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Variability of Essential Oils of Various Parts of *Satureja sahendica* Bornm. and Their Antioxidant Activity

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Abstract: Hydro-distilled essential oils from the aerial parts of *Satureja sahendica* Bornm. and their antioxidant effects were investigated, mainly by a combination of GC/MS. *S. sahendica* is one of the endemic species of *Satureja* in Iran. The plant materials separated to two different parts, 1-inflorescence (S1) 2- leaf and stem (S2). Thirty to forty gram of each samples were subjected to hydro-distillation for 4 h using a cleverger-type apparatus to produce oils. Hydro distillation was repeated thrice for each sample and average was calculated. The oils were found to be yellow liquid and were obtained in yields of 1.66 for S1 and 1.5 for S2 based on v/w. Twenty nine compounds were identified in the oil of S1 that Thymol (32.5%), γ -Terpinen (29.33%) and P-Cymene (23.48) are main constituents and 23 compounds were characterized in the oil of S2 that main constituents were P-Cymene (44.88%), Thymol (28.22%) and γ -Terpinen (10.07%). The antioxidant effect of S1 and S2 compounds were assessed by DPPH assay and RC50 values were and mg mL⁻¹, respectively.

Key words: *Satureja sahendica* Bornm., essential oil, cleverger, antioxidant, DPPH assay

INTRODUCTION

Satureja (Labiatae) species are present in mountainous areas in Iran, mainly in Western and Northern Parts. *Satureja sahendica* Bornm. is an endemic species of Iran distributed in Western and Northwestern and existence were reported from Zanjan, East Azarbayejan and Kordestan provinces.

It is a late flowering species (late summer and fall), growing on rockwalls and rocky slopes.

Foliage (1-2 and 8-12 mm), Flowers (6 mm long) white and attractive for bees (Mozaffarian, 1993).

Due to various usages of *Satureja* species or their oils the components of essential oil of plants identified in Iran and overall the world (Tampieri *et al.*, 2005).

The investigations on the composition of essential oils of *S. mutica*, *S. macrantha* and *S. intermedia* in Iran show that the main constituents in the *S. mutica* essential oil were P-Cymene (25.8%) and Limonene (16.3%) and in the essential oil of *S. intermedia*, Thymol (32.3%) and γ -Terpinene (29.3%) are main components.

In the essential oil of *S. macrantha* main component are P-Cymene (25.8%) and Limonene (16.3%) (Sefidkon and Ahmadi, 2000).

Comparison the constituents of essential oil of *S. boliviana* and *S. parvifolia* from Argentina demonstrate that main constituents in first plant are γ -Terpinen (15.4%), β -Caryophyllene (10.2%), Germacrene (8.9%), Bicyclo germacrene (8.3%) and 1,8-Cineol (7.4%), whereas in second plant, main component is Piperitenone oxide (69.8%) (Viturro *et al.*, 2000).

The essential oil of *S. bachtiarica* from Iran mainly have Thymol (44.5%), γ -Terpinen (23.9%), P-Cymene (7.3%), β -Caryophyllene (95.3%) and Borneol (4.2%) and in *S. khuzistanica* from Iran the main constituents are P-Cymene (39.8%), Carvacrol (29.6%), γ -Terpinene (18.9%) (Sefidkon and Ahmadi, 2000).

The essential oil composition of *S. viminea* in Castarica was investigated and the main constituents are P-Menth-3-en-8-ol (40%), Polegone (35.3%), P-Mentha-3,8-di-en (18.9%) (Vila *et al.*, 2000).

The various species of *Satureja* genus are used as a culinary herbs and green leaves and herbaceous sections of stem are used fresh and dried as flavouring agents in seasoning, stews, meat dishes, poultry, sausages and vegetables and as a medicinal plant has been traditionally used as a stimulant, stomachic, carminative, expectorant, antidiarrheic and aphrodisiac (Baser, 2002).

Also researches indicate that the *Satureja* plants have antimicrobial, antifungal and antibacterial effects (Skocibusic *et al.*, 2004). On the other hand the essential oil of this genus plants are natural antioxidants and have great antioxidative effects (Delazar *et al.*, 2005a, b; Radonic and Milos, 2003; Tomohiro *et al.*, 1994).

