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Influence of Harvest Term on the Content of Carvacrol, p-Cymene, γ-Terpinene and β-Caryophyllene in the Essential Oil of *Satureja montana*

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Abstract

The aim of the studies was to determine the optimal term of harvest for *Satureja montana* L. (winter savory) in order to obtain the essential oil rich in antioxidative compounds such as carvacrol, p-cymene, γ-terpinene and β-caryophyllene. Essential oils of *S. montana* aerial parts were obtained by hydrodistillation in Deryng-type apparatus and analyzed by gas chromatography/mass spectrometry (GC/MS). In total, 30 compounds were identified in the savory volatile oil under different harvesting terms (before flowering, during flowering and after the flowering), that represented 94.61 to 97.55% of the oils. The major components were carvacrol (65.43 to 69.99%), its precursors: p-cymene (3.69 to 9.69%) and γ-terpinene (1.51 to 5.92%) as well as β-caryophyllene (2.74 to 4.71%). Moreover, the term of harvest had a significant effect on the content of main essential oil constituents. The highest concentrations of carvacrol, γ-terpinene and β-caryophyllene were observed in the herb collected before flowering, while the highest amounts of p-cymene were noted after the flowering.

Keywords: essential oil composition, GC/MS, hydrodistillation, stage of plant development, winter savory

Introduction

The genus *Satureja* belongs to the family *Lamiaceae* that comprises numerous species growing wild in Mediterranean area. Among them, many are used worldwide as medicinal and spice plants (Vidic *et al.*, 2010).

Satureja montana L., commonly called winter or mountain savory, is a bushy perennial subshrub with woody stems at the base, linear leaves and pale pink flowers (Lawless, 2002). This aromatic plant can be found in nature, but it is also cultivated as a culinary herb having strong and spicy taste (Silva *et al.*, 2009). The leaves and flowering tops are used as flavoring agents in salads, soups, sauces, stews, and lentil dishes (Small and Deutsch, 2001). Savory is one of the best honey plants and its honey is well-known as folk remedy for bronchitis (Mastelic and Jerkovic, 2003). The whole plant is mildly antiseptic, carminative, digestive, expectorant and stomachic (Damjanovic-Vratnica *et al.*, 2011).

Essential oil, obtained from the whole herb, is rich in biologically active phytochemicals such as carvacrol, thymol, β-caryophyllene, γ-terpinene, p-cymene and linalool, which exhibit strong antioxidative activity (Ruberto and Baratta, 2000; Braga *et al.*, 2006). Antimicrobial activity of oil against pathogenic yeasts (*Candida albicans, Cryptococcus neoformas, Filobasidiella neoformas, Tricosporon cutaneum*) and spoilage yeasts (*Brettanomyces* sp., *Saccharomycodes ludwigii, Schizosaccharomyces octosporus, Zygosaccharomyces rouxii*) has been reported by Ciani *et al.* (2000). The antiviral, antispasmodic and antidiarrhoeal activity of savory's oil has been also documented (Yamasaki *et al.*, 1998; Hajhashemi *et al.*, 2000).

Winter savory oil is used in the food industry to flavor condiments, canned meats, sausages, soups, in the making of liqueurs and in the perfumery (Ciani *et al.*, 2000; Small and Deutsch, 2001). It is also applied as natural conservation agent in cosmetic and food industries and as active ingredient in medicinal preparations (Chorianopoulos *et al.*, 2004). According to Lawrence (1979), the value of *S. montana* oil is due to its high carvacrol content and its fresh, spicy notes reminiscent of oregano and thyme oils.

The application of savory oil in different industries as well as its quality and biological activity depends on the oil composition which is affected by vegetative stage of the plant (Kustrak *et al.*, 1996; Milos *et al.*, 2001; Slavkovska *et al.*, 2001).

The aim of this work was to determine the optimal term of harvest for winter savory herb to gain the oil with high content of biologically active compounds, such as carvacrol, p-cymene, γ-terpinene and β-caryophyllene.

