

CHEMICAL POLYMORPHISM OF *SATUREJA HORTENSIS* L. ESSENTIAL OILS DURING DIFFERENT PHENOLOGICAL STAGES AND VEGETATION CYCLES

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Abstract:

Chemical polymorphism is common in the *Lamiaceae* family, where one or more compounds differ quantitatively within the phenological phases, thus modifying the chemotype according to the major component determined in the essential oil. Thymol and carvacrol are the most common chemotypes present in thyme plants, they have the same molecular weight ($M = 150 \text{ g.mol}^{-1}$), but with a different position of the OH group on the phenolic ring (meta and ortho). Usually, thymol and carvacrol are accompanied by two precursors: *p*-cymene and γ -terpinene. Depending on the end purpose of the essential oil and the desired chemical composition, there is a need for specific analysis of the chemical variations according to the phenological stages. For this purpose, essential oils were obtained and analyzed by GC-MS, in two phenological phases, before and after flowering over two years, and their chemical composition was compared.

The main chemical compounds of the four essential oils analyzed were: γ -terpinene, carvacrol, *p*-cymene, α -terpinolene, β -pinene, etc.. The chemical composition and chemotype of the essential oils, can vary and depends on many factors like variety, cultivation area and the phenological phase when harvesting is taking place.

Keywords: essential oils, phenological stages, *Lamiaceae*, summer savory, GC-MS

INTRODUCTION

The *Lamiaceae* or *Labiatae* family comprises a large group of herbaceous, shrubs or subshrub perennial or annual plants. They are strongly aromatic and produce a wide variety of secondary compounds, being mostly recognised for essential oils secreted by glandular trichomes on the surface of leaves and inflorescences (Avram, 1974; Harley et al., 2004; Moisă et al., 2018a, 2018b; Rhnid, 2012; V. Heywood et al., 2007).

This family includes 236 genres and numbers about 7,173 species and is divided into several subfamilies (Harley et al., 2004). Essential oil production is not a general feature of the whole family, but is specific to the subfamily of *Nepetoideae*, consisting mainly of monoterpenoid - menthone in *Mentha*, thymol in *Thymus*, carvacrol in *Satureja*, linalool in *Origanum*; sesquiterpenoids - caryophyllene and eugenol in *Ocimum* (Harley et al., 2004).

Volatile oils obtained from members of the *Lamiaceae* family (lavender, thyme, oregano, mint, sage, etc.) have a high pharmacological potential in the control of bacterial infections (Aguar et al., 2016; Gustavo, 2016; Tripathi et al., 2011).

However, chemical polymorphism is common in the *Lamiaceae* family, when one or more compounds differ quantitatively within the phenological phases, thus modifying the chemotype according to the major component determined in the essential oil (Clarke, 2009; Thompson et al., 1998). Also, there are many differences in the essential oils chemical composition of specific plant organs: leaves, stems, flowers, roots, and bark (Moisa et al., 2018).

For this purpose, essential oils were obtained and analyzed by GC-MS, in two phenological stages, before and after flowering, and their chemical composition was compared.

MATERIALS AND METHODS

Plant material

Aerial plant parts (leaves and inflorescences) of *Satureja hortensis* L. were gathered in two different phenological phases: in late June before flowering and in July after flowering throughout two different vegetation cycles (2017 and 2018) from a local producer in Arad.

Harvested plant material was dried in a ventilated room, avoiding direct sunlight, for two weeks. Dried herba was stored in brown paper bags until further processes were applied. Essential oil extraction and GC-MS analysis were performed. For each phenological phase, voucher specimens were numbered and cataloged.

Essential oil extraction

Leaves and flowers were separated from the aerial plant parts and were subjected to classical hydro-distillation using a 0.5 L Clevenger installation.

The essential oil and aromatic water mixture were separated using a separating funnel, and the collected essential oils were stored at 4 °C in dark glass vials until further GC-MS analysis. Extraction yields have been calculated.

GC-MS analysis

The chemical constituents for all the obtained essential oils were determined by gas chromatography (GC-MS) (Shimadzu 2010, Kyoto, Japan) coupled with a mass spectrometer (TQ 8040, Shimadzu, Kyoto, Japan). The analysis method and parameters were as previously presented by (Moisa et al., 2019).

All significant compounds have been identified by their mass spectra using NIST 14 library and Wiley 09 library.

