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Original Research Article

Compositional Analysis and Antioxidant Activity of Volatile Components of Two *Salvia* spp

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Abstract

Purpose: *To identify and compare the composition of volatile components of two Salvia species, and also their free radical scavenging activity.*
Method: *The essential oil of two Salvia species was analyzed using gas chromatography-mass spectroscopy (GC-MS) techniques while their phenolic contents were analyzed by high performance liquid chromatography (HPLC). The in vitro antioxidant activity of the essential oils was evaluated by 1, 1-diphenyl-2 picryl hydrazyl (DPPH) radical scavenging technique.*
Results: *Seven derivatives were identified for S. verticillata and four derivatives for S. suffruticosa. For both species, the main compounds were 1, 8-cineole (S. suffruticosa: 31.21 % and S. verticillata: 38.26 %) and camphor (S. suffruticosa: 27.11 % and S. verticillata: 22.98 %). The content of the phenolic compounds was: ascorbic acid (S. suffruticosa: 23.98 % and S. verticillata: 33.53 %), p- hydroxyl benzoic acid (S. suffruticosa 11.50 % and S. verticillata 3.83 %), vanilic acid (S. suffruticosa 5.86% and S. verticillata: 6.55 %), syringic acid (S. suffruticosa 6.29 %), ferulic acid (S. suffruticosa: 6.35 % and S. verticillata 6.04 %) and sinapic acid (S. suffruticosa 6.26 % and S. verticillata 4.93%). DPPH radical scavenging ability was 0.548 % for S. suffruticosa for S. suffruticosa and 0.558 % for S. verticillata.*
Conclusion: *The results of this study demonstrated that these two species are rich in 1, 8-cineole, camphor and phenolic compounds. There is no significant difference between the radical scavenging activities of the two essential oils.*

Keywords: *S. verticillata, S. suffruticosa, essential oil, antioxidant activity, GC-MS, HPLC activity*

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INTRODUCTION

Salvia is one of the largest genera of the Labiatae family. This genus includes nearly 700 species which are spread throughout the world [1]. *Salvia* species are aromatic plants, which are rich in essential oil [2]. In the flora of Iran, the genus is represented by about 58 species of which 17 are endemic [3]. The plants are naturally distributed in different parts of Iran and are called ''Maryam goli'' [4] in Persian. The name Salvia comes from the Latin word salvare, which means healer. The salvia species possess antibacterial, carminative, diuretic, hemostatic and spasmolytic activities and are used as herbal teas all around the world [5].

S. verticillata is a herbaceous perennial which can be found in a wide geographic area ranging from central Europe to western Asia [8,9]. The tiny lavender flowers grow tightly packed in whorls, with tiny lime-green and purple calyces [7,8].

S. suffruticosa is a semi-shrub with branches from the base pinnate leaves that grow up to 50

cm (20 inches) in height. It has bilabiate flowers, yellowish-white corolla, a galeiform upper lip, a tripartite lower lip, greenish calyx, tooth at the margins and thick stipulate glandules [10]. It is a well-known fact that the curative properties of many plants are due to their high contents of phenolics, which act as free radicals scavengers [10]. Thus, the objective of this study was to analyze the phenolic compounds present in the volatile of the studied plants.

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

EXPERIMENTAL

Plant material

Aerial parts of *S. verticillata* (code no: 9539) and *S. suffroticosa* (code No: 9529) were collected at the beginning of flowering and their locations were marked by a Global Positioning System GPS system. This plant is a permanent herb which belongs to the Labiatae family and grows wild in some regions of Iran, including West Azerbaijan Province. The *Salvia* specimens were authenticated and stored in the herbarium of the West Azerbaijani Agricultural Research Center (Table 1).

Extraction of volatile components

One hundred grams portions of each air-dried samples was ground in a Waring 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 to remove traces of moisture and stored in closed sterilized glass vials at +4 ^{o}C in the dark until being analyzed and screened [11].

Gas chromatography-mass spectrometry

Analysis was performed on an Agilent 5973 gas chromatograph equipped with an ion-trap mass spectrometer detector (Varian Saturn 2100), using a ZB-5 (5 % of phenyl-dimethylpolysiloxane), fused-silica capillary column (30 m x 0.25 mm i.d., 0.25 µm film thicknesses). Helium was used as a carrier gas. The injection volume was 1 µL. The column temperature was 120 ^{o}C, with a 5 min initial hold and then it was increased to 260 ^{o}C at 10 ^{o}C/min rate. The injector and detector temperatures were 250 and 200 ^{o}C, respectively and manifold at 70 ºC with line transfer at 240 ºC. The capillary column was coupled to a mass selective detector; the ionization energy voltage was 70 eV, electron multiplier voltage was 3000 v and ion resource temperature 200 ^{o}C. Mass spectra were scanned in the range of 30 - 300 amu [12].