The main characteristic of an antioxidant is ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources.

These free radicals may oxidize nucleic acids, proteins, lipids or DNA and initiate degenerative disease.

The highly susceptibility to oxidation of the fat and oil polyunsaturated fatty acids used in human foods or animal feed requires the application of antioxidants.

The antioxidants can be either synthetic or natural products with scavenge oxygen free radicals, thereby inhibiting or delaying oxidation.

Interest in natural antioxidants has increased dramatically in recent times due to concerns regarding the safety of the chronic consumption of antioxidants (Butylated hydroxyl toluene and Butylated hydroxyl lanisole) the antioxidative efficacy of a variety phytochemicals. The consensus that foods rich in certain phytochemicals can affect the aetiology and pathology of Chronic disease and aging process and the publics conceived belief that natural compounds are innately safer than synthetic compounds and are thus more commercially acceptable.

MATERIALS AND METHODS

Plant materials: The aerial parts of *Satureja sahendica* Bornm. were collected in flowering stage (late summer), from Mazarnashib, near the Sahand hillside at East Azarbayejan (N: 37° 43' 52.07½, E: 46° 10' 3.6' with 2048 heigh), herbarium specimens were deposited at Azarbayejan botanical garden in Tabriz Forest and Rangelands Research institute (the herbarium code was: 5395, *Satureja sahendica*, Sahand-Sufichai-1700 m). The experiments were conducted at the autumn of 2006 in Medicinal Plants Lab, Drug Applied Research Center, Tabriz University of Medical Science.

The air parts of collected plants separated to two parts: inflorescence (S1), leaf and stem (S2) and air dried then storage for 3 month in 2°C at light and air impervious pockets. The plant materials were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus to produce oils.

The oils were dried over Anhydrous sodium chloride and stored in sealed vials at low temperature (2°C) and umbrage before analysis.

Gas chromatography: GC analysis was performed using a Shimadzu GC-17A-Ver. 3. Gas chromatograph equipped with a DB-5 fused column (60 m 0.25 mm i.d., film thickness 1.4 µm).

Oven temperature was held at 50°C for 3 min and then programmed to 250°C at a rate of 3°C min⁻¹.

Injector and detector temperature were 260°C, helium was used as carrier gas with a linear velocity of 32 m sec⁻¹.

Gas chromatography-mass spectrometry: GC-MS analysis were carried out on a Shimadzu GC-17A-Ver. 3 equipped with Quadropole (QP5050) MS with DB-5 column (60 m 0.25 mm i.d.,).

Oven temperature was 50-250°C at a rate of 3°C min⁻¹, transfer line temperature 260°C, carrier gas helium with a linear velocity of 32 cm sec⁻¹.

Identification of components: The components of the oil were identified by comparison of their mass spectra with those of computer library or with authentic compounds and confirmed by comparison their retention indices, either with those of authentic compounds or with data published in the literature (Adams, 2004).

DPPH assay: DPPH assay is a rapid, simple and inexpensive method to measure antioxidant capacity, involves the use of the free radical 2,2-Diphenyl-1-picrylhydrazyl.

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and purple in color. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H.

In this research DPPH was obtained from Sigma Ltd. and stock solutions (1%) of the plant essential oils were papered in CHCl₃. Serial dilutions were carried out to obtain concentration of 3.12×10⁻², 1.56×10⁻², 7.8×10⁻³, 3.9×10⁻³ and 1.9×10⁻³ mg mL⁻¹. Diluted solutions (5 mL each) were mixed with DPPH (5 mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration.

RESULTS AND DISCUSSION

The oils isolated by hydrodistillation from two distinct aerial parts of *S. sahendica* (S1 ad S2) were found to be yellow liquids were obtained in yields of 1.66% (v/w) and 1.5% (v/w), respectively. The components are listed in order of their elution on the DB-5 column (Table 1).

