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Effect of Different Ontogenesis Conditions on Essential Oil Composition of *Satureja Hortensis* L. Cultivated in Iran

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Farshad Sadeghi Ghotbabadi, Ardalan Alizadeh, Masood Zadehbagheri, Mohammad Mojtaba Kamelmanesh, and Majid Shaabani; Effect of Different Ontogenesis Conditions on Essential Oil Composition of *Satureja Hortensis* L. Cultivated in Iran

ABSTRACT

The essential oils were isolated by hydro-distillation from the aerial parts of *Satureja hortensis* at different ontogenesis conditions (pre-flowering, full-flowering and post-flowering stage). The highest oil yield was obtained in full-flowering (2.78%) and post-flowering (2.53%) than pre-flowering (1.95%) stage. The oils were analyzed by capillary GC and GC–MS. In total, 28 components were identified in the essential oil of *S. hortensis* under different ontogenesis conditions, that represented 98.28% - 98.87% of the oils. The major components were carvacrol (46.78-65.54%), γ-terpinene (21.63-32.25%), *P*-cymene (2.85-5.87%) and α-terpinene (2.42-4.73). The results show that different ontogenesis conditions caused significant effect on essential oil yield and composition of *S. hortensis*.

Key words: Summer Savory, Essential oil, GC/MS, Harvest time.

Introduction

The use of traditional herbs and medicinal plants has recently become very popular because they contain large amounts of natural products with biological properties. Plants are now one of the important sources of new pharmaceuticals and healthcare products [13].

The genus *Satureja* (Lamiaceae) consistitutes about 200 species of herbs and shrubs, often aromatic, widly distributed in Mediterranean areas, Asia and boreal America [9].

Satureja hortensis L. is an annual, herbaceous aromatic and medicinal plant that widely cultivated in many parts of the world [18]. This plant is traditionally used as carminative, digestive, antispasmodic and antitussive in Iran [23]. The aerial parts of some *Satureja* plants have been widely used in foods for herbal tea and flavor component and in folk and traditional medicine, to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases [7,10,11,23].

The main constituents of the essential oil of *S. hortensis* are the phenols, carvacrol and thymol, as well as *p*-cymene, β-caryophyllene, linalool and other terpenoids [2,5,6,16,18,19].

The objective of this study was to investigate the changes in essential oil components of *S. hortensis*, under different ontogenesis conditions.

Materials and Methods

Plant Material:

Seed of *S. hortensis* was obtained from Research Institute of Forests & Rangelands Tehran, Iran and were grown in Medicinal and Aromatic Plants Garden in Estahban branch, Islamic Azad University. Aerial parts of *S. hortensis* were collected at three different ontogenesis (pre-flowering, full-flowering and post-flowering) conditions. The harvested plants were dried at room temperature (25°C) for 2 weeks, then, air-dried plants (in each harvesting time) were ground and powdered with mixer for essential oil extraction.

Essential Oil Extraction:

Essential oil was obtained from dried aerial parts (100g) from *S. hortensis* by hydrodistillation using

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Clevenger- type apparatus according to the method recommended in British Pharmacopoeia [8] during 3 h. The distilled dark-yellow essential oil was dried with anhydrous sodium sulfate. Then, the oil was weighed and stored in tightly closed dark vials at 4 °C until analysis.

Essential Oil Analysis:

Gas chromatography (GC):

The analysis of the essential oils was performed on a Agilent technologies model (6990 USA) series II gas chromatograph equipped with flame ionization detector and capillary column DB-5 (30 m ×0.25 mm, 0.25 µm film thickness). The chromatographic conditions were as follows: The oven temperature increased from 60 ºC to 240 ºC at a rate of 3 ºC/min. The injector and detector temperatures were 240 ºC and 250 ºC, respectively. Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s. Injection volume of samples were 2 µl at 1:20 split ratio and quantitative data was obtained from electronic integration of peck areas without the use of correction factors.

Gas Chromatography- Mass Spectrometry (GC/MS):

The analysis of the essential oils was performed on a GC–MS Hewlett-Packard (model 6890 series II) inert MSD (Agilent Technologies, J&W Scientific Products, Palo Alto, CA, USA), quipped with an Agilent Technologies capillary DB-5MS column (30 m length; 0.25 mm i.d.;0.25 mm film thickness), and coupled to a mass selective detector (MSD5975B, ionization voltage 70 eV; all Agilent, Santa Clara, CA). The carrier gas was Helium and was used at linear velocity of 32 cm/s. The oven temperature program was as follows:1 min at 60 °C ramped from 60 to 250 °C at 3 °C min_1 and 10 min at 260 °C. The chromatograph was equipped with a split/split less injector used in the split mode. The split ratio was 1:50. Identification of components was assigned according to the Van Den Doll method using n-alkanes as standard [20]. The compounds were identified by comparison of retention indices (RRI-HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and mass finder 3 libraries or with the published mass spectra [1].

