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SEASONAL AND PHENOLOGICAL DYNAMICS OF ESSENTIAL OIL CONSTITUENTS IN CULTIVATED *Satureja montana* L.

Grażyna Zawiślak https://orcid.org/0000-0002-4332-8394
Ewa Dorota Zalewska✉ https://orcid.org/0000-0001-7445-9808

Department of Vegetable and Herb Crops, Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin, Doświadczalna 50A, 20-280 Lublin, Poland

ABSTRACT

Research on the qualitative and quantitative composition of essential oil was conducted in south-eastern Poland. The aim of the study was to evaluate the chemical composition of essential oil from *Satureja montana* L. depending on the plant's developmental stage. Seedlings of winter savory were planted in the field at a spacing of 30 × 30 cm. The herb was collected on the following dates: June (vegetative stage of the plant), mid-July (onset of flowering), mid-August (full flowering), and September (senescence). The highest essential oil content was found in plants at full flowering (1.88–2.20%) and at the beginning of flowering (1.63–2.20%). The oil from *S. montana* contained high levels of phenolic compounds, mainly carvacrol and thymol. The highest levels of carvacrol were found at the beginning of flowering (60.97–72.03%) and at full flowering (62.36–73.54%). The highest thymol content was found in 2018 – 19.47% – in the herb collected from plants that had finished flowering and 14.90% – in the herb from plants collected during the vegetative phase.

Keywords: winter savory, carvacrol, thymol, plant phenological stages

INTRODUCTION

Plants belonging to the Lamiaceae family represent a rich source of herbal raw materials containing essential oils (EO). Essential oils are the biologically active substances – secondary metabolites of many plants species whose properties are used in health prevention. These biologically active substances obtained from plants of the Lamiaceae family are of significant importance in phytotherapy, the pharmaceutical and cosmetics industries, as well as in food processing [Chorianopoulos et al. 2004, Jafari et al. 2016, Zawiślak and Nurzyńska-Wierdak 2017a, Vrancheva et al. 2022, Vilmosh et al. 2023, 2024, Jakupović et al. 2025, Kartal et al. 2025]. The genus *Satureja* includes approximately 200 species, native to the Middle East and Mediterranean Europe, as well as Western Asia, North Africa, and South America [Chorianopoulos et al. 2004]. One of the most interesting is *Satureja montana* L., whose essential oil demonstrates antiseptic, antioxidant, antifungal, carminative, and digestive properties [Momtaz and Abdollahi 2010, Maccelli et al. 2020, Abbad et al. 2025]. The herb of *S. montana* is commonly used in Mediterranean cuisine [Kartal et al. 2025], and its essential oil serves as a natural antibacterial agent in food packaging applications [Dima and Dima 2015]. Extracts from *S. montana* containing rosmarinic acid are effective against Gram-positive and Gram-negative bacteria [Gomes et al. 2020]. The biological activity of winter savory is mainly due to the presence of essential oil [Nurzyńska-Wierdak 2016, Rezende et al. 2022, Abbad et al. 2025]. According

✉ ewa.zalewska@up.lublin.pl

to literature data the main component of *S. montana* essential oil is carvacrol [Skočibušić and Bezić 2004, Bezić et al. 2009, Miladi et al. 2013, Pokajewicz et al. 2023] or thymol [Damjanović-Vratnica et al. 2011, de Oliveira 2011]. The potent antimicrobial activity of this essential oils has been attributed primarily to its high content of carvacrol and thymol, phenolic compound exhibiting synergistic antimicrobial effect [Maccelli et al. 2020, Šimunović et al. 2020].

Said-Al Ahl et al. [2024] pointed to the growing interest in essential oil from winter savory and its compounds, which have a beneficial effect on human health. However, *S. montana* does not occur naturally in Poland and is rarely cultivated in our country.

The yield and chemical composition of essential oils obtained from plants of the Lamiaceae family are variable and influenced by climatic conditions, cultivation practices, and harvest timing [Zawiślak 2013, Zawiślak and Nurzyńska-Wierdak 2017b, Drozd et al. 2024].

