Agricultural Science Research Journal Vol. 7(12): 362 – 373, December 2017
Available online at http://resjournals.com/journals/agricultural-science-research-journal.html
ISSN: 2026 –6073 ©2017 International Research Journals

Full Length Research Paper

An investigation of phytochemical, total phenolic and flavonoid contents and antioxidant activity in aerial parts of two species of salvia and the effect of ecological factors on their distribution in Behshahr Hezarjarib area

***Isa Jafari foutami[1], Moosa Akbarlou[2], Masoumeh Mazandarani[3] and Mohammad Rahim Foroozeh[4]**

[1]PhD. Student in Rangeland Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[2]Associate Professor, Department of rangeland, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[3]Assistant Professor, Department of Biology, Islamic Azad University, Gorgan Branch, Gorgan, Iran
[4]Assistant Professor, Department of rangeland, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

*****Corresponding Author's E-mail: Isa.jafari84@gmail.com, Tel.: 00989112580834

Abstract

The purpose of this study was an investigation of phytochemical and antioxidant characteristics in aerial parts of *Salvia multicaulis* Vahl and *Salvia sclarea* and the effect of ecological factors on their distribution in Behshahr Hezarjarib area. *Salvia* species are well known in folk medicine and are widely used for therapeutic purposes. *Salvia* grows wildly in north of Iran. Literature review has shown that there is no report on phytochemical investigation about aerial parts of *Salvia* in north of Iran. The aim of the present study was to investigate essential oil constituents, total flavonoid, total fennel and evaluation of antioxidant activities of the essential oils and extracts of *Salvia multicaulis* Vahl and *Salvia sclarea* L. and in order to understand the relationship between vegetation and environmental factor, PCA (Principle Component Analysis) methods has been adopted. Essential oil of the aerial part of *Salvia* was analyzed by gas chromatography-mass spectroscopy (GC/MS). Antioxidant activities of the extracts were also evaluated by DPPH test.

Keywords: *Salvia multicaulis* Vahl, *Salvia sclarea* L., Antioxidant activity, Essential oil composition, PCA, North of Iran

Introduction

Different kinds of Salvia have a large variety of essential oils and the combination of their constituents. Salvia species are important because of their multiple uses like: anti-bacterial, anti-virus, anti-oxidant, Anti-malarial, anti-inflammatory, anti-diabetic, cardiovascular, anti-tumor and anti-cancer. Each species contains lots of flavonoids and tannin (Caffeic acid, chlorogenic acid, gallic acid) (Szentmihályi *et al.,* 2004). Aerial parts of *S.sclarea* are recommended as energy providers and the anti-seizure. Their flowering branches are used as aromatic and

Sweetener. Essential oil of clary sage is used in making Perfume and cologne (Yousefzadi *et al.,* 2007). Most components of essential oils of *S.sclarea* are Linalyl acetate, Linalool, α-Terpineol, D- germacrene, β – caryophyllene, B-cyclo germacrene, Sclareol, Geranyl (Feorkas *et al.,* 2005; Gulcin *et al.,* 2004). *S.sclarea, S.multicaulis* and *S.verticillata* has moderate-to-high antimicrobial effects (Vogiatzakis *et al.,* 2009).

Song *et al* (2006) analyzed grassland plant communities in the Baloung Mountain. They adopted TWINSPAN and DCA for classification and ordination. Munhoz *et al* (2008) investigated association between plant species and environment factors in humid savannah, central area of Brazil. Significant correlation was found by CCA between soil texture and moisture with distribution of plant species. Yibing (2008) applied CA and PCA in China and found that soil physical and chemical properties like nutrients, moisture, salinity and pH affected on Homogeneous habitat, controlled distribution of plant communities in these areas. Mills *et al* (2009) conducted a study in the semi-arid regions Caro in South Africa to evaluate the permeability of the soil as an influential factor in diversity and richness of vegetation in these areas. Regression results data proved that the highest species richness was found in places that permeability and pH were low and the soil has high nitrogen content.

Zhong-hua *et al* (2013) while studying factors affecting distribution of plant species in the forests of southwest China indicated that topographic factors: elevation, slope and the protrusions rocks, and soil parameters like total phosphorous, potassium and exchangeable calcium affect the distribution of plant communities. Vogiatzakis *et al* (2009) while assessing macrophyte community structure and the presence of species in relation to environmental constraints in habitats with Ephemeral covers in Greece, concluded that the most substantial environmental factor on this situation is Water storage in the soil and water holding capacity.

In the present study, we investigate phytochemical, antioxidant characteristics in aerial parts of *Salvia multicaulis* Vahl and *Salvia sclarea* L. and the effect of ecological factors on their distribution in Behshahr Hezarjarib area.

Material and Methods

General

The aerial parts of *Salvia multicaulis* Vahl and *Salvia sclarea* L. were collected at full flowering stage in May 2016 from Hezar Jerib, Mazandaran province, north of Iran. Study area is located in north of Iran and is part of the Hezar Jerib highland. Longitude is 54°03’48“ to 53°58’ 16“east and Latitude is 36° 34’ 22“to 36°24’18“north. Sampling area length is about 19 km, which is mountainous. Its altitudinal range is between 1600 to 2800 meters above sea level and is the highest part of the Hezar Jerib. The air-dried and powdered plant samples (10 g) were extracted for 48 h using 200 mL n-hexane, ethyl acetate and methanol, successively by maceration on a shaker at room temperature. The extracts were filtered and concentrated using a rotary evaporator at 40 °C. The filtered extracts were stored at -20 °C until the experiment. Essential oils were obtained from air-dried and comminuted aerial parts and roots (100 g each) by hydro distillation using a Clevenger-type apparatus for 4 h. The oils were then dried over anhydrous sodium sulfate and stored in umber glasses at 4 °C until analysis.