Statistical Analysis:

All data were expressed as mean ± standard deviation. Analysis of variance was performed by ANOVA by the software SAS (version 9.2 for windows). Significant differences between means were determined by Duncan's new multiple-range test. A significant difference was considered at the level of $P < 0.05$.

Results and Discussion

The essential oil isolated by hydrodistillation of the aerial part of *S. hortensis*, under different ontogenesis conditions, was found to be a yellow oil, obtained in yield of 1.95 - 2.78 (w/w), based on dry weight. The components are listed in order of their elution on the DB-5 column. The yield of essential oil in different harvesting times was in order of: flowering (2.78%) > post-flowering(2.53%) > pre-flowering (1.95) stage. The result show that different ontogenesis conditions caused significant affect on *S. hortensis* essential oil yield (Table 1). According to this result, recent studies of some plants from lamiaceae family shown that, the highest oil yield was obtained in flowering stage in these plants [4,14,15,17]. In total, 28 components were identified in the essential oil of *S. hortensis* under different harvesting times, that represented 98.28% - 98.87% of the oils. The major components were carvacrol (46.78-65.54%), γ-terpinene (21.63-32.25%), *P*-cymene (2.85-5.87%) and α-terpinene (2.42-4.73) (Table 3).

This finding is similar to the results of previous research in *S. hortensis* essential oil [2,5,6,16,18,19], but the amount of some components are different, These differences in the essential oil compositions can be attributed to several environmental factors such as climatic, seasonal and geographical or ontogenesis variations. The results depicted in Table 3 show that, ontogenesis conditions in *S. hortensis* caused significant change in main components on the essential oil. The highest carvacrol content as major phenolic compounds was observed in the pre-flowering and the lowest in the post-flowering and full-flowering stage. In contrast, the highest γ-terpinene and *p*-cymene contents as precursors of carvacrol and thymol were observed in post-flowering and full-flowering and and the lowest in pre-flowering stage, but not significant differences are there in the total amount of the main components in different ontogenesis condition (Table, 3). Also we have found a high correlation between carvacrol and γ-terpinene (0.93), carvacrol and *p*-cymene (0.90) and γ-terpinene and *p*-cymene (0.84) (Table, 4). This results according to carvacrol and thymol biosynthesis pathway. Mikio and Taeko, [12] and Yamaura *et al*., [21], proposed that carvacrol biosynthesis pathway renders as follows: γ-terpinene is the component involved in the aromatization process which results in the formation of *p*-cymene, the precursor of possible oxygenated derivatives, thymol or carvacrol. Thymol and carvacrol are structurally very similar, having the hydroxyl group at a different location on the phenolic ring. It may be assumed that the sequence in this process is as follows: γ-terpinene, *p*-cymene, thymol or carvacrol

(Fig.1). Alizadeh *et al*, [2,3] and Yanive and Palevitch [22] showed that different ontogenesis conditions and environmental factors influence on qualitative and quantitative characteristics of active substances in medicinal and aromatic plants.

Table 1: Simple ANOVA between traits in *S. hortensis*.

Traits		Essential oil yield (%W)	Carvacrol (%)	γ-Terpinene (%)	*P*-Cymene (%)	*α*-Terpinene (%)
Sov	df			MS		
Rep.	3	0.02 ^{ns}	6.45 *	0.32^{ns}	0.04 ^{ns}	0.04 ^{ns}
Treatment	2	0.54 **	266.89 **	84.86**	8.08 **	4.57 **
Error	6	0.01	1.87	2.51	0.21	0.15
CV%		4.84	2.46	5.93	9.65	11.68

Ns: Not significant. * : Significant at 5% probability. ** : Significant at 1% probability.

Table 2: Essential oil compositions in *S. hortensis* under different ontogenesis conditions.

