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RESEARCH ARTICLE

COMPARISON OF ESSENTIAL OIL COMPONENTS AND ANTIOXIDANT ACTIVITY AMONG *SALVIA ATROPATANA*, *SALVIA SYRIACA*, *SALVIA NEMOROSA* AND *SALVIA ARISTATA* IN THEIR NATURAL HABITATS IN WEST AZERBAIJAN PROVINCE, IRAN

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ABSTRACT

The essential oil of four salvia species was studied by means of GC-MS analysis. Total of 25 compounds were identified: 11 compounds were for S. syriaca with total oil of 90.98%; 7 compounds were for *S. aristata* with total oil of 98.23%; 11 compounds were for S. atropatana with total oil of 72.04%; 3 compounds were for S. nemorosa with total oil of 88.53%. The invitro antioxidant activities of the essential oil of four salvia species were examined. S. atropatana had the most antioxidant activity (42%). It was followed by *S. aristata* (31%), S.syriaca (24%) and S. nemorosa (21%).

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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% (Rechinger, 1982; Mozaffarian, 1996). Due to its antioxidant, antibacterial, antifungal, and anti-inflammatory properties sage extracts or tinctures are applied orally or dermally (Sokmen *et al*., 2008; Peana *et al*., 1997). 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 (quaas *et al*., 2012). The aim of this work is to compare volatile components composition among four Salvia species and testing of target compounds for their free radical scavenging activity by using DPPH in West Azerbaijan.

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MATERIALS AND METHODS

Plant material

Aerial parts of *S. nemorosa, S. atropatana, S. aristata, and S. syriaca* were gathered at the beginning stage of flowering on June 2013. 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 (Table 1).

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˚c in dark until being tested and analyzed.

Table 1. Percentage of essential oil composition of *S. syriaca, S. aristata, S.atropatana and S. nemorosa*

	RI	*S.Syriaca*	*S.Aristata*	*S.atropatana*	*S. nemorosa*
1,8-cineole	1059	46.45		25.19	**44.42**
Camphor	1121	27.58		27.52	
Bicyclo3,1,1,heptan-3-one	1109	1.54			
Bicyclo2,1,1,heptan-2-ol	912	0.10			
borneol	1138	0.25		0.24	
Bornyl acetate	1277	4.66			
Sabinyl acetate	1224	3.18			
Ethanone, 1,2,3,4-trihydroxy	1691	1.51		1.65	
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		
transcarophyllene	1494		0.77	1.26	
Delta-cadinene naphthalene	1580		0.06		
1,3-benzodioxole,4-methoxy,6-2	2527		0.98	1.30	
Alpha-cadinol 1-naphthalenol	1641		0.12		
Gamma-terpinene 1,4-cyclo	998			0.75	
Camphene bicyclo heptan	943			0.71	
Caryophyllene oxide	1507			8.94	
7,8-epoxy-alpha-ionone	1473			1.30	
Naphthalene, 1,2,3,4	1185			3.18	
Bicyclo,2,2,1 heptan-2-one					**25.56**
α-pinene	948				**18.55**

	RI	*S.Syriaca*	*S.Aristata*	*S.atropatana*	*S. nemorosa*
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RI= retention index

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 × 0.25 mm id, 0.25 µm film thicknesses). Column temperature was 120 ˚c, with 5 min initial hold and then it was increased to 260 ˚c at 10 ˚C/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 3000v 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).

Identification of components

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

Antioxidant activity assessment

A rapid, simple and inexpensive method to measure antioxidant capacity involves the use of the free radical, 2, 2-diphenyl-1-picrylhydrazil (DPPH). DPPH 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. In this study, DPPH was obtained from CIGMA-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 saliva 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 517nm using ethanol as blank on WPA Biowave S2100 Diode Array Spectrophotometer. The DPPH free radical scavenging activity was calculated using the following formula:

% scavenging = [A control – A sample/A control] X 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 were calculated from the results. The results were expressed as percentage scavenging of DPPH radical.

RESULTS AND DISCUSSION

The essential oils of four 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%; 7 compounds were for *S. aristata* with total oil of 98.23%; 11 compounds were for *S. atropatana* with total oil of 72.04%; 3 compounds were for *S. nemorosa* with total oil of 88.53%. *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%).In *S. atropatana*, the main compounds were 1, 8-cineole (25.19%) and camphor (27.52%). In *S. nemorosa*, the main compounds were camphor (44.42%) and bicycle, 2, 2, 1 heptan-2-one (25.56%) (Table 1). Baser *et al.* (1996) reported more than 50 compounds in aerial parts of *S. syriaca*. Germacrene D (33.83%) and Bicyclogermacrene (12.47%) were found as major constituents (Demircakmak *et al*., 1996).

Chalchat *et al*. (1999) reported P-cymene (33.6%), carvacrol (14.1%), limonene (8.4%) and cis-sabinene hydrate (5.5%) as the major constituent of *S. aristata* from Eastern Serbia (Gorunovic *et al*., 1999). As Chizzola (2012) reported, the flower oils of *S. nemorosa* growing at different sites in the outskirts of Vienna, Austria, contained mainly Sabinene (37.44%), Gemacrene D (9- 14%), β- caryophyllene (8-12%) and caryophyllene oxide (2.6-4.4%). Leaf samples had β-caryophyllene (14-41%), Germacrene D (14-38%) and caryophyllene oxide (5- 20%) as main compounds (Chizzola, 2012). Mirza and Ahmadi (2000) reported 29 compounds in the essential oil from aerial parts of *S. atropatana* Bunge. The major components were β-caryophyllene (16.3%), Sclareol (13.3%), hexyl octanoate (12.2%) and Germacrene B (10.0%) (Ahmadi and Mirza, 2000).

Nadaf *et al*. (2012) reported oxabicyclo[2,2,1] heptanes, 1,3,3,7-tetramethyl (11.5%), bis(2- ethylhexyl) phthalate (8.6%), 9, 12, 15- octadecatrien-1-ol (8.3%), palmitic acid (7.2%), bromocyclohexane (5.0%), 1-hexadecanol (4.7%), dimethyl sufoxide (3.4%) as the major components of *S. nemorosa* from Darkesh area of Bojnord, Iran in May 2011 (Nasrabadi *et al*., 2012). 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) (Affara *et al*., 2001). Qualitative and quantitative differences in essential oil composition can also relate to extraction procedure (Schellenberg and Richer, 2007).

One of the quick methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical and widely used index (Patel Natvar and Patel Rajesh, 2011). In this study, the invitro antioxidant activity of the essential oil of four salvia species were examined (prepared by using ethanol 99.5% solvent). Among the four species screened, S. atropatana had the most antioxidant activity (42%). It was followed by *S. aristata* (31%), *S.syriaca* (24%) and *S. nemorosa* (21%).

RI= retention index

Conclusion

Our results showed that *S. aristata* had the most concentration of essential oil among these species. All species had radical scavenging ability but *S. atropatana* had the most antioxidant activity (42%).

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