GC and GC/MS analysis

The essential oil of *salvia* was analyzed by GC-MS using an Agilent 7890 A. Film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium. Inlet temperature was 230°C and injector temperature was 280°C (Pourmorad *et al.,* 2006).

Total phenolic contents evaluation

The total phenolic contents of the plant extracts were estimated by using Folin-Ciocalteu assay (Karamian *et al,* 2013). 2.5 mL of sample was mixed with 2.5 mL of Folin-Ciocalteu reagent. Then 50 µL of sodium carbonate (7%) was added to the mixture and the volume was adjusted to 250 mL by adding distilled water. The mixture was mixed thoroughly for 30 min at room temperature in the dark. Absorbance of the sample solutions against a blank was determined at 765 nm using a micro plate reader. Total phenolic contents were expressed as mg of gallic acid equivalents per gram of dry extract (mg GAE/g of extract). Different concentrations of gallic acid as standard (12.5, 25, 50, 100, 200 µg/mL) were used to construct a calibration curve. All measurements were carried out in triplicate (Pourmorad *et al.,* 2006).

Total flavonoid contents evaluation

The total flavonoid content of the extracts was estimated according to a previously described method (14). The absorbance was measured against a blank at 510 nm. Results were expressed as mg of quercetin equivalents per gram of dried extract. Different concentrations of quercetin as standard (12.5, 25, 50, 100, 200 µg/mL) were used to construct a calibration curve. All measurements were carried out in triplicate (Pourmorad *et al.,* 2006).

Antioxidant activity

DPPH free radical scavenging assay

DPPH radical scavenging activity of the six extracts were measured according to the method described by (16). 50 µL of various concentrations (5, 10, 20, 40, 80 µg/mL) of the extract solutions in methanol were added to 200 µL of 100 µM DPPH solution in methanol. BHT was used as the standard antioxidant. The reaction mixture was incubated for 30 min at room temperature in darkness, and then absorbance was determined at 517 nm with a microplate reader spectrophotometer (BioTek XS2 model). The control contained 50 µL of methanol in place of the test sample, and the blank contained pure methanol instead of DPPH solution. Experiments were carried out in triplicates. The percentage of inhibition for each concentration was calculated according to the following equation:

%inhibition= [1- (As – Ab)/Ac] × 100

While absorbing from the mixture in the presence of the samples, Ab is absorbing of blank and Ac is absorbing of control. A lower absorbance of the mixture indicated a higher DPPH radical scavenging potential. IC50 value (µg extract/mL) is the concentration at which 50% of DPPH radicals are inhibited and is obtained by interpolation from linear regression calculation (Pourmorad *et al.,* 2006).

Factors affecting distribution and Soil experiment

For each species, 5 soil samples were collected, Soil samples were taken from depths of 0 to 30 cm. Soil texture was determined by the hydrometer methods (Bouyoucos, 1962). Soil acidity in the saturation paste was determined by using a pH meter (Bouyoucos, 1962). EC was determined by electrical conductivity meter (Bouyoucos, 1962). Soil organic carbon was measured by titration (Sun *et al,.* 2009). The lime was obtained from the reaction of hydrochloric acid normal with soil calcium carbonate profit by titration (Jafari Haghighi, 2003). Bulk density was measured by paraffin method (Jafari Haghighi, 2003). Soil moisture was measured by weight methods (Famiglietti *et al*,. 1998). Potassium and sodium were determined by flame photometer and calcium and magnesium were determined by titration method (Shaidai Karkaj, 2011). Classification of vegetation has different methods that in this study were adopted the conventional method called hierarchical cumulative (cluster analysis). SPSS 19 software was adopted and Edwards's method was adopted to calculate the distance between the clusters in the cluster analysis. The Euclidean index was considered as distance index. The output of this analysis was interpreted as dendrogram.

Statistical analysis

PCA analysis was adopted to analyze the relationship between species and samples in two space dimensions.

Results and Discussion

Evaluation of ecological requirements

Results shown that *Salvia sclarea* grow in 1620 m height, in 75 km distance from Behshahr City, and in its habitat average annual rainfall is 363.93mm, average annual temperature is 10.16 °C and soil properties like: Organic matter, Caco3, Ph and EC are 5.78, 29.43, 7.48 and 0.60, respectively (Table 1).

Results shown that *Salvia multicaulis* Vahl grow in Hezar Jerib summer rangeland, in 2400 m height, in 150 km distance from Behshahr City, and in its habitat average annual rainfall is 184.65mm, average annual temperature is 7.213°C and soil properties like: Organic matter, Caco3, Ph and EC are 3.61, 37.23, 7.64 and 0.60, respectively (Table 1).

Table 1: Soil properties

	O.M %	Coco3 %	Ph	EC (ds/mm)	Moisture %	K (ppm)	Na (ppm)	Ca (ppm)	Mg (ppm)	Clay %	Silt %	Sand %
Salvia sclarea L.	5.78	29.43	7.48	0.60	14.01	7.58	9.36	16.52	0.80	16.93	11.4	71.66
Salvia multicaulis Vahl	3.61	37.23	7.64	0.60	13.85	5.4	8.59	18.00	0.57	17.06	18.13	64.93

46 species belong to 42 genius and 16 family were identified in the habitats of *Salvia sclarea.* Gramineae family has more species and also Hemicryptophyes is the most frequent (Table 2).

