UDK 665.5
Original scientific paper

CHEMICAL COMPOSITION AND ANTIOXIDANT CAPACITY OF THE ESSENTIAL OILS FROM TWO HEMOTYPES OF *SATUREJA MONTANA* L.

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Abstract

Medicinal and aromatic plants have been traditionally used as an important source of therapeutic constituents. The genus Satureja contains about 200 aromatic and medicinal plant species, which grow in the Middle East and Mediterranean European regions, West Asia, North Africa, and South America. Satureja montana L. commonly contains around 5% of the essenytial oil that shows antioxidant, antimicrobial, diuretic, antidiarrheal, anticholinesterase, carminative, digestive, and cytotoxic activities. The essential oil provides the basis for a wide range of biological and industrial applications due to a high content of biologically active constituents. In the present study, the essential oils from two chemotypes of S. montana, ct. carvacrol (SM_c), and ct. thymol (SM_t) were isolated using a conventional hydro-distillation method (100 g of plant/1500 mL of dH_2O, 3 h). The areal parts of S. montana flowering plants were harvested from the experimental field of the Institute for Medicinal Plants Research "Dr Josif Pančić" in Pančevo, South Banat, Serbia. The chemical composition of the obtained essential oils was determined using GC-MS analysis, while antioxidant capacity was examined using two antioxidant assays (ABTS and DPPH methods). The GC-MS analysis showed that in the SM_c essential oil 43 compounds were identified and quantified, while in the SM_t essential oil there were 45 compounds. The most dominant compound in SM_c essential oil was carvacrol (39.49%), followed by thymol (30.43%), γ-terpinene (9.68%), p-cimene (5.71%), trans-caryophyllene (2.42%), β-bisabolene (1.96%), α-terpinene (1.41%) borneol (1.35%), myrcene (1.08%), and 1-octen-3-ol (1.04%), while the remaining constituents were presented in the amounts below 1% of the total content. The most abundant component in SM_t essential oil was thymol (70.69%), followed by carvacrol (7.06%), p-cimene (5.71%), γ-terpinene (3.85%), β-bisabolene (2.44%), and borneol (1.10%), whereas other oil constituents ranged from 0.03 to 0.89%. For SM_c and SM_t essential oils, the total content of monoterpene hydrocarbons was 19.61 and 12.99%, of oxygenated monoterpenes, 74.83 and 82.13%, of sesquiterpene hydrocarbons, 4.91 and 3.95%, and of oxygenated sesquiterpenes, 0.36 and 0.51%, respectively. According to the ABTS experiment, SM_c and SM_t had antioxidant capacities of 96.5 and 98.2%, respectively, whereas their respective capacities to neutralize DPPH radicals were 92.0 and 89.8%. The research presented here provides evidence on the chemical composition as well as the ABTS and DPPH radical scavenging potential of two different chemotypes of S. montana essential oils, as well as a foundation for their potential encapsulation and further application.

Key words: *Winter savory, carvacrol, thymol, essential oil properties.*

Introduction

Medicinal and aromatic plants have been traditionally used as an important source of therapeutic constituents. The genus Satureja contains about 200 aromatic and medicinal plant species, which grow in the Middle East and Mediterranean European regions, West Asia, North Africa, and South America. *Satureja montana* L. (winter savory, Lamiaceae) is a bristly perennial subshrub with a woody base and oblong-linear leaves, native to the Mediterranean region and widely cultivated (Khan & Abourashed, 2011). This plant species contains an abundant and varied secondary metabolite composition, as well as a variety of biological activities (Serrano et al., 2015). The plant contains the essential oil composed mainly of carvacrol, *p*-cymene, and thymol with a lower content of α- and β-pinenes, limonene, cineole, borneol, and α-terpineol, triterpenic acids, including ursolic and oleanolic acids, and flavonoids, including apigenin, apigenin-4-methyl ether, scutellarein-6,7 dimethyl ether, etc. (Čutović et al., 2022; Khan & Abourashed, 2011; Radonic & Milos, 2003). The chemical composition of *S. montana* depends on many ecological and agro-ecological factors, such as climatic features, natural habitat, principles of cultivation, and the phenophase stage at harvesting time (Fierascu et al., 2018; Hassanein et al., 2014; Miladi et al., 2013). In traditional medicine, the plant is used as a tonic, carminative, astringent, and expectorant in treating stomach and intestinal disorders, diarrhea, and sore throat, and as an aphrodisiac (Khan & Abourashed, 2011). Also, it exhibits antiviral potential against a variety of viruses, including cucumber mosaic virus and human immunodeficiency virus type 1 (Tepe et al., 2015). *S. montana* commonly contains around 5% of the essential oil that shows antioxidant, antimicrobial, diuretic, antidiarrheal, anticholinesterase, carminative, digestive, and cytotoxic activities. Essential oil provides the basis for a wide range of biological and industrial applications due to a high content of biologically active constituents (Čutović et al., 2022; Jafari et al., 2016). Essential oil of *S. montana* significantly decreased the aflatoxin production by mold named *Aspergillus flavus* (García-Díaz et al., 2020). In the study of Ćavar et al. (2008), two *Satureja* essential oils inhibited *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa*, and *Bacillus subtilis*. In the same study, it was shown that both essential oils possessed significant DPPH antioxidant activity. Antioxidants from essential oils can postpone, limit, or completely halt the oxidation reactions through scavenging free radicals, chelating ions, and lowering oxidative stress, thus the identification and determination of essential oil components, as well as their antioxidant capacity can provide important information on essential oil influence on the improving the quality of food and human health (Shukla et al., 2009; Zheng & Wang, 2001).