Moreover, many scientists have demonstrated that essential oil composition obtained from *S. montana* depends on the phenological stage of the plant and environmental conditions [Milos et al. 2001, Damjanović-Vratnica et al. 2011]. This relationship has also been confirmed for many other species of plants from the Lamiaceae family [Jordán et al. 2013, Moghaddam et al. 2015, Türkmen 2021]. However, studies by Miladi et al. [2013] showed that the carvacrol content in the essential oil of *S. montana* herb, collected in France at the peak of flowering, was 53.35%. A similar carvacrol content (52.4%) was found in essential oil from raw material harvested before flowering in Croatia [Mirjana and Nada 2004].

Therefore, this study aimed to evaluate the chemical composition of essential oil from *S. montana* cultivated in southeastern Poland, examining variations related to plant phenological stages at harvest during three years of the study.

MATERIALS AND METHODS

Plant material

The experiments were carried out at the Experimental Farm of the University of Life Sciences in Lublin, located in southeastern Poland (51.23° N; 22.56° E), in 2017–2019. The Lublin vicinity is a region with a long traditions of herbs cultivations. The average long-term temperatures in May are 13.2 °C, gradually increasing in the following months (June: 15.6 °C, July: 18.1 °C), slightly decreasing in August to 17.5 °C, and in September reaching 12.9 °C. The moderate summer temperature determines, among other the possibility of cultivating essential oil plants. Seeds of *S. montana* were sourced from the Botanical Garden collection of Maria Curie-Skłodowska University, Lublin, Poland. Seedlings were cultivated under greenhouse conditions, with sowing conducted on March 20 into trays containing peat-based substrate. Seed germination occurred within two weeks. Seedlings were subsequently transplanted into multi-cell trays, and then planted in the field in mid-May at a spacing of 30 × 30 cm. The cultivation plot was established following standard agronomic practices for *Satureja* sp. Soil analysis indicated grey-brown podzolic soil derived from loess deposits, containing approximately 1.6% organic matter. Based on this analysis, fertilization consisted of 60 kg N, 50 kg P_2O_5, and 100 kg K_2O per hectare. Weed control and soil aeration were performed manually. Chemical plant protection products were not applied, as pests and diseases were not observed throughout the experiment. The plants were harvested in the second year of cultivation at four developmental stages for three consecutive years.

1. June (vegetative stage),
2. Mid-July (onset of flowering),
3. Mid-August (full flowering),
4. September (senescence).

Plants were harvested by cutting approximately 8 cm above ground level. Collected material was dried in a thermal dryer at 30 °C. The mountain savory herb was placed in a Leśniczanka-type drying chamber. Not all parts of the raw material dried evenly, so the drying time was 8 days. Furthermore, the layer of fresh herb on a single sieve was approximately 15 cm thick, which also extended the drying time. In the case of the *S. montana* herb, the stems remained moist for a long time. When the stems broke with a characteristic cracking sound and the leaves rustled, the drying process was complete. The drying conditions in the conducted tests were consistent with the manufacturer's recommendations [Kołodziej 2018].

The dried herb was sieved (mesh size 4–5 mm) to obtain rubbed herb material, comprising leaves and

shoot tips, from which the essential oil was extracted. The water content in raw material intendent for testing depending on the harvest data from 6% to 14%.

Chemical analyses of plant material

Essential oil extraction. Essential oils were extracted by hydrodistillation using a Clevenger-type apparatus. Samples of 20 g dried herb were placed into a 1000 mL round-bottom flask, and 400 mL distilled water was added. The mixture was brought to boiling, and distillation was carried out for 3 hours, maintaining a consistent distillation rate throughout the extraction. After distillation, heating was stopped, and following a 15-minute cooling period, essential oil volumes were measured using a calibrated receiver according to standard protocols described in the Polish Pharmacopoeia IX [Farmakopea Polska IX 2011].

GC-MS analysis of essential oil. Chemical constituents of the essential oil were identified using a Varian 4000 ITMS GC-MS/MS system (Varian, USA), equipped with a CP-8410 auto-injector and a VF-5ms capillary column (30 × 0.25 mm i.d., 0.25 µm film thickness; Varian, USA). Helium was employed as the carrier gas at a flow rate of 0.5 mL/min. Injector and detector temperatures were maintained at 220 °C and 200 °C, respectively. A split ratio of 1:20 was used, and injection volume was 1 µL. The temperature program was as follows: initial temperature of 60 °C held for 0.5 min, increased by 3 °C/min to 246 °C, and maintained at 246 °C for 10 minutes. Ionization was performed at 70 eV, and mass spectra were recorded in the mass range of 40–1000 Da with a scan time of 0.80 s. Qualitative identification of compounds was performed by comparing obtained mass spectra to reference spectra in the NIST Mass Spectral Library, supplemented with confirmation by comparing calculated retention indices with literature values [Adams 2007]. The retention index was determined according to Van Den Dool and Kratz [1963].

