which grows wild in Iran. Antioxidant properties, neuroprotective effect, antiglycation activity, total phenolic and flavonoid components of this herb have been reported previously [3-5]. The results demonstrated that *S. macilenta* has high values of neuroprotective effects in addition to high antioxidant activities that imply the possibility of this plant as candidates for treating neurodegenerative diseases like Alzheimer's disease. Composition of the essential oil of *S. macilenta* growing wild in Hormozgan: Hajiabad, Golzar protected area, were investigated previously. In this investigation 31 components were characterized and the major components of oil were determined as *α*-pinene (60.0%), *γ*-elemene (6.1%), thymol (5.2%), elemol (4.7%), *β*-caryophyllene (4.1%) and bornyl acetate (2.2%) [3].

The dissimilarity in essential oil profile within the species offers encouragement for the prospects of breeding homogeneous plant material of *S. macilenta* with favorable commercial characteristics for particular uses. To verify this presumption, furthers studies under controlled conditions should be conducted in order to select interesting *S. macilenta* genotypes. In this study, we contrasted essential oil contents and components in two wild populations under two different ecological situations (Kerman and Baluchistan Provinces, Center and South East of Iran respectively). For the first time the antibacterial and the antimycotic properties of these essential oils were also evaluated against seven bacterial and fungal strains extracted from different origins. This selection is surely linked to its future success as industrial crop. This work should be made jointly with that aiming at the selection of suitable plants with high percentage of the desired compounds.

3. Present Study

The identified essential oils ingredients with their percents' are listed in order of their elution from a DB-5 column in Table 1 [6]. Our results demonstrated that "Baluchistan" population of *S. macilenta* contains more essential oils than "Kerman" population (densities of oils were 0.93 g/mL and 0.92 g/mL respectively). A comparison of the essential oil components in the two *S. macilenta* populations proved that there is discrepancy between these two populations as far as essential oils content and constituents are concerned. Main ingredients like *α*-eudesmol, *α*-pinene, *p*-cymene and bornyl acetate previously reported in this genus. For example, *α*-pinene constituted the most abundant compound in the essential oils of *S. rosifolia* [7] (19.7%), *S. divaricata* [8] (10.2%) and *S. recognita* [9] (7. 6%). Also, *α*-eudesmol was one of the main compounds identified in *S. microphylla* [10] (14.1%) and *S. Africana* [11] (10.7%). Other main components like *p*-cymene was extracted and identified in *S. verticillata* [12] (23.0%) and *S. spinosa* [13] (7.9%). To our knowledge, no registered reports on variety of chemical compounds and oil yield of the essential oil of diverse populations of *Salvia macilenta* in the alpine areas in Southeastern and Centeral Iran (Baluchistan and Kerman province) are available. Presence of relatively large amounts of (*E*)-nerolidol and veridiflorol as oxygenated sesquiterpenes as well as *p*-cymene as monoterpene hydrocarbon in the essential oil of the aerial parts *S. macilenta* is reported for the first time in the present investigation.

In a study conducted by Sonboli and colleagues on the essential oil of this plant it was observed that concentrations of some ingredients were different than our research [3]. Because the essential oil components and their biological properties were found to be influenced by ecological factors. However, genetic agents also influence terpene biosynthesis pathways and consequently the principal characteristic ingredients and their percentages. These data permit an inventory, broadening of the variability of essential oil profiles of *S. macilenta* populations in Iran. Generally, it is proposed that this discrepancy in the major components of the essential oils of these plants may be attributed to differences in enviromental factors (climatic conditions, seasonal and geographic conditions, edaphic, elevation and topography, handling method, harvest period) and genetic (species, subspecies, ecotype,

Table 1. Chemical composition of the oils of two populations of *Salvia macilenta* Boiss.