The traditional method for recovering essential oils, like hydrodistillation, has been widely used, although it is frequently associated with some limitations. The mentioned technique can be successfully used for the separation of pure essential oils from other non-volatile organic compounds. However, its application is followed by a longer extraction period, huge energy consumption, and chemical alternation of thermolabile molecules at elevated temperatures (Pourmortazavi and Hajimirsadeghi, 2007). Gas chromatography (GC) is a highly effective separation technique for analyses of various mixtures. The resolving power can be increased when mass spectrometry (MS) is coupled to GC and equipped with a flame ionization detector (FID). GC-MS is one of the most widespread analytical techniques in many scientific fields, including all disciplines of chemistry, environmental protection, food technology, and pharmacy. due to high sensitivity, low limit of detection, and the possibility of analyzing a great number of analytes and their identification using mass spectra. On the other hand, several factors can disturb the quantification process. Namely, the factors such as baseline drift, spectral background, peak shift deformation, homoscedastic and heteroscedastic noise, low S/N, and coelution can significantly change the direction of the experimental analysis (Heravi et al., 2015; Parastoli et al., 2012).

The aim of the present study was the isolation of the essential oils from two chemotypes of *S. montana* ct. carvacrol (SM_c), and ct. thymol (SM_t), using hydrodistillation, chemical

characterization by GC-MS analysis, and determination of ABTS and DPPH radical scavenging activities of the obtained essential oils.

Materials and Methods

Plant material and reagents

The areal parts of *S. montana* flowering plants were harvested from the experimental field of the Institute for Medicinal Plants Research "Dr Josif Pančić" in Pančevo, South Banat, Serbia. The following reagents were used: ethanol (Fisher Scientific, UK), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) - ABTS (Roche Diagnostics GmbH, Germany), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid - Trolox and 2,2-diphenyl-1-picrylhydrazyl - DPPH, and sodium sulfate (Sigma-Aldrich, USA), and ultrapure water.

Isolation of essential oils

The weight of 100 g of air-dried aerial parts of *S. montana* was ground in the laboratory mill with water cooling, and mixed with 1500 mL of distilled water. The obtained extract was subjected to hydrodistillation for 3 h in a Clevenger-type apparatus. *S. montana* essential oil was dried over anhydrous Na_2SO_4, and stored in a dark glass at -18 ºC until analysis. The yield of *S. montana* essential oil (v/w, %) was calculated on a dry weight basis.

GC/MS analysis of essential oils

The chemical composition of *S. montana* essential oil was analyzed using the GC-MS technique. GC-MS analyses were performed on a Shimadzu GCMS-QP2010 ultra mass spectrometer fitted with a flame ionic detector (FID) and coupled with a GC2010 gas chromatography. The low polarity capillary column, the InertCap5, with dimensions: 60 m×0.25 mm×0.25 m was used for separation. Firstly, the sample preparation was performed by dissolving 10 μL of essential oil in 1000 μL of ethyl alcohol. After dissolution, 1 μL of the obtained aliquot was injected into the apparatus, in the split mode. The carrier gas was helium with a flow of 1 mL min^{-1}. The injector and detector temperature was adjusted at 250 and 280 ºC, while the column temperature was linearly programmed in the range from 40 to 260 ºC, with a rate of 4 ºC min^{-1}, and a holding time of 10 min. The identification of the volatile organic compounds was performed by comparing their mass spectra and retention indices (RIs) with those obtained from available electronic MS-libraries (NIST Mass Spectral Library, Wiley Register).