Tab. 1. Meteorological data for the period of *Satureja montana* L. growing in 2010-2011

Years	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Mean daily air temperature (°C)												
2010	-5.6	-0.4	4.0	8.8	11.1	16.5	21.7	18.5	13.1	7.6	4.8	-4.6
2011	0.9	-1.0	3.8	11.6	14.2	17.8	17.6	18.0	15.1	9.8	4.3	4.4
Total rainfall (mm)												
2010	35.1	22.6	42.8	26.7	74.9	21.2	62.6	172.4	54.6	32.3	109.2	61.1
2011	22.9	31.7	27.7	16.9	37.1	49.8	184.6	51.7	76.5	52.6	11.0	77.5
Insolation (h)												
2010	27.6	34.5	82.1	213.9	115.5	301.0	334.7	151.5	144.7	119.9	10.0	14.5
2011	35.6	88.3	161.2	225.5	309.5	300.6	186.0	204.4	180.2	129.2	87.0	23.8

Materials and methods

Plant material

The experiment was conducted at the Horticultural Experimental Station near Szczecin (north-western Poland), which belongs to the West Pomeranian University of Technology Szczecin. The plants of *Satureja montana* L. (winter savory) were grown in experimental plots of area of 1.44 m^2, in four replications.

The savory seeds (purchased from Herb Factory 'Kawon-Hurt', Gostyń, Poland) were sown on seedbed at the first decade of April 2008-2009. In the same years, the obtained seedlings were planted into the open field in the first decade of June, at row spacing of 40×30 cm. For laboratory analyses a herb from two-year old plants was collected before flowering (harvest dates: 11 July 2010 and 13 July 2011), during flowering (harvest dates: 6 August 2010 and 5 August 2011), and after the flowering (harvest dates: 13 September 2010 and 15 September 2011). The field was prepared according to agrotechnique proper for perennial plants from *Lamiaceae* family. Mineral fertilization was quantified according to the results of the chemical analysis of the soil samples. In the first year nitrogen fertilizer (80 kg N ha^{-1}) was applied in three equal doses: ⅓ before sowing, ⅓ three weeks after sowing and ⅓ after harvest. Phosphorous fertilizer (60 kg P_2O_5 ha^{-1}) and potassium fertilizer (100 kg K_2O ha^{-1}) were applied once, during spring cultivation treatment. In the second year of cultivation the whole phosphorous fertilizer and potassium fertilizer were applied once (on early spring) before plants vegetation. Nitrogen fertilizer was applied in two doses: ½ before plants vegetation and ½ after harvest.

The experiment was performed on sandy clay soil which is characterized by low water-holding capacity. During the growing season manual weeding and irrigation were performed.

After the harvest, plant material was dried in a shady and well ventilated place at room temperature (drying room). Dry herb was cut into small pieces and stored (in paper bags in a dry and cool place) until chemical analyses were performed.

Essential Oil Extraction

The essential oil was isolated from dry winter savory herb (collected before flowering, during flowering and after the flowering, separately) by hydrodistillation for 4 hours (after which no more essential oil was obtained) using a Deryng-type apparatus (Polish Pharmacopoeia VI, 2002). Each sample (30 g) was placed in 1 liter round-bottomed flask containing 500 mL of distilled water. The measurements of the distillation time started after the falling of the first drop of distillate. The essential oil obtained this way was separated from water (due to its immiscibility with water) and then dried over anhydrous sodium sulphate, filtered, and stored in sealed vial at 4 °C until GC-MS analysis.

Tree replicates were carried out. Essential oil percentage was calculated based on dry weight of plant material and expressed as (% v/w) in Tab. 2.

Gas Chromatography/Mass Spectrometry (GC/MS) analyses of essential oils

The qualitative GC-MS analysis of the volatile oils was performed using an HP 6890 gas chromatograph coupled with HP 5973 Mass Selective Detector operating at 70 eV mode. Compounds were separated on 30 m long capillary column (HP-5MS), 0.25 mm in diameter and with 0.25 μm thick stationary phase film ((5% phenyl)-methylpolysiloxane).