Compounds	RI[a]	RI[b]	%B	%K	Compounds	RI[a]	RI[b]	%B	%K	Compounds	RI[a]	RI[b]	%B	%K	Compounds	RI[a]	RI[b]	%B	%K
(3*Z*)-hexenal	797	797	0.1	0.4	(2*E*)-octenol acetate	1211	1208	0.1	t	γ-muurolene	1473	1479	1.7	1.3	α-bisabolol acetate	1799	1798	0.1	t
tricyclene	925	921	t[c]	0.1	trans-carveol	1220	1215	t	0.1	germacrene D	1480	1479[d]	t	0.1	phenyl ethyl octanoate	1841	1846	0.4	0.1
α-pinene	936	939[e]	7.7	29.0	nerol	1225	1227	0.02	0.1	β-selinene	1491	1489	1.1	0.7	laurenene	1882	1879	0.2	t
camphene	951	952[e]	1.0	3.9	tetrahydro-linalool acetate	1229	1231	0.03	t	bicyclegermacrene	1499	1500	t	1.1	epi-laurenene	1908	1901	0.2	t
thuja-2,4(10)-diene	956	953	0.04	0.1	tymol, methyl ether	1236	1232	0.1	t	α-selinene	1503	1498	1.0	t	(5*E*,9*E*)-farnesyl acetone	1921	1913	0.1	t
sabinene	968	969	0.01	t	pulegone	1237	1233	0.3	1.0	α-muurolene	1507	1500	0.5	0.4	phytol	1945	1942	0.2	t
β-pinene	980	983[d]	0.4	0.9	trans-2-hydroxy-pinocamphone	1243	1247	0.1	t	β-bisabolene	1510	1505[f]	0.1	t	cuparenic acid	1929	1932	0.04	0.2
myrcene	992	992[e]	0.1	0.5	cis-carvone oxide	1251	1259	0.03	t	γ-cadinene	1517	1513	0.6	0.6	nootkatin	1962	1959	0.3	t
ethyl-(3*E*)-hexenoate	1001	1003	0.02	t	geranial	1259	1264	0.1	t	eugenol acetate	1528	1521	t	0.1	(*Z*)-methyl-isoprenyl cinnamate	1975	1970	0.1	0.1
δ-3-carene	1006	1008[e]	t	0.1	trans-anethol	1278	1283	0.01	t	δ-cadinene	1531	1523[g]	2.1	2.3	(*Z*,*E*)-geranyl linalool	1990	1998[f]	0.1	0.1
limonene	1020	1024	0.04	t	bornyl acetate	1287	1287	7.6	7.3	α-cadinene	1536	1534	0.6	0.9	manool oxide	1995	1987[f]	0.2	t
α-terpinene	1015	1018[d]	0.01	0.3	thymol	1294	1289	0.2	0.3	α-calacorene	1539	1544	0.6	t	13-epi-manool oxide	2004	2009	0.1	t
p-cymene	1020	1026	0.03	10.7	carvacrol	1297	1298	0.2	0.3	elemol	1552	1548	t	0.1	abieta-8,12- diene	2018	2022	0.04	t
ortho-cymene	1023	1022	2.4	t	terpinen-4-ol acetate	1303	1299	0.1	0.2	germacrene B	1559	1562	1.2	t	(*E*,*E*)-geranyl linalool	2021	2026	0.4	0.1
sylvestrene	1027	1025	1.5	t	iso-verbanol acetate	1310	1308	0.2	0.3	(*E*)-nerolidol	1565	1561	6.5	t	4-hydroxy- stilbene	2038	2042	0.4	t
(*Z*)-β-ocimene	1033	1032	t	0.1	4-hydroxy-cryptone	1319	1314	0.1	t	caryophyllene oxide	1587	1582	0.2	0.1	kaurene	2045	2042	0.04	0.1
(*E*)-β-ocimene	1048	1044	t	0.2	myrtenyl acetate	1322	1324	0.1	t	cis-β-elemenone	1595	1589	t	0.1	manool	2051	2056	0.1	t
γ-terpinene	1052	1054	0.1	0.5	δ-elemene	1329	1335	0.03	t	globulol	1598	1590	0.4	0.1	sclarcolide	2059	2065	0.4	0.2