Determination of antioxidant capacity of essential oils (ABTS and DPPH assays)

The ABTS assay was based on the procedure described by Re et al. (1999) with a slight modification, and the absorbance was measured at 734 nm. The antioxidant activity of *S. montana* essential oils was expressed as a percentage of the scavenging of ABTS free radicals (%).

The antioxidant capacity of *S. montana* essential oils was determined using the DPPH assay as well. The mentioned method was based on the procedure given by Mensor et al. (2001) with some modifications. The absorbance was measured at 517 nm, and the results were expressed as a percentage of the neutralization of DPPH free radicals (%).

All spectrophotometric measurements were performed in a UV-1800 spectrophotometer (Shimadzu, Japan).

Statistical analysis

The statistical analysis of the results from antioxidant assays was performed using analysis of variance (one-way ANOVA) and Duncan's *post hoc* test in STATISTICA 7.0. The differences were considered statistically significant at $p<0.05$.

Results and Discussion

Chemical characterization and measurement of ABTS and DPPH radical scavenging potential of the the essential oils from two chemotypes of *S. montana*, ct. carvacrol (SM_c), and ct. thymol (SM_t) were performed and the results are presented in Table 1 (chemical composition) and Table 2 (antioxidant activity).

Table 1. Chemical composition of essential oils from two chemotypes of Satureja montana, ct. carvacrol (SM_c), and ct. thymol (SM_t)

RI_L*	RI_E	Compounds	SM_c (%)	SM_t (%)
924	931	α-Thujene	0.61	0.52
932	939	α-Pinene	0.41	0.36
946	955	Camphene	0.24	0.20
974	981	1-Octen-3-ol	1.04	0.71
974	983	β-Pinene	/	0.11
988	993	Myrcene	1.08	0.85
988	997	3-Octanol	0.03	0.03
1002	1010	α-Phellandrene	0.13	0.11
1008	1016	δ-3-Carene	0.04	0.03
1014	1022	α-Terpinene	1.41	0.95
1020	1031	*para*-Cymene	5.71	5.71
1024	1035	Limonene	0.20	0.22
1026	1038	1,8-Cineol	0.29	0.15
1044	1050	*trans*-β-Ocimene	0.04	0.04
1054	1065	γ-Terpinene	9.68	3.85
1065	1075	*cis*-Sabinene hydrate	0.73	0.79
1177	1082	Dec-1-en-3-ol	0.11	0.14
1086	1094	Terpinolene	0.06	0.05
1095	1103	Linalool	0.33	0.25
1098	1106	*trans*-Sabinene hydrate	0.16	0.17
1118	1129	*cis*-*para*-Menth-2-en-1-ol	0.07	0.07
1174	1178	Borneol	1.35	1.10
1179	1187	Terpinen-4-ol	0.45	0.34
1186	1201	*para*-Cymen-8-ol	0.10	0.15
1199	1209	γ-Terpineol	0.20	0.36
1232	1239	Thymol, methyl ether	/	0.07
1248	1268	Thymoquinone	0.03	/
1289	1301	Thymol	30.43	70.69
1298	1319	Carvacrol	39.49	7.06
1349	1362	Thymol acetate	/	0.05
1417	1443	*trans*-Caryophyllene	2.42	0.89
1432	1449	*trans*-α-Bergamotene	0.03	0.04
1439	1462	Aromadendrene	0.07	0.07
1452	1477	α-Humulene	0.07	0.04
1478	1495	γ-Muurolene	0.06	0.09
1484	1503	Germacrene D	0.09	0.13
1500	1516	Viridiflorene	0.05	/
1505	1522	β-Bisabolene	1.96	2.44