The initial temperature of the column was 40 °C for 5 minutes, then increased to 60 °C at a rate of 20 °C min^{-1}, next to 230 °C at a rate of 5 °C min^{-1} (kept constant for 20 min), and then increased to a final temperature of 280 °C at a rate of 10 °C min^{-1}.

The flow rate of helium through the column was kept at 1.2 mL min^{-1}. Samples of 2 μL (30 mg of oil dissolved in 1.5 mL of dichloromethane) were injected with a split ratio of 5:1. The temperatures of the injector, transfer line and ion source were maintained at 250, 280 and 230 °C, respectively. The solvent delay was 3 min. The scan range of the MSD was set from 40 to 550 m/z. The total running time for a sample was 65 minutes.

The identification of the components in the sample was based on the computer matching with the Wiley NBS75K.L and NIST/EPA/NIH (2002 version) mass spectral libraries, as well as by comparison of their mass spectra with authentic compounds available in our laboratory (thymol, carvacrol and p-cymene), purchased from Fluka and Sigma-Aldrich. The identity of compounds was also confirmed by retention indices from literature data (Adams, 2007; Cavar *et al.*, 2008).

Retention indices (RI) values were measured on HP-5MS column. For RI calculation, a mixture of n-alkanes (C_8-C_{20}) was used, under the same chromatographic conditions which were used for the analysis of the essential oils.

The relative percentage amounts of the essential oil constituents were evaluated from the total peak area (TIC) by apparatus software.

Statistical analysis

Several results of the study (Tab. 2, 4) were subjected to an analysis of variance which was performed with AWAR software, made by Department of Applied Informatics,

Tab. 2. Essential oil content in *Satureja montana* L. according to the term of harvest

Term of harvest	Essential oil content (% v/w)		
	2010	2011	Mean
Pre-flowering	1.93±0.25	1.93±0.45	1.93
During flowering	2.37±0.33	2.17±0.26	2.27
Post-flowering	1.67±0.05	1.70±0.22	1.69
Mean	1.99	1.93	1.96
LSD $_{\alpha=0.05}$	0.668	n.s.	0.494

± standard deviation (n=3)
n.s. – not significant

Tab. 3. Relative percentage composition of *Satureja montana* L. essential oil in dependence on the term of harvest (2010-2011)

Compounds	RI	Term of harvest					
		Pre-flowering		During flowering		Post-flowering	
		2010	2011	2010	2011	2010	2011
α-Thujene	932	0.14	0.18	0.21	0.33	0.25	0.23
α-Pinene	938	0.39	0.45	0.43	0.49	0.44	0.40
β-Pinene	982	0.29	0.11	0.11	0.14	0.13	0.11
β-Myrcene	997	1.14	1.15	0.68	0.68	0.39	0.42
α-Phellandrene	1010	0.22	0.21	0.15	0.14	0.08	0.08
α-Terpinene	1023	1.23	1.32	0.97	0.98	0.49	0.48
p-Cymene	1030	3.69	4.22	8.65	8.01	9.69	9.48
m-Cymene	1036	1.85	1.61	2.12	1.50	2.08	2.64
γ-Terpinene	1065	5.68	5.92	3.48	2.78	1.51	1.92
(E)-Sabinene hydrate	1075	0.38	0.53	0.56	0.75	0.34	0.61
(Z)-Sabinene hydrate	1096	0.24	0.16	0.14	0.12	0.12	0.11
Borneol	1176	0.14	0.15	0.28	0.17	0.34	0.37
m-Cymen-8-ol	1183	0.70	-	-	-	-	-
Terpinen-4-ol	1188	0.57	0.55	0.57	0.47	0.65	0.67
α-Terpineol	1202	0.14	0.15	0.16	0.15	0.19	0.25
Thymoquinone	1260	-	-	1.39	0.78	2.86	2.46
Thymol	1305	0.50	0.23	0.33	0.15	0.14	0.22
Carvacrol	1318	69.99	69.54	66.20	68.65	66.65	65.43
β-Bourbonene	1387	0.40	0.68	0.74	0.78	0.59	0.64
β-Elemene	1397	0.20	0.68	0.64	0.95	0.38	0.54
β-Copaene	1422	0.30	0.41	0.35	0.64	0.20	0.19
β-Caryophyllene	1433	4.71	4.65	3.37	4.36	2.74	2.89
Aromadendrene	1453	0.51	0.60	0.48	0.68	0.24	0.30
α-Caryophyllene	1467	0.24	0.30	0.29	0.33	0.10	0.19
γ-Muurolene	1488	0.23	0.31	0.46	0.48	0.35	0.31
α-Selinene	1502	0.15	0.09	0.13	0.18	0.09	0.08
Virdiflorene	1509	0.54	0.48	0.48	0.63	0.30	0.34
β-Bisabolene	1519	1.41	0.73	0.47	0.49	0.61	0.80
γ-Cadinene	1527	0.48	0.38	0.46	0.45	0.38	0.38
δ-Cadinene	1535	0.59	0.59	0.75	0.83	0.49	0.47
Caryophyllene oxide	1600	0.45	0.31	1.29	0.46	1.80	1.60
Total identified		97.50	96.48	96.34	97.55	94.62	94.61
Monoterpene hydrocarbons		14.63	14.96	16.80	15.05	15.06	15.76
Oxygenated monoterpenes		72.66	71.31	69.63	71.24	71.29	70.12
Sesquiterpene hydrocarbons		9.76	9.90	8.62	10.80	6.47	7.13
Oxygenated sesquiterpenes		0.45	0.31	1.29	0.46	1.80	1.60