Table 2: Associated species of *Salvia sclarea* L., as well as life forms, growth forms

Botanical name	Family	Life form	Growth form
Agropyron aucheri	*Gramineae*	p	He
Agropyron trichophorum	*Gramineae*	p	He
Alliaria officinalis	*Brasicaceae*	A	He
Amygdalus lycioides	*Rosaeae*	p	Ch
Ballota nigra	*Labiatea*	p	He
Berberis vulgaris	*Berberidaceae*	p	Ch
Bromus dontonieae	*Gramineae*	p	He
Bromus tomentellus	*Gramineae*	p	He
Centaurea sp	*Compositae*	p	He
Corronilla sp	*Leguminoseae*	p	He
Cosinia sp	*Compositae*	p	He
Crataegus melanocapra	*Rosaceae*	p	Ph
Cynodon dactylon	*Poaceae*	p	He
Dactylis glomerota	*Gramineae*	p	He
Delphinium elbursens	*Ranunculaceae*	p	He
Dianthus crinitus	*Caryophyllaceae*	p	He
Echinops orientalis	*Compositae*	p	He
Festuca ovina	*Gramineae*	p	He
Festuca.aroundinaceae	*Gramineae*	p	He
Hordeum fragile	*Gramineae*	p	He
Hulthemia persica	*Scrophulariaceae*	A	Ge
Melica persica	*Gramineae*	p	He
Menta pulegium	*Labiateae*	p	He
Nepeta racemosa	*Labiateae*	p	He
Origanum vulgar	*Labiateae*	p	He
Phlomis anisodonta	*Labiateae*	p	He
Plantago sp	*Plantaginaceae*	p	He
Poa pratensis	*Gramineae*	p	He
Poa annua	*Gramineae*	A	Th
Potenilla canescens	*Rosaceae*	p	He
Prangous uloptra	*Umbellifereae*	p	Ch
Primula acaulis	*Primulaceae*	p	He
Rubus idaeus	*Rosaceae*	p	Ch
Salvia sclearia	*Labiatea*	A	Th
Sedum acer	*Crassulaceae*	p	Ch
Sencio vulgar	*Compositeae*	A	Th
Setaria veridis	*Gramineae*	A	He

Silene pruinosa	*Caryophyllacee*	A	Th
Stachys inflata	*Labiateae*	p	He
Stellaria veridis	*Gramineae*	A	He
Stipa barbata	*Gramineae*	p	He
Taraxacum monthanum	*Compositeae*	A	Th
Tragopogon officinalis	*Compositeae*	A	Th
Trifolium repens	*Leguminoseae*	A	He
Valeriana sisymbrifolia	*Valerianaceae*	p	He
Ziziphora clinopodioides	*Labiaceae*	p	He

P :,perennial A :,annual He :,Hemicryptophytes Te :,Throphytes, Ch :,Chameophytes Ph :,Phanerophytes Ge :Geophyte

Hemicryptophytes with 71.73% abundance has been recognized as the dominant growth forms. And Geophytes with 2.17%, Phanerophytes with 2.17%, Throphytes with 13.4% and Chameophytes with 10.86% are another forms (fig, 1).

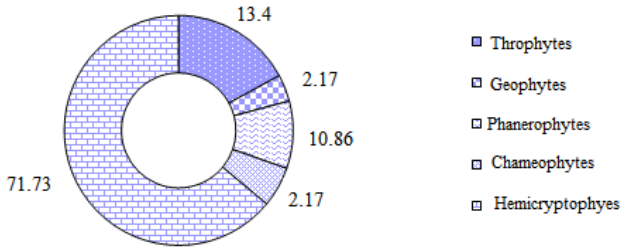

Figure 1: The frequency of species according to growth forms

Perennial plants are the dominant life forms with 76.08%. Another species are annual with 23.92% (fig. 2).

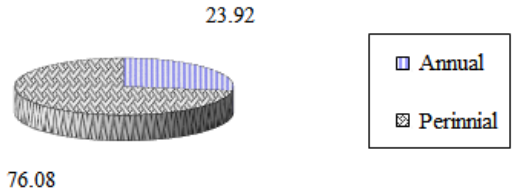

Figure 2: The frequency of species according to life forms

36 species belong to 27 genius and 15 family were identified in the habitats of *Salvia multicaulis* Vahl. Leguminoseae family has more species and also Hemicryptophyes is the most frequent (Table 3).

Table 3: Associated species of *Salvia multicaulis* Vahl, as well as life forms, growth forms