Statistical analysis

The obtained results are presented as the means and were statistically analyzed by ANOVA, and the averages were compared using Tukey's HSD test at the probability level $\alpha = 0.05$. Statistical analyses were calculated with Statistica 13.3 PL software (StatSof Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The essential oil content in *S. montana* herb collected at the beginning of flowering (1.63–2.20%) and at full flowering (1.88–2.20%) was indeed higher than in other term of extraction. This trend persisted throughout all years of the study (Table 1). According to Zawiślak and Nurzyńska [2017 a], the oil content in winter savory herb collected at the beginning of flowering ranged from 1.44 to 2.04%. In the studies by Skočibušić and Bezić [2004], the oil content in flowering plants was 1.7%. In the studies conducted, the herb collected from plants in the vegetative phase contained the least amount of essential oil (1.39–1.51%). A decrease in oil content was observed in *S. montana* at the last stage of the study (in plants that had finished flowering). The oil content in this phase ranged from 1.47 to 1.55% (Table 1).

A total of 44 chemical compounds were identified (Tables 2–4), aligning closely with previously reported ranges of 29–47 [Čavar et al. 2013, Miladi et al. 2013, Hudz et al. 2020, Kovačević et al. 2021, Górska-Drabik et al. 2024]. The essential oil from winter savory herb was dominated by oxygenated monoterpenes and

Table 1. Essential oil content in winter savory herb depending on the plant development stage (%)

Plant development stage	2017	2018	2019
Vegetative stage	1.51 c	1.47 b	1.39 c
Onset of flowering	2.20 a	1.94 a	1.63 b
Full flowering	1.88 b	2.01 a	2.20 a
Senescence	1.54 c	1.47 b	1.55 b

Values marked with the same letter in column do not differ significantly

Table 2. Chemical composition (%) of essential oil from the herb of *Satureja montana* L. (2017)