RI: retention indices relative to n-alkanes (C_8-C_{20}) on HP-5MS column

Institute of Soil Science and Plant Cultivation in Puławy. The means of two years were separated by the Tukey's test at p=0.05.

Results and discussions

The essential oil isolated by hydrodistillation from plant material collected at different growth stages (pre-flowering, during flowering and post-flowering) was found to be yellow liquid with strong, characteristic smell. As shown in Tab. 2, the term of harvest had a significant effect on the content of essential oil in *Satureja montana* L. herb. Statistical analysis of the results obtained in the first year of the study as well as the synthesis of the two year study results showed that *Satureja montana* L. herb collected during the flowering phase and before flowering was characterized by significantly higher concentration of the essential oil in comparison with the herb collected after plant flowering. However, the differences between the content of essential oil in herb collected before and after flowering were not significant. In the second year of the study there were no significant differences found between the content of the essential oil according to the term of harvest.

Available literature data indicates that the essential oil content in *S. montana* herb varies depending on the place of origin. Skocibuasic and Bezic (2004) reported that the content of essential oil in some Croatian winter savories varied from 1.2 to 2.2%. In the study conducted by Damjanovic-Vratnica *et al.* (2011), the content of essential oil in the herb of *S. montana* from Montenegro ranged from 1.1 to 1.9%. Savory plants collected from north part of Albania (Ibraliu *et al.*, 2010) contained from 0.22 to 1.61% of essential oil, while plants harvested in the central part of Italy contains 0.59% of oil (Fraternale *et al.*, 2007). The content of essential oil in our plants, especially in these collected during flowering stage, was higher compared to cited literature. Only winter savory cultivated in Egypt (Hassanein *et al.*, 2014) had higher essential oil content (about 3%), in contrast to our results.

The chemical composition of isolated essential oil is shown in Tab. 3. The components are listed in order of their elution on the HP-5MS column.

Thirty compounds accounting for 96.48 to 97.50% of the total composition were identified in pre-flowering stage. The major components were carvacrol (69.99 and 69.54% in 2010 and 2011, respectively), γ-terpinene (5.68 and 5.92%), β-caryophyllene (4.71 and 4.65%) and p-cymene (3.69 and 4.22%). In the volatile oil obtained from the flowering stage, thirty components were characterized, which represented 96.34-97.55% of the total composition. Carvacrol (66.20 and 68.65%), p-cymene (8.65 and 8.01%), β-caryophyllene (3.37 and 4.36%) and γ-terpinene (3.48 and 2.78%) were the principal components of this oil.