Botanical name	Family	Life form	Growth form
Acantholimon embergeri	*Plumbaginaceae*	p	Ch
Achillea sp	*Composite*	p	He
Anemone sp	*Ranuunculaceae*	A	Ge
Astragalus barrassari	*Leguminoseae*	p	Ge
Astragalus capaiti	*Leguminoseae*	p	He
Astragalus confusus	*Leguminoseae*	A	Th
Astragalus delutulus	*Leguminoseae*	p	He
Astragalus platysematus	*Leguminoseae*	A	Th
Astragalus rahensis	*Leguminoseae*	p	He
Astragalus rasellusas	*Leguminoseae*	p	He
Astragalus seragensis	*Leguminoseae*	p	He
Astragalus stenalepis	*Leguminoseae*	A	He
Astragalus subsecundus	*Leguminoseae*	p	He
Bromus tomentellus	*Gramineae*	p	He
Carpinus betulus	*Corylaceae*	p	Ph
Ceratacarpus sp	*Chenopodiaceae*	A	He
Chenopodium	*Chenopodiaceae*	p	He
Cuscuta sp	*Cuscutaceae*	p	He
Dianthus crinitus	*Caryophyllaceae*	p	He
Lactuca scariolla	*Composite*	p	He
Melica persica	*Gramineae*	p	He
Nepeta racemosa	*Labiateae*	p	He
Noea macronata	*Chenopodiaceae*	A	Th
Onobrichys coruta	*Papilionaceae*	p	He
Onopordon sp	*Compositae*	p	He
Piper bettle	*Piperaceae*	A	Th
Rhamnus catharica	*Rhamnaceae*	p	Ph
Salsola sp	*Chenopodiacae*	A	Th
salvia multicalis Vahl	*Labiatae*	A	Th
Secale ceral	*Gramineae*	p	He
Secale montunom	*Gramineae*	p	He
Silene pruinosa	*Caryophyllacee*	A	Th
Sorbus boissieri	*Rosaceae*	p	Ph
Teucrium chamaedris	*Labiatae*	p	Ch
Valeriana sisymbrifolia	*Valerianaceae*	p	He
Xanthium strumarium	*Compositeae*	A	Th

P :,perennial A :,annual He :,Hemicryptophyes Te :,Throphytes, Ch :,Chameophytes Ph :,Phanerophytes Ge :Geophytes Hemicryptophytes with 58.33% abundance has been recognized as the dominant growth forms. And Geophytes with 5.5%, Phanerophytes with 8.33%, Throphytes with 22.22% and Chameophytes with 5.5% are other forms (fig, 3).

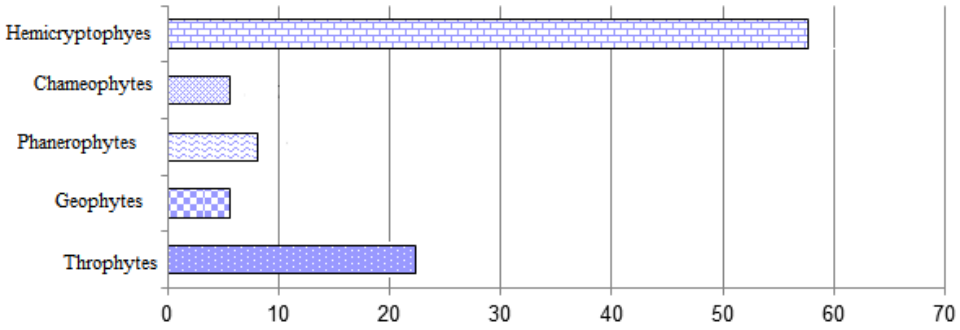

Figure 3: The frequency of species according to growth forms

Perennial plants are the dominant life forms with 69.44%. Another species are annual with 30.55% (fig. 4).

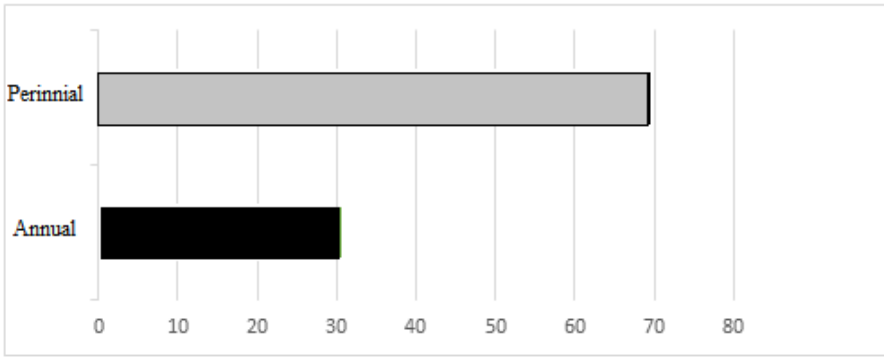

Figure 4: The frequency of species according to life form

Essential oil composition

Table 4: Constituents of the essential oils from aerial part of *Salvia sclarea* L.

NO	Compounds	RI	%Area
1	Copaene	1475	6.25
2	β-Caryophyllene	1565	36.10
3	1,6-Cyclodecadiene	1733	41.95
4	Germacrene B (CAS)	1219	9.77
5	Naphthalene	963	5.92
	Total		99.99

Results of GC/MS indicated that 5 compounds were identified in the essential oil from the aerial parts of *Salvia sclarea* L.. The results indicated that the major components were 1,6-Cyclodecadiene (41.95%) and β-Caryophyllene (36.10%) (Table 4).

The chemical composition of S. sclarea essential oil is summarized in Table 5. A total of 5 compounds were identified representing 99.9% of the total composition. The essential oil was dominated by 1,6-Cyclodecadiene and β-Caryophyllene. Soković (2001) done a research on the chemical composition of wild S. sclarea in Serbia and shown that main component is the diterpene sclareol (28.29%). Pitarokili *et al*., (2002) recognized 66 compounds and linalyl acetate, linalool, geranyl acetate, and terpineol were the main components. The results of Fraternale (2005) showed that linalool, linalyl acetate, geranyl acetate, trans-β-ocimene, and caryophyllene oxide are the dominant components. DŽAMIĆ *et al*., (2008) concluded that the main components of *salvia sclarea* L. were linalyl acetate (52.83%), linalool (18.18%),

α-terpineol (5%), α-pinene (4.57%), 1.8-cineole (2.29%), limonene (1.55%), β-caryophyllene (1.83%) and β-terpineol (1.19%) and identified 34 components. Ghani *et al.,* (2010) reported the major constituents of the essential oil of (*Salvia sclarea* L.) cultivated in Mashhad climatic conditions were linalool (30.03%), linalyl acetate (23.08%) and α-terpineol (11.13%). In another study in Tajikistan, Sharopov and Setzer (2012), the main composition of essential oil were the monoterpenic ester linalyl acetate and alcohol linalool. The result of all above researches are different from our result, based on GC/MS result 1,6-Cyclodecadiene and β-Caryophyllene are main components, While in other researches has never been mentioned about them.