Compounds	RI	2017			
		vegetative stage	onset of flowering	full flowering	senescence
α-thujene	856	1.16 ±0.02	1.02 ±0.08	0.95 ±0.03	1.29 ±0.06
α-pinene	862	0.73 ±0.00	0.65 ±0.03	0.61 ±0.01	0.88 ±0.03
camphene	877	0.25 ±0.00	0.29 ±0.01	0.26 ±0.00	0.53 ±0.02
sabinene	897	0.14 ±0.00	0.13 ±0.00	0.13 ±0.00	0.16 ±0.01
β-pinene	901	0.11 ±0.01	0.14 ±0.02	0.08 ±0.02	0.32 ±0.01
myrcene	909	1.48 ±0.04	1.16 ±0.05	1.47 ±0.00	1.27 ±0.10
α-phellandrene	923	0.26 ±0.06	0.27 ±0.04	0.25 ±0.02	0.25 ±0.04
δ-2-carene	924	tr	tr	–	–
α-terpinene	931	1.77 ±0.01	1.95 ±0.07	1.73 ±0.02	1.88 ±0.06
p-cymene	938	3.76 ±0.07	5.28 ±0.17	3.89 ±0.04	9.72 ±0.28
limonene	941	0.10 ±0.01	0.14 ±0.00	0.10 ±0.00	0.18 ±0.01
β-phellandrene	943	tr	tr	tr	tr
(Z)-β-ocimene	945	1.94 ±0.06	0.12 ±0.07	3.37 ±0.07	0.15 ±0.00
(E)-β-ocimene	954	0.33 ±0.03	0.45 ±0.01	0.56 ±0.05	0.50 ±0.01
γ-terpinene	964	9.39 ±0.13	9.98 ±0.26	9.51 ±0.15	9.23 ±0.22
cis-sabinene hydrate	977	0.54 ±0.01	0.63 ±0.03	0.52 ±0.01	0.88 ±0.01
terpinolene	988	0.08 ±0.00	0.06 ±0.01	0.07 ±0.00	0.09 ±0.01
trans-sabinene hydrate	1006	0.17 ±0.02	0.39 ±0.02	0.17 ±0.01	0.43 ±0.01
borneol	1092	0.68 ±0.01	0.89 ±0.01	0.83 ±0.02	1.67 ±0.01
terpinen-4-ol	1103	0.33 ±0.01	0.21 ±0.02	0.28 ±0.01	0.37 ±0.02
α-terpineol	1117	tr	tr	tr	0.10 ±0.01
thymol	1196	6.99 ±0.02	2.61 ±0.02	5.91 ±0.02	2.94 ±0.02
carvacrol	1206	63.58 ±0.54	70.36 ±0.58	62.36 ±0.37	63.71 ±0.86
α-copaene	1289	0.07 ±0.01	0.06 ±0.01	0.11 ±0.01	0.07 ±0.01
β-bourbonene	1299	tr	tr	tr	tr
α-gurjunene	1319	tr	tr	tr	tr
trans-caryophyllene	1331	2.64 ±0.05	0.20 ±0.01	2.76 ±0.02	0.31 ±0.01
β-gurjunene	1338	tr	tr	tr	tr
β-copaene	1340	tr	tr	0.06 ±0.00	tr
aromadendrene	1347	0.50 ±0.00	0.62 ±0.01	0.63 ±0.00	0.63 ±0.00
α-humulene	1362	0.10 ±0.00	0.10 ±0.00	0.10 ±0.00	0.10 ±0.01
allo-aromadendrene	1366	0.06 ±0.00	0.06 ±0.00	0.08 ±0.00	0.06 ±0.00
γ-muurolene	1377	0.22 ±0.01	0.18 ±0.02	0.33 ±0.01	0.15 ±0.00
germacrene d	1384	0.14 ±0.03	0.11 ±0.01	0.18 ±0.02	0.10 ±0.01
viridiflorene	1392	0.58 ±0.01	0.59 ±0.01	0.79 ±0.00	0.54 ±0.01
bicyclogermacrene	1396	0.51 ±0.01	0.55 ±0.01	0.63 ±0.01	0.46 ±0.01
epizonarene	1402	tr	tr	tr	tr
β-bisabolene	1407	0.32 ±0.00	tr	0.05 ±0.02	0.07 ±0.00
γ-cadinene	1414	0.16 ±0.00	0.14 ±0.01	0.20 ±0.01	0.10 ±0.00
δ-amorphene	1418	tr	tr	–	–
α-cadinene	1440	0.39 ±0.00	0.36 ±0.01	0.52 ±0.02	0.27 ±0.01
spathulenol	1486	0.14 ±0.00	0.17 ±0.02	0.20 ±0.00	0.24 ±0.02
caryophyllene oxide	1492	0.07 ±0.00	0.08 ±0.01	0.06 ±0.00	0.20 ±0.01
globulol	1496	tr	tr	0.06 ±0.00	0.06 ±0.00
Total	–	99.69	99.95	99.81	99.91

tr (trace) <0.05%

RI – non-isothermal Kovats'a retention indices (from temperature – programming using definition of Van Den Dool and Kratz [1963]), for series of n-alkanes C_6–C_{40}

Table 3. Chemical composition (%) of essential oil from the herb of *Satureja montana* L. (2018)