terpinolene	1079	1086	t	0.3	α-terpenylacetate	1354	1349[g]	0.03	t	guaiol	1603	1600	0.3	t	methyl linoleate	2098	2095	0.1	t
p-cymenene	1083	1089	0.1	t	eugenol	1365	1356	0.04	t	geranyl isovalerate	1608	1606	5.9	9.1	laurenan-2-one	2119	2115	0.1	t
linalool	1099	1097[g]	0.4	0.9	carvacrol acetate	1366	1370	0.1	0.3	trans-β-elemenone	1609	1601	t	0.1	7- isoprenyl oxycoumarin	2122	2115	0.04	t
6-camphenol	1115	1111	t	0.3	α-ylangene	1375	1373	0.3	t	5-epi-7-epi-α-eudesmol	1612	1607	0.4	t	methyl octadecanoate	2128	2124	0.1	t
endo-fenchol	1119	1114	0.1	t	α-copaene	1381	1374	1.4	1.4	geranyl isovalerate	1614	1606	t	0.1	grandiflorene	2171	2174	0.1	t
myrcenol	1121	1119	t	0.2	geranyl acetate	1384	1379	t	0.1	10-epi-γ-eudesmol	1619	1622	1.7	0.4	phenethyl cinnamate	2178	2179	0.4	0.1
3-octanol acetate	1120	1120	0.1	t	β-bourbonene	1386	1387	0.2	t	γ-eudesmol	1633	1630	1.5	t	ethyl octadecanoate	2199	2196	0.04	t
α-capholenal	1123	1122	0.2	0.3	β-elemene	1395	1389	0.1	t	epi-α-muuroiol	1642	1640	t	0.5	incensole oxide	2282	2279	0.1	t
dihydro-linalool	1133	1131	t	0.34	sesquithujene	1411	1405	0.6	t	hinesol	1645	1640	1.0	t	trans-totarol	2319	2314	0.2	t
trans-pinocarveol	1138	1135	0.1	t	(*Z*)-caryophyllene	1413	1408	0.1	t	β-eudesmol	1653	1649	t	0.2	methyl dehydroabietate	2338	2341	0.1	t
camphor	1143	1141	0.3	t	cis-nerone	1420	1412	t	0.3	vulgarone B	1656	1649	t	0.1	abietol	2397	2401	0.1	t
isopulegol	1147	1145	0.1	0.3	(*E*)-caryophyllene	1423	1417	1.1	0.8	α-eudesmol	1658	1652	35.6	8.7	totarolone	2544	2542	0.1	t
(*E*)-2-nonenal	1152	1155	t	0.5	β-humulene	1439	1436	0.1	0.1	epi-β-bisabolol	1671	1670	0.1	t	Monoterpene Hydrocarbons			13.4	46.6
pinocarvone	1157	1160	0.1	t	(*Z*)-β-farnesene	1445	1440	0.04	t	cadalene	1679	1675	0.6	t	Oxygenated Monoterpenes			12.7	20.1
borneol	1168	1165	1.2	4.9	selina-3,7(11)-diene	1451	1445	t	1.2	elemol acetate	1684	1680	0.1	0.3	Sesquiterpene Hydrocarbons			15.0	9.9
umbellulone	1169	1167	t	0.1	geranyl acetone	1456	1453	0.4	t	germacrone	1689	1693	0.1	0.1	Oxygenated Sesquiterpenes			53.0	21.8
terpinen-4-ol	1180	1174	0.3	1.1	trans-prenyl limonene	1464	1457	t	0.1	caryophyllene acetate	1696	1701	0.1	0.1	Others			4.1	0.8
α-terpineol	1182	1186	0.2	0.9	sesquisabinene	1466	1457	t	0.1	(2*E*,6*Z*)-farnesol	1711	1714	0.1	t					
verbenone	1199	1204[f]	0.03	0.03	allo-aromadendrene	1467	1458	t	0.1	(2*E*,6*E*)-farnesol	1739	1742	0.1	t	Total			99.1	99.1
n-decanal	1205	1201	0.18	t	cabreuva oxide B	1467	1462	0.28	t	(*Z*)-α-santalol acetate	1770	1777	0.07	0.09					