The chemical composition of plants is known to be influenced by several external factors including harvest time and local, climatic and seasonal factors, as some compounds may be accumulated at a particular period to respond to environmental changes. Plant material collected at different times of the year may contain different novel compounds with other bioactivities. The effects of seasonal variations on the chemical and biological characteristics of some essential oils of the family Lamiaceae have been reported in the literature (Kofidis *et al.,* 2004).

Table 5: Constituents of the essential oils from aerial part of *Salvia multicaulis* Vahl

NO	Compounds	RI	%Area
1	α-Pinene	1112	29.82
2	Camphene	1036	11.63
3	β -Pinene	1018	2.16
4	Limonene	1103	2.67
5	1,8-Cineole	1013	23.84
6	Camphor	1232	19.93
7	Bicyclo[2.2.1]heptane	983	5.13
8	Isoxazole	863	0.52
9	γ-*Terpinene*	1030	1.65
10	Camphene	1126	0.80
11	α.-Humulene	1439	0.57
12	α -Amorphene	834	0.47
13	delta.-Cadinene	1773	0.82
	Total		94.01

Results of GC/MS indicated that 13 compounds were identified in the essential oil from the aerial parts of *Salvia multicaulis* Vahl. The results indicated that the major components were α-Pinene (29.82%), 1,8-Cineole (23.84%) and Camphor (19.93%) (Table 5).

The result of our study is different from the other world surveys. According to Ahmadi and Mirza (1999), bornyl-acetate (18.1%) and camphor (10.0%) were among the main components of *S.multicaulis,* while we have detected only high amounts of camphor (19.93%) and there is no bornyl-acetate in our results. Also Senatore *et al.,* (2004) reported the major components the essential oils of *Salvia multicaulis* growing wild in Lebanon were α-copaene (8.0%), α-pinene (6.6%), myrtenol (5.7%) and *trans*-sabinyl acetate (5.3%). Feo et al., (2009) reported *Salvia multicaulis* var. *simplicifolia* oil is rich of monoterpenes (34.5%) and sesquiterpenes (46.9%), and our results are also contradictory with these results. In similar examples, Rustaiyan et al., (1999) identified high amounts of α-pinene (26.0%), 1,8-cineole + limonene (20%) and camphor (10.0%), Bagci and Kocak (2008) reported the major constituents of the essential oil of *Salvia multicaulis* from east Anatolian region (Turkey) were 1,8-cineole (17.0%), camphor (13.2%), α-pinene (9.3%), valeranone(8.5%) and alpha-eudesmol (5.7%). Tepe *et al.,* (2004) found 47 compounds in *Salvia multicaulis* Vahl essential oil and α-pinene and Eucalyptol are the main constituents.

Comparison between these results and the results of the other reports showed differences, probably due to that plant varieties or sites, as well as the time of harvesting. The variations in chemical composition of the essential oils with respect to season might have been due to the influence of phenological status, and environmental conditions can influence the regulation of the biosynthesis of essential oil (Masotti *et al.* 2003).

Antioxidant activity and total flavonoids and phenolic compounds

As you can in table 6, antioxidant activity, total flavonoids and phenolic compounds of *salvia multicaulis* Vahl are higher than *salvia sclarea* L.. Total flavonoids, total phenolic and free radical scavenging capacities measured by DPPH are shown in Table 6.

Table 6: Antioxidant activity and the amount of flavonoids and phenolic compounds of aerial part of *Salvia sclarea* L. and *Salvia multicaulis* Vahl

	DPPT (ppm)	Total flavonoids)mg CUE g ^{01} DW(	Total phenolic compounds)mg GAE g ^{01} DW(
Salvia sclarea	52.3	2.35	41.23
Salvia multicaulis	54.34	3.13	89.88

Peng *et al.,* (2005), antioxidant activity has important role in foods and biological systems as preventing free radicals from damaging role. Karamian *et al.,* 2013 showed that DPPH activity, total phenol and flavonoid contents of *salvia multicaulis* Vahl are, 0.112, 3.70 and 1.12, respectively, but in our study these values are different. Other researchers like; Nickavar *et al.,* 2007; Esmaeili *et al.,* 2008 and Tepe *et al.,* 2007, done studies on DPPH radical-scavenging activity of *salvia multicaulis* Vahl but their results are different. Lamiaceae family is very significant in terms of antioxidant activity (Lamaison *et al.,* 1996; Zheng *et al.,* 2001; Shan *et al.,* 2005).The reasons for these differences may be due to differences in soil and climatic. Phenolic compounds act as a free radical terminators and they are an antioxidative agents (Shahidi *et al.,* 1992). Flavonoids have considerable role on human health and their activities are as an antioxidant (Karamian *et al.,* 2013). Result showed that *S. multicaulis* Vahl has a higher content of total phenol and flavonoid contents. Asadi *et al*., 2010, represented 85.10 (mg GAE g 01 DW) and 46.21 (mg CUE g 01 DW) for total phenols and total flavonoid in *salvia multicaulis* Vahl. Karamian *et al.,* (2013) results showed that total phenol and flavonoids compounds are 3.70±0.25 (mg/g dw) and 1.12±0.12 (mg/g dw), respectively.