HPLC analysis

A 20 µL aliquot of the extracted solution was separated using a HPLC Knauer system equipped with UV-Vis detector and a Eurospher 100-5 C-18 column (25 cm × 4.6 mm; 5 µm). The mobile phase consisted of HPLC grade water with 2 % acetic acid (A) and acetonitrile (B). Solvent gradient was used as follows: from 0 to 5 min isocratic 85 % A flow, from 5 to 19 min (14 min) a linear gradient of 85 % A to 100 % B. After termination of the cycle, 15 min of column equilibration (85 % A) were allowed prior next injection. Phenolic compounds were detected at a wavelength of 280 nm and identified by comparing their relative retention times and UV spectra with authentic compounds; they were detected using an external standard method [14].

Evaluation of antioxidant activity

The measurement of DPPH radical scavenging activity was carried out according to the method of Barros *et al* [15]. A total of 10 µL of the essential oil of *S. suffruticosa* and *S. verticillata* was added to 2 mL of methalonic DPPH (0.0023 mol/L) solution. The mixture was incubated in room temperature for 1 h before the change in absorbance at 517 nm was measured. The radical scavenging activity (D) was calculated as in Eq 1:

$$(D\ (\%) = \{(A_0 - A_1)/A_0\} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ (1)$$

where A_0 is the absorbance of the DPPH solution and A_1 is the absorbance of the sample.

Table 1: Geographic sampling location (UTM system) of the plant species

Salvia species	UTM system	Altitude (m)	Collection date	Voucher no.
S. suffruticosa	38 S 496689 4209523	1657	4 June 2013	9539
S. verticillata	38 S 0570079 4129194	1438	7 June 2013	9529

Data analysis

Constituents were identified by GC-MS by comparison of their Kovats retention indices (RI) and also by comparison of the constituents' mass spectra with those of the Wiley libraries using NIST ver. 02 software [16]. For antioxidant activity, all data represent an average of three replicates. Mean values and standard deviation (SD, n = 3) were calculated from the results. Comparison between groups was performed by one-way ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

The essential oils of two *Salvia* species were extracted by hydro-distillation in a Clevenger apparatus and analyzed by GC - MS. Seven compounds were determined for *S. verticillata* with total essential oil content of 71.27 %; and 4 compounds were determined for *S. suffruticosa* with total essential oil content of 61.11 %. In *S. suffruticosa*, the main compounds were 1, 8-cineole (31.21%), camphor (27.11%), dimethyl sulfone (13.17 %) and bornylacetate (8.8 %). In *S. verticillata*, the main compounds were 1, 8-cineole (38.26 %), and camphor (22.98 %). Other compounds with low concentrations were bicycloheptan (5.52 %), cyclohexane (1.67 %), α-pinene (1.77 %), camphene (0.54 %) and borneol (2.29 %) (Table 2).

As the result, there was no significant difference between radical scavenging ability of *S. suffruticosa* and *S. verticillata*.

The concentrations of phenolic compounds in the *Salvia* samples are reported in Table 3.

Table 2: Essential oil composition of *S. suffroticosa and S. verticillata*

Compounds	RI	*S. suffroticosa*	*S. verticillata*
1,8-cineole	1059	31.21	38.26
camphor	1121	27.11	22.98
bornyl acetate	1277	8.81	-
dimethyl sulfone	727	13.17	-
geranyl acetate	1352	-	-
α- Pinene	948	-	1.77
camphene	943	-	0.54
borneol	1167	-	2.29
bicycloheptan	1581	-	5.52
cyclohexane	719	-	1.67

RI = Retention index

Table 3: Content of phenolic compounds in *Salvia* species

Plant material	mg/100g dried plant material								
	AA	RU	CA	*p -HBA*	VA	*p - CA*	SA	FA	SA*
S. suffruticosa	23.29	n.d	n.d	11.50	5.86	n.d	6.29	6.34	6.26
S. verticillata	33.52	n.d	n.d	3.83	6.55	n.d	n.d	6.04	4.93

n.d = not detected; AA = Ascorbic acid; RU = Rutin; CA = Caffeic acid; p –HBA = p-Hydroxy benzoic acid; VA=Vanillic acid; p – CA = p-Coumaric acid; SA= Syringic acid; FA = Ferulic acid; SA = Sinapic acid*