Compounds	RI	2018			
		vegetative stage	onset of flowering	full flowering	senescence
α-thujene	856	1.04 ±0.09	0.98 ±0.05	1.12 ±0.08	1.24 ±0.04
α-pinene	862	0.73 ±0.02	0.62 ±0.03	0.66 ±0.03	0.70 ±0.02
camphene	877	0.46 ±0.00	0.20 ±0.02	0.27 ±0.01	0.22 ±0.01
sabinene	897	0.14 ±0.00	0.14 ±0.00	0.15 ±0.00	0.15 ±0.00
β-pinene	901	0.24 ±0.01	0.12 ±0.09	0.29 ±0.01	0.10 ±0.07
myrcene	909	1.38 ±0.02	1.48 ±0.01	1.54 ±0.01	1.43 ±0.01
α-phellandrene	923	0.19 ±0.08	0.20 ±0.11	0.24 ±0.00	0.33 ±0.00
δ-2-carene	924	tr	tr	–	–
α-terpinene	931	1.49 ±0.01	1.68 ±0.01	1.34 ±0.03	1.81 ±0.03
p-cymene	938	6.05 ±0.07	4.01 ±0.07	3.61 ±0.07	4.82 ±0.08
limonene	941	0.13 ±0.00	0.09 ±0.00	0.08 ±0.00	0.14 ±0.01
β-phellandrene	943	tr	tr	tr	tr
(Z)-β-ocimene	945	3.31 ±0.02	2.76 ±0.06	3.07 ±0.04	0.93 ±0.01
(E)-β-ocimene	954	0.40 ±0.00	0.44 ±0.10	0.52 ±0.00	0.15 ±0.01
γ-terpinene	964	6.93 ±0.02	9.23 ±0.19	7.28 ±0.12	8.21 ±0.02
cis-sabinene hydrate	977	0.97 ±0.02	0.57 ±0.01	0.60 ±0.00	0.63 ±0.03
terpinolene	988	0.07 ±0.00	0.07 ±0.01	0.06 ±0.00	0.06 ±0.00
trans-sabinene hydrate	1006	0.23 ±0.01	0.24 ±0.02	0.16 ±0.05	0.32 ±0.02
borneol	1092	1.62 ±0.09	0.71 ±0.01	0.82 ±0.03	0.63 ±0.06
terpinen-4-ol	1103	0.39 ±0.08	0.36 ±0.03	0.29 ±0.01	0.27 ±0.04
α-terpineol	1117	0.10 ±0.01	0.08 ±0.00	0.07 ±0.00	0.09 ±0.04
thymol	1196	14.90 ±0.17	8.94 ±0.26	9.02 ±0.15	19.47 ±0.03
carvacrol	1206	51.43 ±0.79	60.97 ±0.69	62.37 ±0.72	52.58 ±0.19
α-copaene	1289	0.08 ±0.00	0.06 ±0.00	0.06 ±0.00	0.06 ±0.00
β-bourbonene	1299	0.09 ±0.01	tr	tr	0.06 ±0.04
α-gurjunene	1319	tr	tr	tr	tr
trans-caryophyllene	1331	2.56 ±0.14	2.75 ±0.00	3.13 ±0.04	2.61 ±0.10
β-gurjunene	1338	tr	tr	tr	tr
β-copaene	1340	0.08 ±0.01	0.05 ±0.00	0.06 ±0.00	tr
aromadendrene	1347	0.45 ±0.02	0.37 ±0.01	0.31 ±0.00	0.24 ±0.01
α-humulene	1362	0.10 ±0.00	0.11 ±0.00	0.12 ±0.00	0.10 ±0.00
allo-aromadendrene	1366	0.06 ±0.00	tr	–	–
γ-muurolene	1377	0.31 ±0.01	0.22 ±0.01	0.21 ±0.00	0.11 ±0.02
germacrene D	1384	0.36 ±0.00	0.18 ±0.00	0.19 ±0.01	0.15 ±0.01
viridiflorene	1392	0.54 ±0.00	0.43 ±0.02	0.39 ±0.02	0.27 ±0.00
bicyclogermacrene	1396	0.79 ±0.01	0.52 ±0.03	0.56 ±0.03	0.37 ±0.00
epizonarene	1402	tr	tr	tr	tr
β-bisabolene	1407	0.46 ±0.00	0.25 ±0.01	0.37 ±0.03	0.91 ±0.00
γ-cadinene	1414	0.22 ±0.01	0.16 ±0.02	0.16 ±0.02	0.13 ±0.01
δ-amorphene	1418	0.50 ±0.02	0.35 ±0.03	0.33 ±0.04	0.24 ±0.02
α-cadinene	1440	tr	tr	tr	–
spathulenol	1486	0.65 ±0.04	0.22 ±0.01	0.18 ±0.02	0.15 ±0.02
caryophyllene oxide	1492	0.35 ±0.01	0.22 ±0.01	0.22 ±0.04	0.16 ±0.03
globulol	1496	0.09 ±0.01	0.06 ±0.01	–	tr
Total	–	99.89	99.84	99.85	99.84

tr (trace) <0.05%

RI – non-isothermal Kovats' a retention indices (from temperature – programming using definition of Van Den Dool and Kratz [1963]), for series of n-alkanes C_6–C_{40}

Table 4. Chemical composition (%) of essential oil from the herb of *Satureja montana* L. (2019)