Ben taarit *el al.,* (2012) conducted that Total phenolic and antioxidant activity of the methanol extracts of *S.sclarea* under different NaCl concentrations are different, and NaCl affect total phenolic and antioxidant activity differently. Kharazian *et al.,* (2013), compared the amount of total flavonoids in seven wild growing salvia and concluded that between these salvia, *S.multicaulis* Vahl has the most amount of flavonoids.

The results of the relationship between vegetation and environmental factors by PCA

The results of this method are in Table 5 that including Eigen values and variance Percentage for each of the components (axes). As you can see, three components are substantial include component one 45.81%, component two 30.36% and component three 23.82%.

Table 6 shows ordination habitats based on the first and second components. In first component (first axis) pH, Caco3 and elevation decrease, organic matter increase from left to right. In second component (second axis) Silt, Ca and Aspect decrease but Sand increase from down to up.

Table 7: The results of PCA analysis to determine the most substantial environmental factors

Components	Eigen value	Percentage of variance	Cumulative variance
1	7.788	45.811	45.811
2	5.162	30.368	76.179
3	4.050	23.821	100.000
4	0.000	0.000	100.000
5	0.000	0.000	100.000
6	0.000	0.000	100.000
7	0.000	0.000	100.000
8	0.000	0.000	100.000
9	0.000	0.000	100.000
10	0.000	0.000	100.000

Table 8: Specific vector values of each component

Factors	First component	Second component	Third component	Forth component	Fifth component	Sixth component
Sand	0.0546	**0.3732**	0.2523	-0.4993	0.0953	0.0391
Silt	-0.1107	**-0.3675**	-0.2262	-0.2187	0.1060	-0.0090
Clay	0.1959	-0.2828	-0.2668	-0.6369	-0.0856	-0.0966
Mg	0.2479	0.2403	-0.2347	0.2569	0.0024	-0.0046
Ca	0.1559	**-0.3347**	0.2396	0.0689	0.2836	0.1369
Na	0.0863	0.0435	-0.4798	-0.2192	-0.2343	0.0883
K	0.3201	0.0367	0.2195	0.0031	0.0972	-0.2947
Moisture %	0.2561	-0.2699	-0.1672	0.0281	0.0458	0.4448
EC	0.2451	-0.2796	-0.1783	0.1996	0.0081	-0.3654
PH	**-0.3580**	0.0072	-0.0208	-0.0855	-0.0076	0.1719
Caco3	**-0.3481**	0.0850	-0.0680	-0.0622	0.1204	0.3062
Organic matter	**0.3418**	-0.1150	0.0730	0.0622	-0.1356	0.1681
Temp	0.2345	0.3226	-0.0921	-0.1482	0.0210	-0.0561
Rain	0.2345	0.3226	-0.0921	-0.0118	0.4637	0.2724
Slop	0.1630	-0.0457	0.4395	-0.3022	0.0770	-0.1507
Elevation	**-0.3493**	-0.0919	-0.0383	-0.0479	0.5523	-0.4230
Aspect	0.0159	**-0.3758**	0.2873	0.0291	0.2612	-0.3373

The PCA method was adopted to determine the most substantial factor or environmental factors that cause changes in species distribution. The eigenvalues and percent variance of each component is in Table 7.

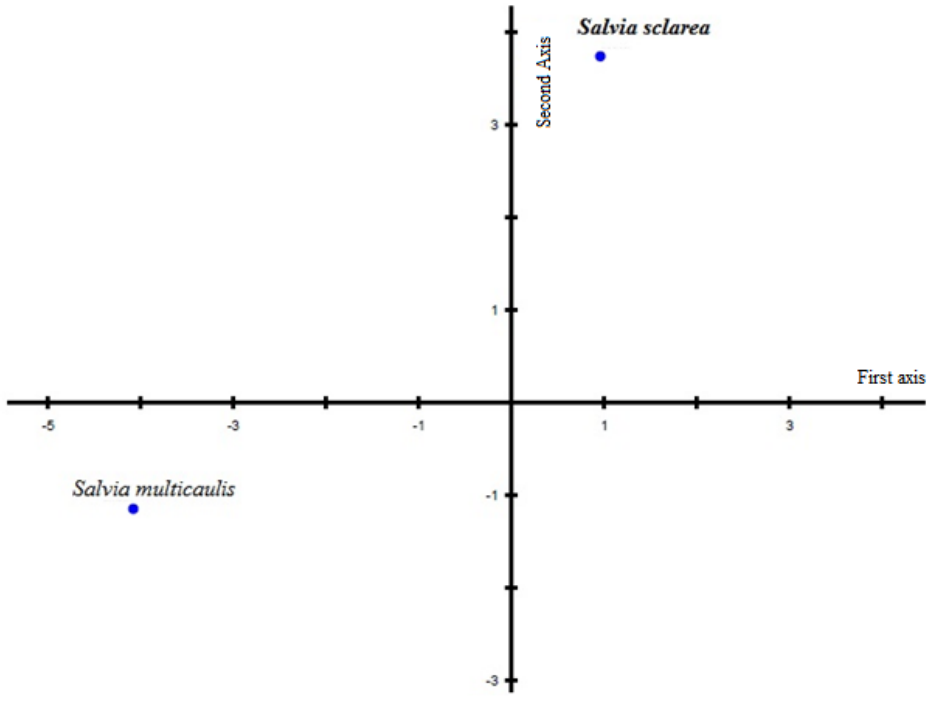

Fig. 5: Ordination diagram sites by using principal components analysis

The main components of the first and second graph (Figure 7) show distribution of species in relation to environmental factors by PCA analysis. The figure shows that the distribution of species affected by environmental factors and soil properties.