Table 1: Percentage composition of essential oils of S1 and S2 samples

Compounds	RI	S1 sample	S2 sample
α -Thujen	930	0.80	0.82
α -Pinene	939	0.41	0.50
Camphene	954		0.13
β -Pinene	979	0.15	0.14
Myrcene	991	1.87	1.57
α -Phellandrene	1003	0.22	0.11
α -Terpinene	1017	3.30	0.91
Para-Cymene	1025	23.48	44.88
Limonene	1029	0.43	0.52
δ -Carene	1031	0.19	
Cis-Ocimene	1037	0.32	
β -Ocimene	1050	0.10	0.32
γ -Terpinene	1060	29.33	10.70
Para-Cymenene	1091		0.27
Linalool	1097	0.28	0.54
Sabinene hydrate	1098		0.24
Borneol	1169	0.28	0.63
Terpinene-4-ol	1177	0.66	0.84
Para-Cymene-8-ol	1183	0.16	0.84
α -Terpineol	1189	0.09	
Thymol	1290	32.57	28.22
Carvacrol	1299	0.94	1.69
Thymol acetate	1352	0.11	
β -Caryophyllene	1419	1.01	0.53
δ -Elemene	1437	0.23	
β -Pharnesene	1457	0.25	1.16
β -Chamigrene	1478	0.14	
Germacrene D	1485	0.13	
δ -Cadinene	1514	0.10	
δ -Cadinene	1523	0.16	
Spathulenol	1578	0.65	1.16
Caryophyllene oxide	1583	0.24	66.00

Twenty nine components of the S1 sample oil, representing 98.6% of total oil and 23 components of the S2 sample representing 97.38% were identified. The main components of the S1 samples oil were Thymol (32.57%), γ -Terpinene (29.33%) and P-Cymene (23.48%). The major constituents of the S2 samples oil were P-Cymene (44.8%), Thymole (28.22%) and γ -Terpinene (10.07%).

The DPPH antioxidant assay is based on the ability of 2,2, diphenyl, 1-picrylhydrazyl (DPPH) a stable free radical contains an odd electron which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound the DPPH is decolourised which can be quantitatively measured from the changes in absorbance. The S1 and S2 samples displayed significant free radical scavenging activity in the DPPH assay.

Comparing the oil composition of S1 and S2 essential oils showed some differences (Fig. 1). The first major compound of S1 is Thymol, while the first major compound of S2 is P-Cymene. Some minor or trace components such as δ -Carene, γ -Elemene, β -Camigrene, β -Farnesene, γ -Cadinene that was found in S1 was not observed in the oil of S2 and some minor components such as P-Cymene, Sabinene hydrate that was found in S1 essential oil was not observed in the oil of S1. Investigations showed that the oil composition of

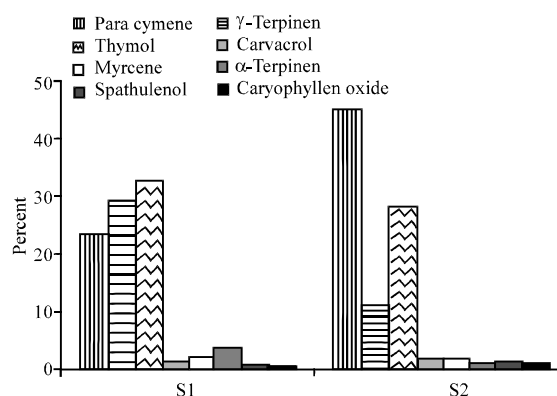


Fig. 1: Comparison between some main components of essential oils of samples

S. sahendica is like that of *S. bachtiarica*, *S. spicigera* and *S. cuneifolia* (Biavati and Ozcan, 2004; Sefidkon and Jamzad, 2000). Also, the investigations on the antioxidant activity of S1 and S2 samples and their RC_{50} values showed that essential oil of aerial parts of *S. sahendica* had a great antioxidant effect and due to high amount of Thymole and P-Cymene and other terpenoids in the oil, it can be used as folk remedies for many diseases (with bactericidal, carminative, digestive, expectorants, fungicidal, laxative, antidiuretic, sedative and antioxidant activities). The essential oil of this plant can be applied in flavouring condiments, relishes, soups, sausages, canned meats and in spicy table sauces.

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