RESULTS AND DISCUSSIONS

Essential oil composition

The essential oil extraction yields, before and after flowering stages, were calculated and better results were recorded after the flowering stage. In 2017 before flowering the yield was ~0.9% and after flowering, it was ~1.9%, in 2018 the situation was similar with 1.3% before and 2.3% after flowering. The essential oils chemical compositions are presented in table 1.

Table 1. *Satureja hortensis* L. essential oils chemical composition in different phenological stages and vegetation cycles

Nr. Crt	Compound	2017		2018	
		BF %	AF %	BF %	AF %
1	α -Thujene	1.17	1.51	0.96	1.53
2	α -Pinene	0.47	0.95	0.58	1.1
3	Camphene	0.13	0.22	0.16	0.22
4	Sabinene	0.27	0.43	0.42	0.54
5	1-Octen-3-ol	-	0.14	-	-
6	β -Pinene	1.49	1.98	2.01	1.83
7	α -Phellandrene	0.3	0.42	0.45	0.53
8	3-Carene	0.1	0.17	-	-
9	α -Terpinolene	4.16	4.49	4.41	3.68
10	<i>p</i> -Cymene	3.59	5.55	6.22	7.08
11	D-Limonene	0.49	0.73	0.65	0.13
12	<i>trans</i> -beta-Ocimene	0.15	0.10	0.35	0.15
13	γ -terpinene	40.14	44.9	50.45	37.31
14	<i>cis</i> -Sabinene hydrate	0.27	0.69	0.41	0.66
15	α -Terpinolen	0.07	0.19	-	-
16	<i>trans</i> -Sabinene hydrate	0.07	0.39	-	-
17	Terpinen-4-ol	0.14	0.34	0.42	0.33
18	α -terpineol	0.08	0.08	-	-
19	Carvacrol methyl ether	0.18	0.47	0.15	0.12
20	Dihydroedulan	-	-	0.21	-
21	Thymol	0.17	0.07	0.16	-
22	Carvacrol	43.03	31.02	24.14	41.34
23	β -Bourbonene	-	-	0.15	-
24	Caryophyllene	1.87	3.47	1.77	2.16
25	α -Humulene	0.06	0.20	0.17	0.15
26	Germacrene D	0.27	0.26	2.7	-
27	γ -Elemene	-	-	0.5	0.26
28	β -Bisabolene	1.15	1.10	0.94	-
29	α -Farnesene	-	-	0.36	0.6
30	δ -Cadinene	-	-	0.24	-
31	α -Bisabolene	0.18	0.13	0.18	0.02
32	Isogermacrene D	-	-	0.19	0.26
33	<i>cis</i> -Carveol	-	-	0.12	-

BF – before flowering, AF – after flowering

Between all four essential oils, a number of 33 compounds were identified, with 18 being present in all phenological phases. Two major compounds make around 70-80% of the total composition, γ -terpinene, and carvacrol, exchanging the oils chemotype between phenological phases.

As depicted in figure 1, in 2017, carvacrol is the primary compound found in the essential oil before flowering with 43.03% and decreases to 31.02% after flowering. In contrast, most compounds increased percentage after flowering: γ -terpinene (40.14% to 44.9%) becoming the dominant compound after flowering, *p*-cymene (3.59% to 5.55%), α -

terpinolen (4.16% to 4.49%) among many others with different percentages.

In 2018 (figure 2), γ -terpinene, before flowering, is the major compound, accounting for 50.45% of all constituents. Immediately after blooming, its content drops to 37.31%, leaving carvacrol to become the new major compound, increasing from 24.14% to 41.34%. The quantitative differences of the other compounds

are minor, some increasing or decreasing with small percentages, such as α -Terpinolene who dropped from 4.41% to 3.68% and Germacrene D from 1.77% before flowering to completely disappearing during flowering. The compounds that grew during the flowering stage were: α -Thujene, α -Pinene, *p*-Cymene, Caryophyllene.