Compounds	RI	2019			
		vegetative stage	onset of flowering	full flowering	senescence
α-thujene	856	0.83 ±0.01	1.13 ±0.04	1.33 ±0.04	1.1 ±0.06
α-pinene	862	0.55 ±0.01	0.63 ±0.02	0.7 ±0.03	0.7 ±0.03
camphene	877	0.23 ±0.00	0.17 ±0.00	0.14 ±0.01	0.31 ±0.01
sabinene	897	0.13 ±0.00	0.14 ±0.00	0.16 ±0.01	0.15 ±0.00
β-pinene	901	0.21 ±0.01	0.22 ±0.01	0.22 ±0.01	0.36 ±0.00
myrcene	909	1.38 ±0.02	1.49 ±0.06	1.57 ±0.11	1.23 ±0.01
α-phellandrene	923	0.17 ±0.04	0.29 ±0.02	0.29 ±0.02	0.12 ±0.06
δ-2-carene	924	0.06 ±0.00	0.05 ±0.00	0.06 ±0.01	0.05 ±0.00
α-terpinene	931	1.37 ±0.01	1.44 ±0.04	1.45 ±0.13	1.11 ±0.03
p-cymene	938	5.16 ±0.10	3.37 ±0.10	4.05 ±0.36	5.46 ±0.01
limonene	941	0.08 ±0.00	0.07 ±0.00	0.07 ±0.01	0.07 ±0.01
β-phellandrene	943	tr	tr	tr	tr
(Z)-β-ocimene	945	4.08 ±0.15	1.86 ±0.07	1.6 ±0.12	1.72 ±0.01
(E)-β-ocimene	954	0.65 ±0.03	0.34 ±0.04	0.28 ±0.01	0.31 ±0.06
γ-terpinene	964	5.73 ±0.06	7.71 ±0.24	7.61 ±0.71	5.01 ±0.04
cis-sabinene hydrate	977	0.60 ±0.03	0.67 ±0.06	0.68 ±0.03	0.66 ±0.02
terpinolene	988	tr	tr	–	–
trans-sabinene hydrate	1006	0.48 ±0.01	0.16 ±0.04	0.14 ±0.06	0.48 ±0.01
borneol	1092	0.85 ±0.04	0.51 ±0.02	0.31 ±0.00	0.87 ±0.01
terpinen-4-ol	1103	0.30 ±0.01	0.32 ±0.01	0.34 ±0.01	0.27 ±0.03
α-terpineol	1117	0.12 ±0.03	0.12 ±0.00	0.11 ±0.01	0.12 ±0.05
thymol	1196	3.14 ±0.05	2.77 ±0.11	2.34 ±0.05	2.62 ±0.04
carvacrol	1206	65.49 ±0.19	72.03 ±0.73	73.54 ±1.18	68.8 ±0.06
α-copaene	1289	0.09 ±0.00	0.10 ±0.01	0.05 ±0.03	0.08 ±0.04
β-bourbonene	1299	0.10 ±0.00	tr	tr	0.15 ±0.02
α-gurjunene	1319	tr	tr	tr	tr
trans-caryophyllene	1331	3.20 ±0.11	0.51 ±0.01	0.57 ±0.06	4.5 ±0.04
β-gurjunene	1338	tr	tr	tr	tr
β-copaene	1340	0.08 ±0.00	0.05 ±0.00	–	0.06 ±0.00
aromadendrene	1347	0.43 ±0.01	0.44 ±0.01	0.25 ±0.00	0.17 ±0.00
α-humulene	1362	0.13 ±0.00	0.11 ±0.00	0.07 ±0.01	0.19 ±0.02
allo-aromadendrene	1366	0.06 ±0.01	0.07 ±0.00	–	–
γ-muurolene	1377	0.26 ±0.00	0.22 ±0.00	0.12 ±0.00	0.16 ±0.00
germacrene D	1384	0.23 ±0.00	0.15 ±0.00	0.15 ±0.02	0.30 ±0.01
viridiflorene	1392	0.49 ±0.01	0.50 ±0.01	0.27 ±0.01	0.23 ±0.00
bicyclogermacrene	1396	0.58 ±0.01	0.64 ±0.00	0.62 ±0.05	0.38 ±0.01
epizonarene	1402	tr	tr	tr	tr
β-bisabolene	1407	0.32 ±0.01	0.23 ±0.01	0.25 ±0.05	0.55 ±0.02
γ-cadinene	1414	0.23 ±0.00	0.18 ±0.00	0.11 ±0.01	0.15 ±0.01
δ-amorphene	1418	0.46 ±0.01	0.37 ±0.00	0.18 ±0.01	0.25 ±0.03
α-cadinene	1440	tr	tr	tr	–
spathulenol	1486	0.79 ±0.00	0.31 ±0.01	0.13 ±0.10	0.23 ±0.07
caryophyllene oxide	1492	0.77 ±0.02	0.53 ±0.07	0.11 ±0.09	0.89 ±0.22
globulol	1496	0.12 ±0.02	0.06 ±0.02	0.07 ±0.02	0.07 ±0.00
Total		99.95	99.96	99.94	99.88