1515	1529	Sesquicineole	0.03	0.06
1521	1538	β-Sesquiphellandrene	0.03	0.05
1522	1541	δ-Cadinene	0.07	0.13
1529	1553	*trans*-γ-Bisabolene	0.07	0.08
1577	1605	Spathulenol	0.05	0.07
1608	1612	Caryophyllene oxide	0.20	0.19
1639	1665	α-Muurolol	0.03	0.04
1649	1680	β-Eudesmol	/	0.05
1683	1701	*epi*-α-Bisabolol	0.06	0.11
		Monoterpene hydrocarbons	*19.61*	*12.99*
		Oxygenated monoterpenes	*74.83*	*82.13*
		Sesquiterpene hydrocarbons	*4.91*	*3.95*
		Oxygenated sesquiterpenes	*0.36*	*0.51*
			99.71	***99.58***

**RI_L - retention index obtainded from Mass Spectral Library (Wiley Register); RI_E - retention index obtainded from the essential oil of S. montana.*

The GC-MS analysis showed that in the SM_c essential oil 43 compounds were identified and quantified, while in the SM_t essential oil, there were 45 compounds (Table 1).

The most dominant compound in SM_c essential oil was carvacrol (39.49%), followed by thymol (30.43%), γ-terpinene (9.68%), *p*-cimene (5.71%), *trans*-caryophyllene (2.42%), β-bisabolene (1.96%), α-terpinene (1.41%) borneol (1.35%), myrcene (1.08%), and 1-octen-3-ol (1.04%). The remaining constituents, including α-thujene, α-pinene, δ-3-carene, camphene, 3-octanol, α-phellandrene, δ-3-carene, limonene, 1,8-cineo, *trans*-β-ocimene, *cis*-sabinene hydrate, dec-1-en-3-ol, terpinolene, linalool, *trans*-sabinene hydrate, *cis-para*-menth-2-en-1-ol, terpinen-4-ol, *para*-cymen-8-ol, γ-terpineol, thymoquinone, *trans*-α-bergamotene, β-sesquiphellandrene, aromadendrene, α-humulene, γ-muurolene, germacrene D, viridiflorene, sesquicineole, β-Sesquiphellandrene, δ-cadinene, *trans*-γ-bisabolene, spathulenol, caryophyllene oxide, α-muurolol, and *epi*-α-bisabolol, were presented in the amounts below 1% of the total content. The compounds identified in SM_t essential oil, such as β-pinene, thymol methyl ether, thymol acetate, and β-eudesmol, did not detected in SM_c essential oil.

The most abundant component in SM_t essential oil was thymol (70.69%), followed by carvacrol (7.06%), *p*-cimene (5.71%), γ-terpinene (3.85%), β-bisabolene (2.44%), and borneol (1.10%), whereas other oil constituents (α-thujene, α-pinene, δ-3-carene, camphene, 3-octanol, β-pinene, thymol methyl ether, thymol acetate, β-eudesmol, α-phellandrene, δ-3-carene, limonene, 1,8-cineo, *trans*-β-ocimene, *cis*-sabinene hydrate, dec-1-en-3-ol, terpinolene, linalool, *trans*-sabinene hydrate, *cis-para*-menth-2-en-1-ol, terpinen-4-ol, *para*-cymen-8-ol, γ-terpineol, *trans*-α-bergamotene, β-sesquiphellandrene, aromadendrene, α-humulene, γ-muurolene, germacrene D, sesquicineole, β-Sesquiphellandrene, δ-cadinene, *trans*-γ-bisabolene, spathulenol, caryophyllene oxide, α-muurolol, and *epi*-α-bisabolol) ranged from 0.03 to 0.89%. The compounds identified in SM_c essential oil, such as thymoquinone and viridiflorene, did not detected in SM_t essential oil.

For SM_c and SM_t essential oils, the total content of monoterpene hydrocarbons was 19.61 and 12.99%, of oxygenated monoterpenes, 74.83 and 82.13%, of sesquiterpene hydrocarbons, 4.91 and 3.95%, and of oxygenated sesquiterpenes, 0.36 and 0.51%, respectively.