In the oil isolated from plants collected after the flowering, thirty constituents accounting for 94.62% of the total oil were characterized that included carvacrol (66.65 and 65.43%), p-cymene (9.69 and 9.48%), β-caryophyllene (2.74 and 2.89%) and γ-terpinene (1.51 and 1.92%).

The investigated essential oils consisted mainly from oxygentated monoterpenes (69.63-72.66%), monoterpene hydrocarbons (14.63-16.80%) and sesquiterpene hydrocarbons (6.47-10.80%). Oxygenated sesquiterpenes were present in very low amounts (0.31-1.80%) (Tab. 3).

The obtained results shows that the savory oils obtained from plants collected at different growth stages had similar composition, however, thymoquinone was found only in the oils obtained from savory herb collected during (1.39 and 0.87%) and after the flowering (2.86 and 2.46%) stage.

Because of high content of p-cymene, γ-terpinene, carvacrol and β-caryophyllene in the all oil samples, these major components were analyzed statistically (Tab. 4).

Statistical analysis of the results given in Tab. 4 showed that, in the first and the second year of the study as well as synthesis of both years of the study, that among four main constituents of *Satureja montana* L. essential oil, the highest concentration was noted for carvacrol, significantly lower for p-cymene and the least for β-caryophyllene and γ-terpinene. Moreover, it was found that the content of these components differed significantly according to the stage of plant development. Significantly higher amounts were assessed for herb collected before and during the flowering while the least – for the plant material collected after the flowering. However, the analysis of the interaction between the experimental factors gave us more detailed information. In the case of γ-terpinene, carvacrol and β-caryophyllene significantly higher concentrations were assessed for the herb collected before flowering and the least – after the flowering, while in the case of p-cymene the highest amounts were noted after the flowering and the least – before flowering.

According to literature data, the composition of *Satureja montana* L. oil shows large variations in the relative concentration of major components: carvacrol, linalool, γ-terpinene, p-cymene and β-caryophyllene, depending on the geographic origin and existence of different chemotypes (Cazin *et al.*, 1985).

The essential oil extracted from *S. montana* grown in central Italy (Fraternale *et al.*, 2007) contained carvacrol (18.00%), p-cymene (14.30%), thymol (9.92%), β-phellandrene (5.60%), β-caryophyllene (4.97%), carvacrol methyl ether (4.86%) and linalool (4.81%) as the main components. In the oils isolated from plant material collected from two different localities in Bosnia and Herzegovina (Cavar *et al.*, 2008), thymol (3.8-31.7%), carvacrol (10.6-23.3%), geraniol (0.1-22.3%) and caryophyllene oxide (5.2-7.7%) were found as the most abundant components. Volatile oil isolated from aerial parts of winter savory growing wild at Biokovo Mountain in Croatia (Cavar *et al.*, 2013) contained carvacrol (63.4%), thymol (19.4%) and borneol (4.2%) as the main

Tab. 4. Relative percentage composition of the main *Satureja montana* L. essential oil constituents in dependence on the term of harvest

Essential oil constituent (I)	Term of harvest (II)											
	2010				2011				2010-2011			
	Pre-flowering	During flowering	Post-flowering	Mean	Pre-flowering	During flowering	Post-flowering	Mean	Pre-flowering	During flowering	Post-flowering	Mean
p-Cymene	3.69±0.04	8.65±0.19	9.69±0.19	7.34	4.22±0.23	8.01±0.59	9.48±0.13	7.23	3.96	8.33	9.59	7.29
γ-Terpinene	5.68±0.08	3.48±0.18	1.51±0.34	3.56	5.92±0.24	2.78±0.16	1.92±0.02	3.54	5.80	3.13	1.72	3.55
Carvacrol	69.99±0.44	66.20±0.22	66.65±0.96	67.61	69.54±0.40	68.65±0.98	65.43±0.76	67.87	69.77	67.43	66.04	67.74
β-Caryophyllene	4.71±0.02	3.37±0.07	2.74±0.03	3.60	4.65±0.10	4.36±0.08	2.89±0.11	3.97	4.68	3.87	2.82	3.79
Mean	21.02	20.42	20.15	20.53	21.08	20.95	19.93	20.65	21.05	20.69	20.04	20.59
LSD $_{\alpha=0.05}$ for:												
Essential oil constituent (I)	0.943				1.315				0.581			
Term of harvest (II)	0.781				0.814				0.510			
Interaction IxII	1.562				1.628				1.019			