No	Compound	RI[a]	Pre- Flowering	Full-Flowering	Post- Flowering
1	α-Thujene	928	0.50±0.12	0.80±0.02	1.12 ± 0.33
2	α-Pinene	938	0.40±0.11	1.13±0.26	0.92 ± 0.26
3	Camphene	950	0.05±0.01	1.10±0.23	0.07 ± 0.02
4	Sabinene	978	0.08±0.02	0.07±0.02	0.05 ±0.01
5	β-Pinene	980	0.40±0.14	0.22±0.09	0.72 ± 0.22
6	Myrcene	990	1.33±0.25	0.64±0.21	1.21 ± 0.37
7	α-Phellandrene	1004	0.21±0.08	1.77±0.45	0.33 ± 0.08
8	δ-3-Carene	1010	0.02±0.01	0.24±0.12	0.13 ± 0.03
9	α-Terpipene	1017	2.82±0.32	2.42±0.47	4.73 ± 0.87
10	ρ-Cymene	1025	2.85±0.41	5.47±1.13	5.87 ± 1.23
11	β-Phellandrene	1030	0.05±0.01	0.46±0.19	0.38 ±0.17
12	(E)-β-Ocimene	1050	0.07±0.02	0.08±0.02	0.09 ± 0.02
13	γ-Terpinene	1060	21.63±2.23	26.37±3.34	32.25± 3.53
14	Cis-Sabinene hydrate	1061	0.13±0.04	0.30±0.18	0.43 ± 0.25
15	α-Terpinolene	1089	0.08±0.02	0.31±0.21	0.42 ± 0.18
16	Linalool	1098	0.12±0.03	0.18±0.11	0.21 ± 0.11
17	Camphor	1144	0.12±0.03	0.20±0.09	0.16 ± 0.09
18	Borneol	1164	0.05±0.01	0.06±0.01	0.07 ± 0.02
19	Terpinen-4-ol	1177	0.22±0.09	0.34±0.16	0.42 ± 0.18
20	Thymyl methyl ether	1189	0.05±0.01	0.07±0.02	0.06± 0.02
21	Thymol	1290	0.12±0.05	0.19±0.08	0.58 ± 0.23
22	Carvacrol	1299	65.54±3.38	54.73±3.18	46.78 ± 0.41
23	Carvacryl acetate	1352	0.05±0.01	0.07±0.02	0.06 ± 0.02
24	β- Caryophyllene	1419	0.34±0.12	0.86±0.45	0.94 ± 0.28
25	Bicyclogermacrene	1441	0.42±0.16	0.43±0.23	0.52 ± 0.24
26	B -Bisabolene	1454	0.24±0.11	0.13±0.07	0.08 ± 0.03
27	E(α-)Bisabollen	1492	0.31±0.17	0.16±0.09	0.15± 0.06
28	Caryophyllene oxide	1507	0.08±0.01	0.07±0.02	0.06 ± 0.03
	Oil Yield [b] (%w/w)		1.95 b	2.78 a	2.53 a
	Total		98.28%	%98.87	98.81%

[a] RI, retention indices in elution order from HP-5 column. [b] Oil Yield, Values followed by the row, is significantly different ($p > 0.01$).
Each value in the table was obtained by calculating the average of four experiments ±standard deviation. Data expressed as percentage of total.

Table 3: Effects of different ontogenesis conditions on major components on the essential oil of *S. hortensis*.

Harvesting Times	Carvacrol (%)	γ-Terpinene (%)	*P*-Cymene (%)	α-Terpipene (%)	Total
Pre Flowering Stage	65.54 a	21.63 b	2.85 b	2.82 b	92.84 a
Flowering Stage	54.73 b	26.37 ab	5.47 a	2.42 b	88.99 a
Post Flowering Stage	46.78 c	32.25 a	5.87 a	4.73 a	89.63 a

Each value in the table was obtained by calculating the average of four experiments ± standard deviation.
In each column, means with the same letters are not significantly different at 1% level of Duncan's new multiple range test.

Table 4: Pearson correlation between traits in *S. hortensis*.

Traits	Essential oil Yield (%W)	Carvacrol (%)	γ-Terpinene (%)	*P*-Cymene (%)	α-Terpipene (%)
Essential oil Yield	---				
Carvacrol	0.68*	---			
γ-Terpinene	0.54 ^{ns}	0.93 **	---		
P-Cymene	0.82 **	0.90**	0.84 **	---	
α-Terpipene	0.45 ^{ns}	0.66 *	0.76*	0.47 ^{ns}	---

Ns: Not significant. * : Significant at 5% probability. ** : Significant at 1% probability.

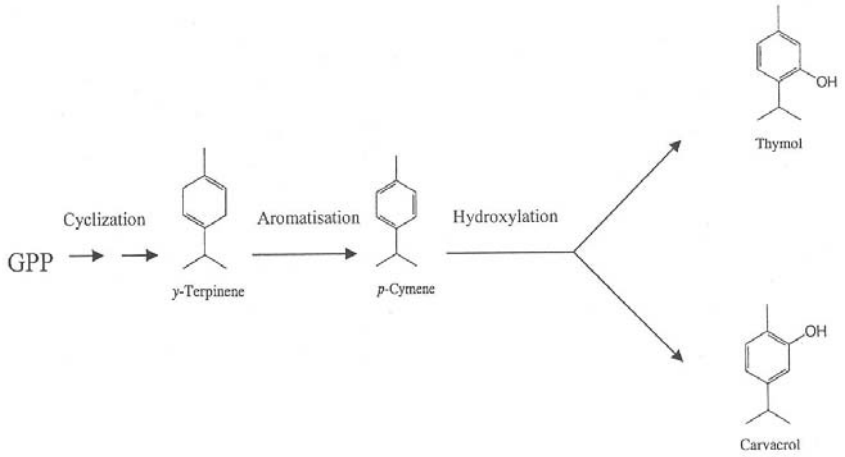

Fig. 1: Thymol and carvacrol biosynthesis pathway [12].

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