The first and second components are the main components and these components constitute 76.179 Percentages of changes in vegetation. The first component is more substantial, it is include 45.811 % of changes and the second component have 30.368 percentages of changes. Table 6 shown Vector eigenvalues for each variable in each of the components. According to coefficients, first component is including elevation, Ph, Caco3 and soil organic matter. Clay, silt, Ca and aspect are the most substantial factors in second component.

Salvia multicaulis Vahl is in the third quarter of coordinate axis and due to the great distance compared to the second axis is more affected by the properties of the first axis and show a trend toward higher acidity, $Caco_3$ and elevation (Table 8).

As you can see, *Salvia sclarea* L. is in the first quarter of coordinate axis and due to the great distance compared to the first axis is more affected by the properties of the second axis and show a trend toward higher Sand (Table 8).

Brauch study results (2005) showed that the amount of sand and elevation are influencing factors to determine Venezuela Savanna.

Topography, and on top of that, other factors such as altitude and even soil and climatic factors affect this location. Orographic rains are strongly influenced by the elevation changes, so as to deal with the mass of moist air to the mountains, saw a mass rally and then cold pressed and eventually will be precipitation. The intensity and how this process is greatly influenced by the shape and intensity of elevation changes. In this area we also saw this event, so that the community first, with the highest average altitude, the least amount of soil organic matter, is that it confirms the above. Generally, it can be acknowledged that the height of impact on the amount of precipitation, temperature, evapotranspiration and soil characteristics play an important role in the establishment of vegetation.

Conclusion

The results of study reveal that, ecological requirements of each species are different and these ecological factors have important effect on plants. *Salvia multicaulis* Vahl show a trend toward higher acidity, $Caco_3$ and elevation but *Salvia sclarea L.* show a trend toward higher Sand. Associated species of *Salvia sclarea* L. and *Salvia multicaulis* Vahl are different. The results showed that the number and type of *Salvia sclarea* L.and *Salvia multicaulis* Vahl components were different. And HPLC detected Camphene in two different times, 6.723 and 19.249.

References

Ahmadi L, Mirza M. Essential oil of Salvia multicaulis Vahl. from Iran. J Essent Oil Res, 1999; 11: 289–290

Bagci E, Kocak A. Essential oil composition of the aerial parts of two Salvia L. (*S. multicaulis* Vahl. Enum and *S. tricochlada* Bentham) species from east Anatolian region (Turkey). International Journal of Science & Technology, 2008; 3(1): 13-18.

Ben Taârit M, Msaada K, Hosni K, Marzouk B. Fatty acids, phenolic changes and antioxidant activity of clary sage (*Salvia sclarea* L.) rosette leaves grown under saline conditions. Industrial Crops and Products, 2012; 38: 58– 63.

Bouyoucos CJ. Hydrometer method improved for making particle size analysis of soil. Agron. J, 1962; 54:464-465.

Brauch, Z., 2005. Vegetation environmental relationships and classification of the seasonal savannas in Venezuela. Journal of flora. 200:49-64.

Dzamic A, Sokovic M, Ristic M, Grujic-Jovanovic S, Vukojevic J, Marin Pd. Chemical Composition and Antifungal activity of Salvia *sclarea* (Lamiaceae) essential oil. Sci., Belgrade, 2008; 60 (2): 233-237.

Esmaeili A, Rustaiyan A, Nadimi M, Larijani K, Nadjafi F, Tabrizi L. Chemical composition and antibacterial activity of essential oils from leaves, stems and flowers of Salvia reuterana Boiss. grown in Iran. Nat Prod Res, 2008; 22: 516–520

Famiglietti JS, Rudnicki JW, Rodell M. Variability in surface moisture content along a hill slope transect: Rattlesnake Hill, Texas. Journal of Hydrology, 1998; 210: 259-281.

Feo VD, Martino LD, Roscigno G, Mancini E, Falco ED. Chemical composition and antigerminative activity of the essential oils from five Salvia species. Molecules, 2010; 15: 735-746.

Feorkas P, Holla M, Vaverkova S. Composition of the essential oils from the flowers and leaves of *Salvia sclarea* L. (Lamiaceae) cultivated in Slovak Republic. JEOR, 2005; 17(2): 141-144.

Fraternale D, Giamperi L, Bucchini A, Ricci D, Epifano F, Genovese S, Curini M. Composition and antifungal activity of essential oil of Salvia sclarea from Italy. Chem. Nat. Comp, 2005; 41(5): 604-606.

Ghani A, Ebrahimpour A, Tehrani-far A, Hassanzadeh-Khayyat M. Evaluation of growth and development adaptability and medicinal ornamental potential of Clary sage (*Salvia sclarea* L.) cultivated in Mashhad climatic conditions. J of Plant Production, 2010; 17(1): 77-90.