± Standard deviation (n=3)

components, while in the oil isolated from plants collected at Kozjak Mountain in Croatia (Bezic *et al.*, 2009) the concentration of carvacrol was much lower (13.7%). Oil contained also p-cymene (11.8%) and γ-terpinene (10.6%) as the main components. Carvacrol (79.75%), o-cymene (4.26%), 1-octen-3-ol (2.33%) and thymol (2.26%) dominated in the essential oil of *S. montana* cultivated in Egypt (Hassanein *et al.*, 2014). The high content of carvacrol in volatile oil (76.16%) was found by Rzepa *et al.* (2012) in winter savory cultivated in south-eastern Poland. The other abundant components were p-cymene (12.51%) and γ-terpinene (6.03%). Similarly, carvacrol (52.2%), p-cymene (12.8%) and γ-terpinene (8.9%) dominated in the essential oil obtained from *S. montana* cultivated in Spain (Silva *et al.*, 2009) and in the essential oils extracted from six *S. montana* populations collected from agro-climatically diverse sites in Albania: carvacrol (2.21-55.95%), p-cymene (1.13-16.22%), γ-terpinene (0.31-8.86%) (Ibraliu *et al.*, 2010).

In contrast, the essential oil of savory from western Serbia contained carvacrol only in 0.4-1.1% (Slavkovska *et al.*, 2001).

The content of carvacrol (65.43-69.99%) found in our oils of *S. montana* was higher as compared to the results obtained by Slavkovska *et al.* (2001), Fraternale *et al.* (2007), Cavar *et al.* (2008 and 2013), Bezic *et al.* (2009), Silva *et al.* (2009) and Ibraliu *et al.* (2010). Only plants cultivated in south-eastern Poland (Rzepa *et al.*, 2012) and Egypt (Hassanein *et al.*, 2014) had higher carvacrol content in the essential oil. However, the content of thymol, p-cymene and γ-terpinene, which we noted in our oils was lower as compared to the results obtained by other researches. Interestingly, β-caryophyllene, which we reported as the main component found in our oils (2.74-4.71%), was not detected in the oils extracted by hydrodistillation from savory cultivated in south-eastern Poland (Rzepa *et al.*, 2012).

The influence of growth stages on essential oil composition of winter savory has been also reported by several authors (Milos *et al.*, 2001; Skocibuasic and Bezic, 2004; Damjanovic-Vratnica *et al.*, 2011).

Milos *et al.* (2001) investigated the essential oil composition of *S. montana* collected from different localities (Biokovo, Brac, Kozjak) in Dalmatia (Croatia) and at three different stages of development: prior to flowering, during flowering and after flowering. They found carvacrol (16.1-52.4%), thymol (1.90-20.6%) and p-cymene (3.00-28.9%) as the most abundant compounds in all oil samples, although, γ-terpinene (4.90-8.1%) – the fourth most abundant compound – was present only in the oils isolated from plants collected prior flowering and during flowering stage. Moreover, β-caryophyllene was not detected in plants collected from Kozjak, while in plants collected from Biokovo, was present only in pre-flowering stage (2.7%). In case of savory oil obtained from plants collected from Brac, the content of β-caryophyllene varied from 1.30 (during flowering and after flowering) to 1.80% (prior to flowering). They also reported, that the highest content of p-cymene (19.10-28.9%) was observed after the flowering, while the highest amount of thymol (11.00-20.60%) was noted prior to flowering. The concentration of carvacrol was variable in dependence on the place of cultivation.