a=Retention Index (RI) calculated using n-alkane series from C_6 to C_{24} confirmed by comparison on DB-5MS; b=Retention Index (RI) from literature data [6]; c= relative percentage less than 0.01%.
B=Baluchistan sample K=Kerman sample; d= [24]; e= [25]; f= [26]; g = [27]

cultivar, chemotype, etc.), and their intraction effects are very effective on the type and percentage of components of essential oils. Many data published in the literature display that the essential oil content and the oil yield can be affected by genetic, climatic, and soil content, as well as by method of propagation, organ development and the date of accumulation of the herb material [14,15]. Also, several reports have demonstrated on the variation in *Salvia* essential oil composition induced by environmental, physiological and morphological factors that all of them state that, the observed inter-population variation in the essential oil composition of sage might be the result of various complex factors, both endogenous and exogenous, such as geographical and climatic conditions [16, 17].

In this study the relevance between the geographical distribution and the essential oil composition was shown and separated these populations from each other. Further investigation of the composition of the essential oils from more populations of the Iran is necessary for the identification of possible chemotypes, while molecular analysis is needed to show the significance of terpenes as taxonomical markers in *S. macilenta* plant.

In vitro antimicrobial properties of essential oils was determined against some microorganisms.This research proved that the *S. macilenta* essential oil displayed antimicrobial activity on some Gram negative and Gram positive bacteria. The examined microorganisms are pathogens or compromiser for human, plants and animal, and they cause pollution and deterioration in food, air and water. The powerful antimicrobial property of the essential oil versus some sensitive microorganisms can be ascribed to the attendance of high concentration of monoterpenes. In the present study, monoterpenes, represented essentially by *α*-pinene (monocyclic hydrocarbon); *ρ*-cymene (monocyclic aromatic); bornyl acetate (bicyclic oxygenated hydrocarbon) and borneol (bicyclic alcohol) were found to be the major compounds. Antimicrobial activities of *α*-pinene, *α*-eudesmol and bornyl acetate were presented only in a few publications [18, 19] but the biological properties of these compounds have been reported in a large number of papers. The MIC values for antimicrobial activity of *α*-pinene against *C. albicans, C. neoformans* and *R. oryzae* were 3125, 117 and 390 µg/mL respectively [19]. As well *p*-cymene is an important intermediate used in pharmaceutical industries and for the production of pesticides, fungicides and as flavoring compound [20]. Previous studies demonstrated that *p*-cymene was able to present analgesic and anti-inflammatory activities in mice [20]. Also, in other research the antinociceptive and anti-inflammatory actions of the *p*-cymene were reported [21]. The anti-neurogenic inflammation action of *α*-eudesmol that becomes useful for the treatment of the neurogenic inflammation in the trigemino-vascular system such as migraine was reported previously [22]. Also, in other report the analgesic and anti-inflammatory effects of bornyl acetate illustrated [23]. It is difficult to ascribe the antimicrobial properties of an essential oil to one or a few active ingredients, but it may be concluded that much more *α*-eudesmol, *α*-pinene and bornyl acetate in the Baluchistan sample caused the more antimicrobial activity of this sample. So, antibacterial activities of *S. macilenta* essential oils demonstrated that this herb has potential for use in aromatherapy and pharmacy. This *in vitro* experimental investigation obviously revealed the efficient bactericidal act of *S. macilenta* essential oil and patronage the freely usage of this natural eco-friendly mixture as a preservative in food and water which are sensitive for producing mellow scent. The results represented here can be considered as the first information on the antimicrobial and antifungal activity of aerial portions essential oil of *S. macilenta*. Antibacterial activity of the essential oil of *S. macilenta* could be considered as a supplementary health elevating factor. Therefore, additional researches are indispensable to evaluate the specifications of each population which grows in various soil and climatic situations. As a conclusion, the main constituents of these essential oils of *S. macilenta,* include *α*-pinene, *p*-cymene, veridiflorol, *α*-eudesmol, bornyl acetate, borneol, *α*-eudesmol and (*E*)-nerolidol. The data presented in this investigation showed a high variation of the essential oil composition among Iranian *S. macilenta* populations harvested from different bioclimatic zones. The essential oil components and their biological properties were found to be influenced by ecological factors. However, genetic agents also influence terpenes biosynthesis pathways and therefore the main components and their percentages. To verify this hypothesis, furthers investigations under controlled situations should be conducted in order to select interesting *S. macilenta* genotypes. This selection is surely linked to its future achievement as industrial crop.

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Supporting Information

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