tr (trace) <0.05%
RI – non-isothermal Kovats'a retention indices (from temperature – programming using definition of Van Den Dool and Kratz [1963]), for series of n-alkanes C_6–C_{40}

Table 5. The content of four main components of EO obtained from winter savory in different stage of vegetation in three years of the study

Compounds	Stage	2017	2018	2019
carvacrol	vegetative stage	63.58 b	51.43 b	65.49 c
	onset of flowering	70.35 a	60.97 a	72.03 a
	full flowering	62.36 c	62.37 a	73.54 a
	senescence	63.71 b	52.58 b	68.80 b
thymol	vegetative stage	6.99 a	14.90 b	3.14 a
	onset of flowering	2.61 b	8.94 c	2.77 b
	full flowering	5.91 a	9.02 c	2.34 b
	senescence	2.94 b	19.47 a	2.62 b
γ-terpinene	vegetative stage	9.39 b	6.93 b	5.73 b
	onset of flowering	9.98 a	9.23 a	7.71 a
	full flowering	9.51 a	7.28 b	7.61 a
	senescence	9.23 b	8.21 a	5.01 b
p-cymene	vegetative stage	3.76 b	6.05 a	5.16 a
	onset of flowering	5.28 b	4.01 b	3.37 b
	full flowering	3.89 b	3.61 b	4.05 a
	senescence	9.72 a	4.82 ab	5.46 a

Values marked with the same letter in the column (for each component separately) do not differ significantly

monoterpene hydrocarbons (Tables 2–4). In studies by many authors, the main components also belonged to these chemical groups [Čavar et al. 2013, Miladi et al. 2013, Hudz et al. 2020, Kovačević et al. 2021, Górska-Drabik et al. 2024]. According to Wesołowska et al. [2017], the distillation time does not affect the amount of components contained in the oil from *S. montana* or their content. In the present experiment, the oil hydrodistillation process lasted 3 hours.

The dominant compounds in all years and developmental stages of *Satureja montana* were: carvacrol (51.43–73.54%), γ-terpinene (5.01–9.98%), thymol (2.34–19.47%) and *p*-cymene (3.37–9.72); see Tables 2–4. Studies by Ćavar et al. [2013] showed that the main components of essential oil obtained from *S. montana* in Croatia were carvacrol (63.4%) and thymol (19.4%). In the study by Trifan et al. [2015], the carvacrol content in the oil from mountain savory grown in Romania was also 63.4%. In the study by Wesołowska et al. [2017], the essential oil isolated from *S. montana* grown in Poland and harvested during flowering contained 54.44–68.53% carvacrol. The present study showed the highest carvacrol content in fully flowering plants (73.54% in 2019) and at the beginning of flowering (72.03% in 2019 and 70.36% in 2017); see Table 5. The lowest carvacrol content was found in herb harvested from plants in the vegetative phase (51.43–65.49%). Essential oils from winter savory were characterized by a high carvacrol content in studies by Abbad et al. [2025] – 50.8% (cultivated in Morocco), Miladi et al. [2013] – 53.35% (natural sites in France) and Kavačević et al. [2021] – 55.01% (cultivated in Serbia). According to Trifan et al. [2015] winter savory, which contains significant amounts of carvacrol in its essential oil, belongs to the carvacrol chemotype. Carvacrol is a phenolic compound and is most often found in the company of its isomer, thymol [Schönknecht et al. 2016].