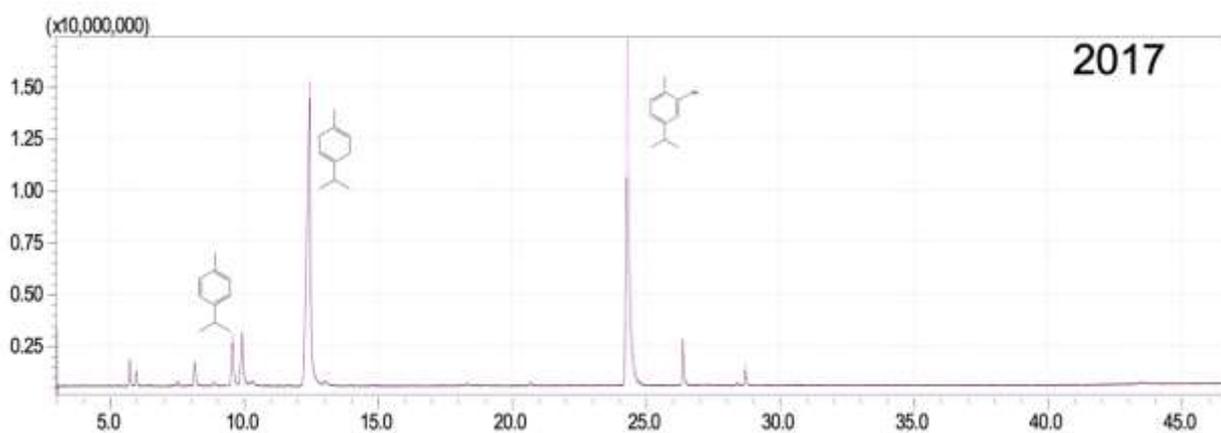


Figure 1. Overlapped chromatogram of *Satureja hortensis* L. essential oil from 2017 (pink - before flowering, black - after flowering)

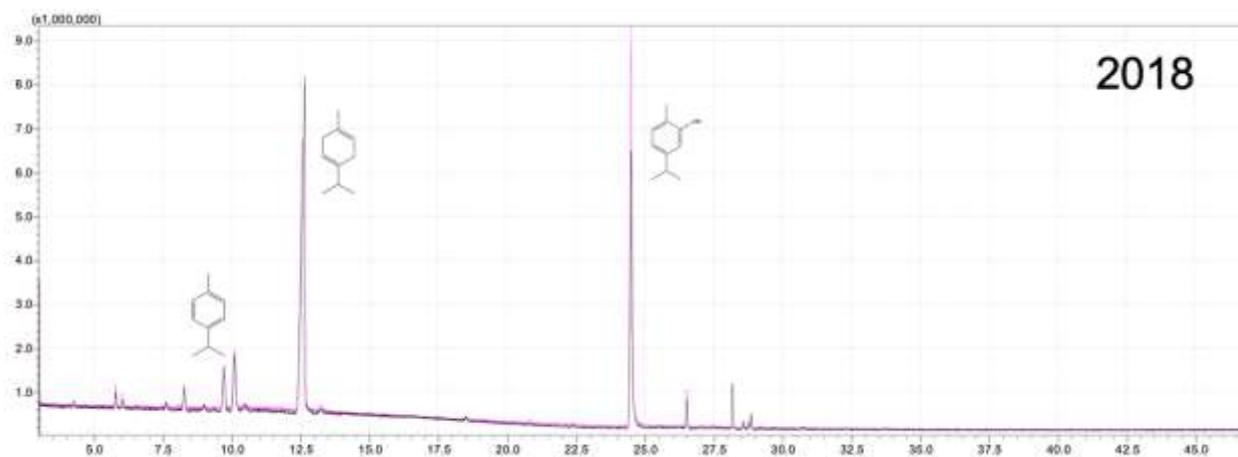


Figure 2. Overlapped chromatogram of *Satureja hortensis* L. essential oil from 2018 (pink - after flowering, black - before flowering).

The major compounds found in *Satureja hortensis* L. essential oils were: γ -terpinene 37.31 - 50.45%, carvacrol 24.14 - 43.03%, and *p*-cymene 3.59 - 7.08%.

For both vegetation cycles (2017 and 2018) and phenological stages (before and after flowering), the chemotype for *Satureja hortensis* L. oscillates between carvacrol and γ -terpinene.

Table 2. The chemotypes of essential oils studied for two years (2017 and 2018) and two phenological phases (before and after flowering).

Essential oil	Phenological stage	Chemotype
<i>Satureja hortensis</i> L. 2017	before flowering	carvacrol
	after flowering	γ -terpinene
<i>Satureja hortensis</i> L. 2018	before flowering	γ -terpinene
	after flowering	carvacrol

CONCLUSIONS

The main chemical compounds of the four essential oils analyzed were: γ -terpinene, carvacrol, *p*-cymene, α -terpinolene, β -pinene, etc.

The chemical composition and chemotype of the essential oils, can vary and depends on many factors like variety, cultivation area and the phenological phase when harvesting is taking place. Thus, for both vegetation cycles (2017 and 2018) and phenological stages (before and after flowering), the chemotype for *Satureja hortensis* L. oscillates between carvacrol and γ -terpinene.

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