Based on the literature data, the content of terpenes and their derivates differs between *S. montana* essential oils from different regions. The main components of *S. montana* essential oil include myrcene, *p*-cymene, γ-terpinene, linalool, thymol, carvacrol, and viridiflorol, and their concentrations differed among the plant varieties and chemotypes. Regarding that, thymol, followed by carvacrol were previously reported as the main constituents from populations originating from the Republics of Albania (Valbona valley national park) and its amount is about 31 and 16%, respectively (Hajdari et al., 2016; Ibraliu et al., 2010). Moreover, the area of the

Tenth village (Albania's Shkodra region) is rich in the population of *S. montana* which includes linalool chemotype, and the same chemotype is present in the Croatian regions of Biokovo, Drač, and Kozjak which stretch along the entire southwest coast of Adriatic Sea (Miloš et al., 2001). Further, the samples of *S. montana* essential oil in which *p*-cimen and linalool were the main chemical constituents were previously found in Serbia (the hill-mountainous belt that forms the border zone between Serbia and Albania) (Slavkovska et al., 2001). Also, there is some report that myrcene and viridiflorol chemotypes are only presented in the foot of the Golesh and Morinë Mountains (Hajdari et al., 2016).

Table 2. Antioxidant activity (ABTS and DPPH assays) of essential oils from two chemotypes of Satureja montana, ct. carvacrol (SM_c), and ct. thymol (SM_t)

sample	ABTS neutralization [%]	DPPH neutralization [%]
SM_c	$96.5\pm0.7^{a*}$	92.0 ± 0.4^{b}
SM_t	98.2 ± 0.6^{b}	89.8 ± 0.3^{a}

**Values with different letters (a-b) in each row showed statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's post-hoc test).*

According to the ABTS experiment, SM_c and SM_t had statistically different antioxidant capacity (96.5±0.7 and 98.2±0.6%, respectively, Table 2). The potential to neutralize DPPH radicals were also statistically significantly different, 92.0±0.4% for SM_c and 89.8±0.3% SM_t (Table 2). SM_c essential oil showed a higher antioxidant activity in DPPH assay, while SM_t essential oil possessed a higher ABTS radical scavenging capacity. Furthermore, winter savory essential oils, along with their principal components, carvacrol, thymol, *p*-cymene, and γ-terpinene among others, exhibit excellent antioxidant activities when used in proper concentrations in a variety of *in vivo* and *in vitro* models (Prieto et al., 2007). This result is in correlation with the findings obtained from the research of Zeljković et al. (2015). Namely, the phenolic compounds in the essential oil of *S. montana (*thymol and carvacrol), are responsible for the overall reactivity of the winter savory towards DPPH radicals. That claim has been confirmed by earlier studies on this topic (Ćavar et al., 2008; Radonić and Miloš, 2003; Vidović et al., 2014). Ćetković et al. (2007) came to the same outcomes as well. According to a literature review, the antioxidant activity of *S. montana* essential oil was concentration dependent. In literature can also be found that some varieties of winter savory possessed a strong reducing capacity, and it was more effective as a DPPH free radical scavenger than as an ABTS inhibitor (Trifan et al., 2015). Finally, in the study by Caprioli et al. (2018), the essential oil of *S. montana* (cultivated in the Central Mediterranean, Northern Italy) showed the IC_{50} about seventy times lower compared to the reference reagent (Trolox), while in ABTS assay, the essential oil was about ten times less active than reference reagent. Therefore, the main conclusions are that varied experimental approaches lead to dispersed results that are hardly comparable, and often contradictory, so the composition of essential oil is fundamentally important in determining the biological activity.

Conclusions

The aim of the present study was the isolation, chemical characterization, and examination of the antioxidant activity of the essential oils from two chemotypes of *S. montana*, ct. carvacrol (SM_c), and ct. thymol (SM_t). The most dominant compounds in SM_c essential oil (in descendent order) were carvacrol, thymol, γ-terpinene, *p*-cimene, *trans*-caryophyllene, β-bisabolene, α-terpinene, borneol, myrcene, and 1-octen-3-ol, whereas in SM_t essential oil were thymol, carvacrol, *p*-cimene, γ-terpinene, β-bisabolene, and borneol. SM_c essential oil showed a

higher antioxidant potential in the DPPH assay, while SM_t essential oil possessed a higher ABTS radical scavenging activity. The research presented here provides evidence on the chemical composition as well as the ABTS and DPPH radical scavenging potential of two different chemotypes of *S. montana* essential oils, as well as a foundation for their potential encapsulation and further application.

Acknowledgments

The authors acknowledge their gratitude to the Ministry of Education, Science and Technological Development of Serbia, contract numbers 451-03-47/2023-01/200003, 451-03-47/2023-01/200135, and 451-03-47/2023-01/200019.

Conflict of interest

The authors declare that they have no financial and commercial conflicts of interest.

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