

Comparison of Essential Oil Components and Antioxidant Activity between *Salvia syriaca* and *Salvia aristata* in Their Natural Habitats in West Azerbaijan Province, Iran

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Abstract: The aerial parts of two *Salvia* species were collected at the beginning of flowering stage in June 2013. The essential oil of the air-dried parts of plants was extracted by hydro distillation and the chemical composition of the essential oils of *S. aristata* and *S. syriaca* collected in West Azerbaijan (Iran) was studied by means of GC-MS analysis. Total of 18 compounds were identified: 11 compounds were for *S. syriaca* with total oil of 90.98% and 7 compounds were for *S. aristata* with total oil of 98.23%. *S. aristata* had the most concentration of essential oil between these species. In *S. syriaca*, the main compounds were 1, 6-cineole (46.45%) and camphor (27.58%). In *S. aristata*, the main compound was benzene, 1, 3-bis m-phenoxyphenoxy (95.42%). Other compounds with low concentrations were transcaryophyllene (0.77%) and 1, 3-benzodioxole, 4-methoxy 6-2 (0.98%). In this study, the *in vitro* antioxidant activities of the essential oil of two *Salvia* species were examined (prepared by using ethanol 99.5% solvent). Between two species screened, *S. aristata* had the most antioxidant activity (31%). It was followed by *S. syriaca* (24%).

Key words: *Salvia aristata*, *Salvia syriaca*, antioxidant activity, GC-MS

1. Introduction

Fifty-eight species of the genus *Salvia* (Lamiaceae) are found in Iran, of which 17 are endemic. The rate of endemism in the genus *Salvia* in Iran is 29% [1, 2]. The leaves of *Salvia* species have reputation as a medicinal plant. Sage leaves are used as tonic, carminative, antispasmodic, antiseptic and hypoglycemic herbal drug [3-7]. Due to its antioxidant, antibacterial, antifungal, and anti-inflammatory properties sage extracts or tinctures are applied orally or dermally [8-11]. For instance, besides its main use as a tea, sage is also processed into drops for alleviating oropharyngeal inflammations. Here, the polyphenols, but also the terpenes, contained in the essential oil are responsible for the characteristic

flavor as well as the antibacterial effect of the herb [12].

Oxygen is essential for the life of all aerobic organisms as it is involved in the vital process to liberate energy. However, along with the release of energy, free radicals which are capable of damaging various biomolecules such as proteins, deoxyribose nucleic acid or polyunsaturated fatty acids are also formed [13]. Research in the recent past has accumulated enormous evidences revealing that the enrichment of body systems with natural antioxidants may prevent, delay or ameliorate many of the disorders caused due to oxidative stress [14]. Plants constitute an important source of natural antioxidants that differ widely in terms of their structures, biological properties and mechanism of action [15].

S. aristata is a perennial, stout root, woody, apically thickened plant and clothed with petiolar remains. Stem is above 6-sided. It is 30-70 cm long, rigid, erect,

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branched at base, long glandular and sparsely short glandular villous. Radical leaves are numerous, pale green, deeply pinnatisect, villous and $6-13 \times 3-4$ cm. Segments are linear-lanceolate and oblong-ovate; basal leaves are long petiolate, petiole is up to 14 cm long. Cauline leaves are in 2-5 whorls (per node usually 3-4, sometimes 2 leaves). Pedicels are 5-20 mm long, erect or spreading. Calyx is slightly inflated and has unequal teeth. Upper lip is tridentate, median tooth is reduced. Tube is straight and gradually dilated toward throat. Upper corolla lip is straight and shorter than lower lip; filaments are longer than staminal connectives, the anterior arm of connective is half as long as the posterior. Lower theca is fertile and slightly shorter than upper theca. Nutlets are $6-7 \times 4-5$ mm, trigonous globose and brown [16].