Gulcin I, Tansu Uguz M, Oktay M, Beydemir S, Kufrevioglu OI. Evaluation of the antioxidant and antimicrobial activities of Clary Sage (*Salvia sclarea* L.).Turk J Agric For, 2004; 2(8): 25-33.

Jafari Haghighi M. Methods of soil analyze- physical and chemical sampling and analysis, published by Nedaye Zoha, 2003; 236 p.

Karamian R, Asadbegy M, Pakzad R. Essential oil compositions and in vitro antioxidant and antibacterial activities of the methanol extracts of two Salvia species (Lamiaceae) from Iran. International Journal of Agriculture and Crop Sciences. 2013; 1171-1182.

Kharazian N. Identification of flavonoids in leaves of seven wild growing Salvia L. (Lamiaceae) species from Iran. Progress in Biological Sciences, 2013; 3 (2): 81-98.

Kofidis G, Bosabalidis A, Kokkini S. Seasonal variation of essential oils in a linalool-rich chemotype of *Mentha spicata* grown wild in Greece. J of essential oil Res, 2006; 16: 469–472.

Lamaison JL, Petitjeanfreytet C. Medicinal Lamiaceae with antioxidant activity: potential sources of rosmarinic acid. Pharmacology Acta Helvetiae, 1996; 66: 185–188.

Masotti V, Juteau F, Bessiere JM, Viano J. Seasonal and phonological variation of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. J of Agricultural and Food Chemistry, 2003; 51: 7115–7121.

Mills A, Fey M, Donaldson J, Todd S, Theron L. Soil infiltrability as a driver of plant cover and species richness in the semi-arid Karoo, South Africa, Plant Soil, 2009; 320:321–332.

Nickavar B, Kamalinejad M, Izadpanah H. In vitro free radical scavenging activity of five Salvia species. Pak J Pharm Science, 2007; 20(4): 291–294

Peng Y, Ye J, Kong J. Determination of phenolic compounds in Perilla frutescens L. by capillary electrophoresis with electrochemical detection. J Agric Food Chem, 2005; 53: 8141–8147

Pitarokili D, Couladis M, Petsikos-Panayotarou N, Tzakou O. Composition and antifungal activity on soil-borne pathogens of the essential oil of Salvia sclarea from Greece. J. Agric. Food Chem, 2002; 6(50): 6688-6691.

Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology, 2006; 5(11): 1142-1145.

Rustaiyan A, Masoudi S, Monfared A, Komeilizadeh H. Volatile constituents of three *Salvia* species grown wild in Iran. Flavour and Fragrance J, 1999; 14: 256-276.

Senatore F, Apostolides A, Piozzi F. Chemical composition of the essential oil of *Salvia multicaulis* Vahl. var. *simplicifolia* Boiss. growing wild in Lebanon. J of Chromatography A, 2004; 237–240

Shahidi F, Wanasundara PKJPD. Phenolic antioxidants. Crit Rev Food Sci Nutr, 1992; 32: 67–103

Shaidai Karkaj E. Carbon sequestration potential redox species of Agropyron elongatum and Atriplex lentiformis (Case Study: Chaparqoymeh the Gonbad). MSc thesis range. Gorgan University of Agricultural Sciences and Natural Resources, 2011; 85 P.

Shan B, Cai YZ, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of the Agricultural and Food Chemistry, 2005; 53: 7749–7759.

Sharopov F, Setzer W. The Essential Oil of Salvia sclarea L. from Tajikistan. Rec. Nat. Prod. 2012; 6(1):75-79.

Soković M. Antifungalna aktivnost etarskih ulja odabranih aromatičnih i lekovitih biljaka in vitro i in vivo. Doctoral Dissertation, University of Belgrade, 2001.

Song A, Liu S, Shi Z, Dong L. Quantitative classification and ordination of subalpine meadow in wolong Nature Reserve. Ying Yong Sheng Tai Xue Bao, 2006; 17(7): 1174-8.

Sun J, Li X, Wang X, Li Z. Latitudinal changes in species diversity of permafrost wetland plant communities in Great Xing'an Mountain valleys of Northeast China. Acta Ecological Sinica, 2009; 29: 272-277.

Szentmihályi K, Then M, Csedő C. Comparative study on tannins, flavonoids, terpenes and mineral elements of some Salvia species. Acta Hort. 2004; 629: 463-470.

Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six Salvia species from Turkey. Food Chemistry, 2006; 95: 200–204.

Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissiou M, Sokmen A. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chemistry, 2004; 84: 519-525.

Vogiatzakis N, Kazakis G, Ghosn D. Macrophyte community structure and species occurrence in relation to environmental determinants in the ephemeral aquatic habitats of Gavdos. Journal of Hidrobiologica, 2009; 630: 127-138.

Yibing Q. Impact of habitat heterogeneity on plant community pattern in Gurbantunggut Desert. Geographical science, 2008; 14(4): 447-455.

Yousefzadi M, Sonboli A, Karimi F, Nejad Ebrahimi S, Asghari B, Zeinali A. Antimicrobial activity of some Salvia species essential oils from Iran. Z Naturforsch C, 2007; 62(7-8): 514-8.

Zargari, Ali. Medicinal plants, Tehran university publication, 1990. 452 pp.

Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. Journal of the Agricultural and Food Chemistry, 2001; 49: 5165–5170.

Zhong-hua Zhang, Gang Hu, Jian Ni. Effects of topographical and edaphic factors on the distribution of plant communities in two subtropical karst forests, southwestern China, Journal of Mountain Science, 2013; 10 (1): 95-104.