Skocibuasic and Bezic (2004) also examined *S. montana* gathered from Brac (Croatia) at the same stages of development. They found carvacrol (52.40%) as the main oil constituent especially before flowering while p-cymene increased through flowering (from 3.80 to 25.60%).

The effect of vegetation cycle on phytochemical composition of the essential oil obtained from wild-growing *S. montana* collected from Podgorica region (central part of Montenegro) was studied by Damjanovic-Vratnica *et al.* (2011). The higher content of thymol (37.36%), carvacrol (15.47%), γ-terpinene (11.75%) and β-caryophyllene (3.96%) they noted in the volatile oil obtained from plants collected before flowering, while content of p-cymene (31.37%) was higher in the essential oil extracted from plants collected during the flowering stage.

The content of carvacrol found in our oils was higher as compared to the results obtained by others (Milos *et al.*, 2001; Skocibuasic and Bezic, 2004; Damjanovic-Vratnica *et al.*, 2011), although its highest concentration we also observed in volatile oil obtained from plants collected before flowering. Similarly, our plants contained more β-caryophyllene at pre-flowering stage. Moreover, β-caryophyllene was detected in all our oil samples in contrast to the results obtained by Milos *et al.* (2001). However, the content of p-cymene noted in our oils isolated from plants collected after the flowering, was lower compared to cited literature (Milos *et al.*, 2001; Skocibuasic and Bezic, 2004). Also, the content of this compound noted in our oils obtained from savory collected during flowering (8.65 and 8.01% in 2010 and 2011, respectively) was much lower compared to the results presented by Damjanovic-Vratnica and co-workers (2011).

Conclusion

The results presented in this study shows that the essential oils obtained from winter savory collected at different growth stages had similar compositions. The major compounds were carvacrol, p-cymene, m-cymene, γ-terpinene and β-caryophyllene. Significantly higher concentrations of carvacrol, γ-terpinene and β-caryophyllene were assessed for the herb collected before flowering, while in the case of p-cymene the highest amounts were noted after the flowering. The optimal time for harvesting of the plants with respect to high carvacrol, γ-terpinene and β-caryophyllene content is before flowering. If essential oil rich in p-cymene is necessary for any reason, then the plants should be harvested after the flowering

It can be also concluded, that winter savory cultivated in north-western Poland may found wide industrial application due to the high content of phenolic compound – carvacrol, which is responsible mainly for high antimicrobial activity of savory's oil.

References

Adams RP (2007). Identification of essential oil components by gas chromatography/mass spectrometry. 4th ed. Allured Publishing, Carol Stream, Illinois, USA.

Bezic N, Samanic I, Dunkic V, Besendorfer V, Puizina J (2009). Essential oil composition and internal transcribed spacer (ITS) sequence variability of four South-Croatian *Satureja* species (Lamiaceae). Molecules 14:925-938.

Braga PC, Dal Sasso M, Culici M, Galastri L, Marceca MT, Guffanti EE (2006). Antioxidant potential of thymol determined by chemiluminescence inhibition in human neutrophilis and cell-free systems. Pharmacology 76:61-68.

Cavar S, Maksimović M, Solic ME, Jerkovic-Mujkic A, Besta R (2008). Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. Food Chem 111:648-653.

Cavar S, Solic ME, Maksimovic M (2013). Chemical composition and antioxidant activity of two *Satureja* species from Mt. Biokovo. Bot Serb 37(2):159-165.

Cazin C, Jonard R, Alain P, Pellecuer J (1985). L'evolution de la composition des essentieles chez divers chemotypes des Sarriette des montangnes (*Satureja montana* L.) obtenus par l'isolement in vitro des apex. CR Acad Sc Paris 6:237-240.

Chorianopoulos N, Kalpoutzakis E, Aligiannis N, Mitkau S, Nychas GJ, Haroutounian SA (2004). Essential oils of *Satureja, Origanum,* and *Thymus* species: chemical composition and antibacterial activities against foodborne pathogens. J Agric Food Chem 52(26):8261-8267.