S. syriaca is a rhizomatous perennial herb. It is 30-60 cm, yellowish green, erect, Branched, eglandular-pubescent below and denser above (and rarely glandular). Leaves are simple, ovate and cordate. Pedicels are 3-4 mm. upper lip is straight and tridentate and nutlets are rounded-trigonous [17].

The aim of this work is to compare volatile components composition among two *Salvia* species and testing of target compounds for their free radical scavenging activity by using DPPH in West Azerbaijan.

2. Materials and Methods

2.1 Plant Material

Aerial parts of *S. aristata*, and *S. syriaca* were gathered at the beginning stage of flowering in June 2013 (Table 1). This plant is a grassy and permanent herb which belongs to Labiatea family and grows wild in some regions of Iran including West Azerbaijan province. *Salvia* specimens were stored in the herbarium of the West Azerbaijani agricultural

research center.

2.2 Isolation of the Volatile Components

30 grams of each air-dried sample were ground in a warring blender and then the essential oils were extracted by hydro-distillation in a Clevenger apparatus for 120 min. The oils were filtered over anhydrous sodium sulphate and stored in closed sterilized glass vials at $+4^{\circ}\text{C}$ in dark until being tested and analyzed.

2.3 Gas Chromatography-Mass Spectrometry

Analysis was performed on an Agilent 6890 gas chromatography with a 30 m to 0.25 mm HP-5MS capillary column (30 m \times 0.25 mm id, 0.25 μm film thickness). Column temperature was 120°C , with 5 min initial hold and then it was increased to 260°C at $10^{\circ}\text{C}/\text{min}$ rate. Injector and detector temperatures were 250°C , respectively. Capillary column was coupled to a mass selective detector; Ionization energy voltage was 70 eV, electron multiplier voltage was 3000 v and ion resource temperature 200°C . Mass spectra were scanned in the range of 30-600 amu. Helium was used as a carrier gas (35 mL/min).

2.4 Identification of Components

Constituents were identified by GC-MS by comparison of their kovats RI (retention indices) and also by comparison of constituents' mass spectra with those of the Wiley libraries using NIST ver.02 software.

2.5 Antioxidant Activity Assessment

A rapid, simple and inexpensive method to measure antioxidant capacity involves the use of the free radical, DPPH (2, 2-diphenyl-1-picrylhydrazil). DPPH

Table 1 Geographic sampling location (UTM system).

Salvia species	Geographic location	Altitude (meter)	Gathering date	Oil Percentage
<i>S. syriaca</i>	38 S 501755 4150560	1436	05 June 2013	4.5%
<i>S. aristata</i>	38 S 501938 4150358	1375	01 June 2013	14.5%

is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. It has also been used to quantify antioxidant in complex biological systems in recent years. DPPH is a well-known radical and a trap ("scavenger") for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction [18].

In this study, DPPH was obtained from Sigma-Aldrich Company, Germany. 0.23 mg DPPH was dissolved in 100 ml ethanol. 2 ml of this solvent was poured in each test tube and then 10 µl of the essential oil of four *Salvia* samples was added. Tests were carried out in triplicate. It was protected from light by covering the test tubes with aluminum foil. Absorbance was taken after 60 min. at 517 nm using ethanol as blank on WPA Biowave S2100 Diode Array Spectrophotometer. The DPPH free radical scavenging activity was calculated using the following formula:

$$\% \text{ scavenging} =$$

$$[A \text{ control} - A \text{ sample}/A \text{ control}] \times 100$$

A control is absorbance of the control reaction

(containing all reagents except the test compound) and a sample is the absorbance of the test compound. All data represent an average of 3 replicates. Mean values and standard deviations were calculated from the results. The results were expressed as percentage scavenging of DPPH radical.