Essential oil from Montenegro extracted from *S. montana* before flowering contained thymol as the main component at a level of 37.36%, and the carvacrol content was 15.47% [Damjanović-Vratnica et al. 2011]. In the study by Górska-Drabik et al. [2024], thymol dominated in the oil from *S. montana* (40.04%). In the present study the oils extracted from plants in different phenological stages had a significantly lower thymol content in each year (Table 5). The herb collected from plants after flowering contained the highest amount of thymol (19.47% in 2018). The thymol content in essential oil from winter savory

in its natural site in Serbia during the flowering phase was 16.7% [Djordjevic et al. 2021], and in the studies by Dimitrijević et al. [2025] was 15.5%. The oil from winter savory, collected during the flowering phase in Ukraine and analyzed by Hudz et al. [2020], had an unusual chemical profile. *P*-thymol, an isomer of thymol and carvacrol, was dominant, with a level of 81.79%.

Based on the conducted research, it was demonstrated that γ-terpinene was present at the highest level at the beginning of *S. montana* flowering (7.71–9.98%) and at full flowering (7.61–9.51%); see Table 5. During the growing season, the γ-terpinene content changed slightly. In the study by Wesołowska et al. [2017], the γ-terpinene content in the oil during the flowering period ranged from 5.21 to 8.67%. In winter savory cultivated in Ukraine, γ-terpinene was present at a level of 0.9–6.6% [Pokajewicz et al. 2023]. In the studies by Górska-Drabik et al. [2024], γ-terpinene was one of the components present in high concentrations in the oil from *S. montana*. In the work of Kavačević et al. [2021], the level of γ-terpinene was 11.09%, in Abbad et al. [2025] was 18.5%, and in the studies of Dimitrijević et al. [2025] was only 3.1%.

The highest concentration of *p*-cymene was found in herbs obtained from plants that had finished flowering in 2017 (9.72%) and 2019 (5.46%); see Table 5. Damjanović-Vratnica et al. [2011] obtained different results. According to these researchers, the oil from *S. montana* herb before and during flowering contained *p*-cymene at levels of 7.86% and 31.37%, respectively. In the studies by Pokajewicz et al. [2023], *p*-cymene was also considered the dominant component of winter savory oil (5.0–8.8%). Similarly, Trifan et al. [2015] identified *p*-cymene (10.97%) as the main compound in *S. montana* oil.

Based on the conducted research, it was demonstrated that the α-terpinene content in *S. montana* essential oil remained relatively stable across all stages of plant development ranging from 1.11% to 1.95% (Tables 2–4). A similar trend was observed for myrcene. It concentration varied only slightly from 1.16% to 1.57%. Particularly noteworthy were the results concerning *trans*-caryophyllene in 2018. In the vegetative phase, *S. montana* essential oil contained 2.56% *trans*-caryophyllene. Its increased during full flowering, reaching 3.13%, followed by a decline in the post-flowering stage to 2.61% (Table 3). In the other years of the study, no such relationship was found in the *trans*-caryophyllene content (Tables 2 and 4).

Essential oil from *S. montana* from sites in Albania contained significant amounts of linalool (11.0%) [de Oliveira et al. 2011]. In the study by Milos et al. [2001], linalool was also the main component of the oil in winter savory at some sites in Croatia. The linalool content varied depending on the environment, with a maximum level of 62%. Čopra-Janićijević et al. [2020], due to the high linalool content in the EO, classified *S. montana* occurring in natural sites in Bosnia and Herzegovina as linalool type. In the present experiment, no linalool was found in cultivated plants (Table 2–4).

CONCLUSIONS

The essential oil obtained from winter savory cultivated in Lublin region was rich in phenolic compounds (carvacrol and thymol). The highest carvacrol content was found in essential oil obtained at the peak of flowering or at the beginning of flowering. However, the thymol content in the essential oil varied during the plant's developmental stages over the years. This proves the high quality of the essential oil and indicates the possibility of obtaining it from *S. montana* throughout the plant's growing season for medicinal purposes. The main components of the essential oil were also γ-terpinene and *p*-cymene, whose content also varied during the different stages of plant development. The results highlight the promising potential for introducing and expanding the cultivation of *S. montana* in Poland, especially for pharmaceutical and therapeutic applications. Further research should focus on optimizing agrotechnical factors in order to obtain a high-quality yield of *S. montana* raw material.

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