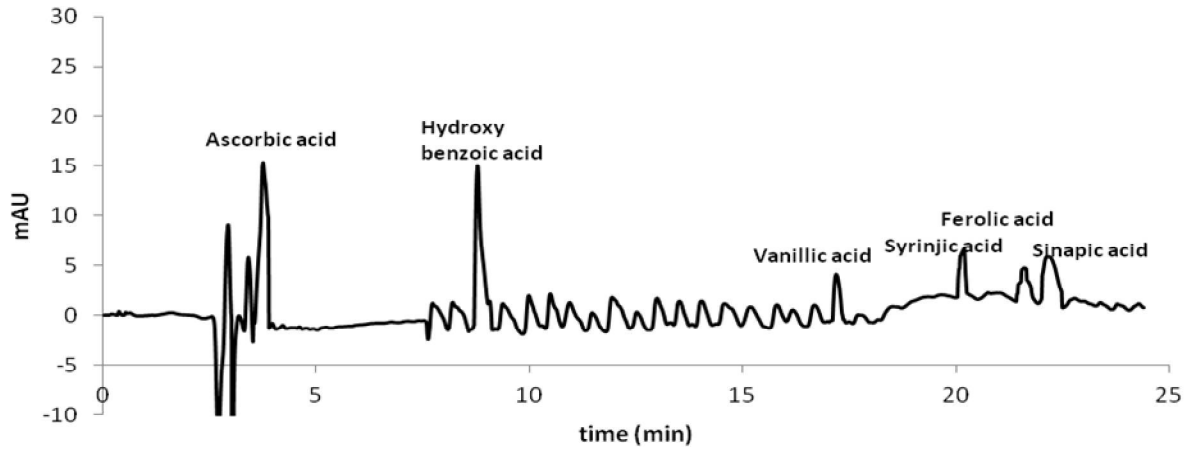

Figure 1: Chromatogram of identified phenolics compounds in *S. suffruticosa*

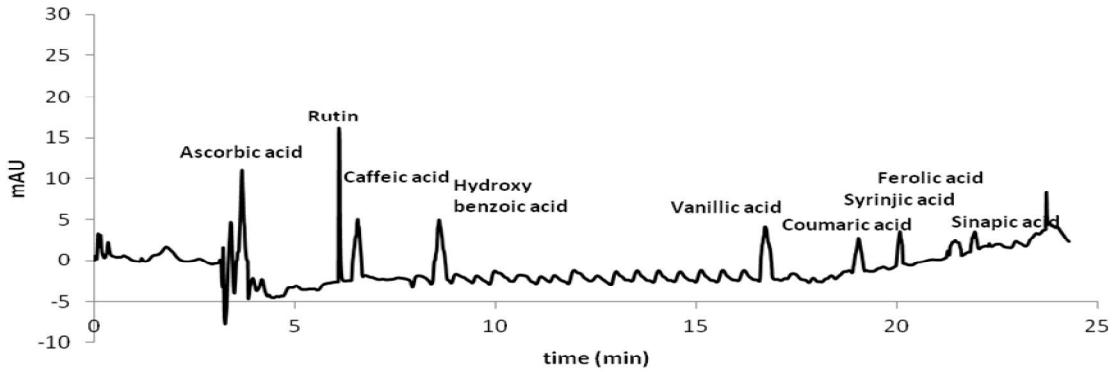

Figure 2: The chromatogram of identified phenolics compounds in *S. suffruticosa*

The DPPH radical scavenging activity of *S. suffruticosa* and *S. verticillata* was 0.548 ± 0.026 and 0.558 ± 0.064, respectively.

DISCUSSION

Each individual essential oil was 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) [17]. Qualitative and quantitative differences in essential oil composition can also relate to its extraction procedure [17].

Nasermoadeli *et al* found that e-caryophyellene, α-gurjunene, germacrene-d, α- humulene, β-phellandrene, β-pinene and bicyclogermacrene are the main components of wild *S. verticillata* [18].

Sefidkon and Khajavi found β-caryophyllene, γ-murolene, limonene and α-humulene as the major constituents of *S. verticillata* oil. Most of the compounds that were identified in essential oil of *S. verticillata* were present in other *Salvia* species but; in contrast to the other studies, no caryophyllene and α-humulene were detected in their sample [19].

Norouzi-Arasi *et al* found 30 components in the essential oil of *S. suffruticosa*, of which the main components were camphor (48.5 %), 1, 8-cineole (18.6 %) and camphene (7.9 %) [20]. As stated previously, in our *S. suffruticosa* samples, the main compounds were 1, 8-cineole (31.21 %), camphor (27.11 %), dimethyl sulfone (13.17 %) and bornyl acetate (8.81 %).

These two species possess antioxidant potential. Extensive studies have been carried out on the antioxidant activity of many species of *Salvia*. They demonstrated that this family species had strong antioxidant capacity. Some authors have demonstrated a linear correlation between the content of total phenolic compounds and their antioxidant capacity [21,22].

Many papers have suggested the identified components presented here as the major derivatives of *Salvia* species [23,24]. It is obvious that these two species is valuable species in terms of biologically active principles content. 1,8-cineole, camphor, ascorbic acid, p-hydroxy benzoic acid, vanilic acid, ferrolic acid and sinapic acid were found in two species are chemical mediators in biochemical interactions among other plants and this could suggest models for lead compounds in the development of some products such as drugs and pesticides [25].

CONCLUSION

S. verticillata has higher total essential oil content than *S. suffruticosa*. *S. verticillata* is rich in 1,8-cineole while *S. suffruticosa* is rich in camphor. Both species have approximately the same level of DPPH radical scavenging ability. The essential oils plants, because they are rich phenolic acids, are potentially a good source of natural antioxidants.

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