3. Results

The essential oils of two *Salvia* species were extracted by hydro-distillation in a Clevenger apparatus and analyzed by GC-MS (HP-5 column). Total of 25 compounds were identified: 11 compounds were for *S. syriaca* with total oil of 90.98% and 7 compounds were for *S. aristata* with total oil of 98.23%. *S. aristata* had the most concentration of essential oil among these species. In *S. syriaca*, the main compounds were 1, 6-cineole (46.45%) and Camphor (27.58%). In *S. aristata*, the main compound was benzene, 1, 3-bis m-pheoxypheoxy (95.42%). Other compounds with low concentrations were transcaryophyllene (0.77%) and 1, 3-benzodioxole, 4-methoxy 6-2 (0.98%) (Table2).

Table 2 Percentage of essential oil composition of *S. syriaca* and *S. aristata*.

	RI	<i>S. Syriaca</i>	<i>S. Aristata</i>
1,8-cineole	1059	46.45	-
Camphor	1121	27.58	-
Bicyclo3,1,1,heptan-3-one	1109	1.54	-
Bicyclo2,1,1,heptan-2-ol	912	0.10	-
Borneol	1138	0.25	-
Bornyl acetate	1277	4.66	-
Sabiny acetate	1224	3.18	-
Ethanone, 1,2,3,4-trihydroxy	1691	1.51	-
3-cyclohexen-1-ol	890	1.93	-
2-propenoic acid,3,4-methoxy	1546	2.56	-
Valencene naphthalene	1474	1.22	-
Benzene,1,3-bis(m-pheoxypheoxy)	2158	-	95.42
2-citral 2,6-octadienal,3,7	2864	-	0.57
2,6-octadienal,3,7-dimethyl	1174	-	0.31
transcaryophyllene	1494	-	0.77
Delta-cadinene naphthalene	1580	-	0.06
1,3-benzodioxole,4-methoxy,6-2	2527	-	0.98
Alpha-cadinol 1-naphthalenol	1641	-	0.12

RI: (Retention index)¹.

¹ In gas chromatography, Kovats retention index (shorter Kovats index, retention index; plural retention indices) is used to convert retention times into system-independent constants. The index is named after the Hungarian-born Swiss chemist Ervin Kováts, who outlined this concept during the 1950s while performing research into the composition of the essential oils (Kovats Ervin, 1950)

One of the quick methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical and widely used index [19]. In this study, the *in vitro* antioxidant activity of the essential oil of two *Salvia* species were examined (prepared by using ethanol 99.5% solvent). Between these two species screened, *S. aristata* had the most antioxidant activity (31%). It was followed by *S. syriaca* (24%).

4. Discussion

Each individual essential oils is composed of several dozen substances, however, usually a single compound is responsible for its flavor and pharmacological activity. The percentage of each individual constituent in the essential oil is variable and it depends on genetics (chemical variability) and environmental factors (climate, insolation, altitude) [20]. Qualitative and quantitative differences in essential oil composition can also relate to extraction procedure [21].

In this paper the essential oil of two *Salvia* species was investigated in West Azerbaijan, Iran. Constituents in the *Salvia* species studied show diversity in world, and they are often differentially distributed. As mentioned above, chemical differentiation might be correlated to the geographical and ecological conditions under which they grow and the large variability of structures [22, 23]. The ecological correlations in the adaptation of plants to habitats apply to the results of chemotaxonomy [24]. The findings of this study support the view that *Salvia* species are promising sources of potential antioxidants and may be efficient as preventive agents in diseases caused due to oxidative stress. In addition, these plant extracts can be used as easily accessible sources of natural antioxidants and as possible food supplements or in pharmaceutical industries. However, there is a need for further studies to be carried out to isolate and identify the components of these two *Salvia* species responsible for their antioxidant activity. Both species has DPPH radical scavenging ability but there is no

significant difference between them. These plants, rich in phenolic acids could be a good source of natural antioxidants. The differences in essential oils and phenolic compounds can be emanated from ecological, genetical and nutritial factors.

5. Conclusions

Our results showed that *S. aristata* had the most concentration of essential oil among these species. Both species had radical scavenging ability but *S. aristata* had the most antioxidant activity (31%).

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