Ciani M, Menghini L, Mariani F, Pagiotti P, Menghini A, Fatichenti F (2000). Antimicrobial properties of essential oil of *Satureja montana* L. on pathogenic and spoilage yeasts. Biotechnol Lett 22:1007-1010.

Damjanovic-Vratnica B, Perovic A, Sukovic D, Perovic S (2011). Effect of vegetation cycle on chemical content and antibacterial activity of *Satureja montana* L. Arch Biol Sci Belgrade 63(4):1173-1179.

Fraternale D, Giamperi L, Bucchini A, Ricci D, Epifano F, Genovese S, Curini M (2007). Chemical composition and antifungal activity of the essential oil of *Satureja montana* from central Italy. Chem Nat Compd 43(5):622-624.

Hassanein HD, Said-Al Ahl HAH, Abdelmohsen MM (2014). Antioxidant polyphenolic constituents of *Satureja montana* L. growing in Egypt. Int J Pharm Sci 6(4):578-581.

Hajhashemi V, Sadraei H, Ghannadi AR, Mohseni M (2000). Antispasmodic and anti-diarrhoeal effect of *Satureja hortensis* L. essential oil. J Ethnopharmacol 71:187-192.

Ibraliu A, Dhillon BS, Faslia N, Stich B (2010). Variability of essential oil composition in Albanian accessions of *Satureja montana* L. J Med Plants Res 4(14):1359-1364.

Kustrak D, Kuftinec J, Blazevic N, Maffei M (1996). Comparison of the essential oil composition of two subspecies of *Satureja montana.* J Essent Oil Res 8:7-13.

Lawless J (2002). The encyclopedia of essential oils. Thorsons, London, Great Britain, 170 p.

Lawrence BM (1979). Essential oils. Allured Publishing, Wheaton, IL.

Mastelic J, Jerkovic I (2003). Gas chromatography-mass spectrometry analysis of free and glycoconjugated aroma compounds of seasonally collected *Satureja montana* L. Food Chem 80:135-140.

Milos M, Radonic A, Bezic N, Dunkic V (2001). Localities and seasonal variations in the chemical composition of essential oils of *Satureja montana* L. and *S. cuneifolia* Ten. Flavour and Fragrance J 16:157-160.

Polish Pharmacopoeia VI (2002). Warszawa, 151 p.

Ruberto G, Baratta MT (2000). Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem 69:167-174.

Rzepa J, Sajewicz M, Baj T, Gorczyca P, Włodarek M, Głowniak K, Waksmundzka-Hajnos M, Kowalska T (2012). A comparison of methodical approaches to fingerprinting of the volatile fraction from winter savory (*Satureja montana*). Chromatogr Res Int 2012:1-8.

Silva FVM, Martins A, Salta J, Neng NR, Nogueira JMF, Mira D, Gaspar N, Justino J, Grosso C, Urieta JS, Palavra AMS, Rauter AP (2009). Phytochemical profile and anticholinesterase and antimicrobial activities of supercritical versus conventional extracts of *Satureja montana*. J Agric Food Chem 57:11557-11563.

Skocibuasic M, Bezic N (2004). Chemical composition and antimicrobial variability of *Satureja montana* L. essential oils produced during ontogenesis. J Essent Oil Res 16:387-391.

Slavkovska V, Jancic R, Bojovic S, Milosavljavic S, Djokovic D (2001). Variability of essential oils of *Satureja montana* L. and *Satureja kitaibelii* Wierzb. Ex Heuff. from the central part of Balkan Peninsula. Phytochemistry 57:71-76.

Small E, Deutsch G (2001). Culinary herbs for short-season gardeners. Ismant Peony Press, Canada, 157-158 p.

Vidic D, Cavar S, Maksimovic M (2010). Antioxidant activity of two *Satureja* species. Planta Med 76:1227.

Yamasaki K, Nakano M, Kawahata T, Mori H, Otake T, Ueba N (1998). Anti-HIV-1 activity of herbs in *Labiatae.* Biol Pharm Bull 21:829-833.