

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL FROM ROMANIAN *Satureja montana* L.

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Abstract

The aim of the present study was to investigate the chemical composition and antioxidant activity of *Satureja montana* essential oil of Romanian origin (*S. montana* EO). The essential oil was obtained by hydrodistillation from dried aerial parts of cultivated plants in northeastern Romania. The chemical composition of *S. montana* EO was analysed by GC and GC-MS. The main compounds were carvacrol (63.40%), *para*-cymene (10.97%) and γ -terpinene (3.70%). *S. montana* EO showed a high reducing capacity ($IC_{50} = 64.35 \mu\text{g/mL}$), and was more effective as DPPH free radical scavenger ($IC_{50} = 243.80 \pm 4.93 \mu\text{g/mL}$) than ABTS⁺ inhibitor ($IC_{50} = 560.86 \pm 23.15 \mu\text{g/mL}$). *S. montana* essential oil of Romanian origin belongs to the carvacrol chemotype and represents a suitable agent for potential applicability in the protection of biological matrices from the free radical-mediated oxidative stress, including ionizing radiation-induced oxidative damage.

Rezumat

Acest studiu a avut drept obiectiv investigarea compoziției chimice și a activității antioxidante a uleiului volatil de *Satureja montana* din România (*S. montana* EO). Uleiul volatil a fost obținut prin hidrodistilarea părților aeriene uscate provenite de la plantele cultivate. Compoziția chimică a *S. montana* EO a fost analizată prin GC și GC-MS. Compușii principali identificați au fost: carvacrol (63,40%), *para*-cimen (10,97%) și γ -terpinen (3,70%). *S. montana* EO a prezentat o capacitate reducătoare ridicată ($CI_{50} = 64,35 \mu\text{g/mL}$), și a fost mai eficient ca *scavenger* de radicali liberi DPPH ($CI_{50} = 243,80 \pm 4,93 \mu\text{g/mL}$) comparativ cu inactivarea ABTS⁺ ($CI_{50} = 560,86 \pm 23,15 \mu\text{g/mL}$). Uleiul volatil de *Satureja montana* din România aparține chemotipului carvacrol și reprezintă un agent cu aplicabilitate potențială în protecția matricelor biologice față de afectarea oxidativă indusă de diferiți factori, inclusiv radiațiile ionizante.

Keywords: *Satureja montana*, essential oil, chemical composition, antioxidant

Introduction

The essential oils (EOs) are vegetal metabolites with a wide range of biological properties and uses. The antioxidant activity of EOs is of great interest in medicine, food and cosmetic industry but also in the development of effective radioprotective agents. *Satureja montana* L. (*Lamiaceae*), commonly known as mountain savoury or winter savoury, is an aromatic, perennial semishrub native to the Mediterranean area. It is found throughout Europe, Russia and Turkey, growing in sunny, stony and rocky regions [11, 16]. Winter savoury is used as medicinal plant, spice, and is one of the best honey plants [17]. The whole plant has antiseptic, stomachic, carminative and expectorant properties [18]. The bioactivity and the aromatic profile of *S. montana* are significantly influenced by the chemical composition of its essential oil (EO). Depending mostly on geographic area and its stages of development,

the occurrence of various *S. montana* EO chemotypes was reported [3, 10]. The prevailing carvacrol/thymol chemotypes determine a strong spicy flavour of the plants. They exhibit a significant antioxidant potential while the linalool or geraniol chemotypes have a lower activity [2]. Despite the fact that *S. montana* is an important medicinal and aromatic plant in Romania, there is little information on the chemical composition and antioxidant activity of EO from the Romanian species. The aim of this study was to investigate the chemical composition and *in vitro* antioxidant activity of *S. montana* EO isolated from cultivated plants of Romanian origin.

Materials and Methods

Chemicals

All chemicals were of analytical grade and were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and Merck (Darmstadt, Germany).

Plant material

The aerial parts of *S. montana* were harvested during flowering stage in July 2013 from the cultivated field of Stejarul Biological Research Centre, Piatra Neamt, a location in northeastern Romania. A voucher specimen was deposited at the Department of Pharmacognosy, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, Romania (SM1/Pharmacog/2013).

Essential oil isolation

100 g of air-dried aerial parts of plants were ground and mixed with 1500 mL of bi distilled water and subjected to hydrodistillation for 3 h in a modified Clevenger-type apparatus. *S. montana* EO was dried over anhydrous sodium sulphate and stored in a sealed dark glass at -4°C until it was analysed. The yield of *S. montana* EO (v/w %) was calculated on a dry weight basis.

GC and GC-MS analysis

The gas chromatography with flame ionization detector (GC-FID) analysis was performed using an Agilent 6890 gas chromatograph equipped with a flame ionization detector and a HP-5MS capillary column (30 m x 0.25 mm internal diameter, 0.25 µm film thickness). A volume of 0.2 µL of *S. montana* EO was injected in the split mode (split ratio 1:50). Helium was used as carrier gas at a flow rate of 1 mL/min. The analysis was performed using the following temperature program: 4°C/min from 40°C to 250°C, 10°C/min from 250°C to 300°C; the final temperature was held for 7.5 min. The GC-MS analysis of the oil was carried out on an Agilent type 7890A gas chromatograph, equipped with an Agilent 5975C mass spectrometer selective detector with electron impact ionization. The column and GC-MS conditions were the same as the ones described above [1].

Volatile compounds identification

The retention indices were determined by analysing a standard solution of *n*-alkanes (C₈–C₂₀) under the same chromatographic conditions. The compounds were identified by comparison of their recorded mass spectral data with those stored in the Wiley 275 mass spectral library from GC-MS database and their retention indices relative to *n*-alkanes with those mentioned in literature. The relative percentages of the essential oil constituents were obtained from the FID peak areas without using correction factors [2, 7].

In vitro antioxidant assays

S. montana EO was diluted with methanol and the concentration range is mentioned for each assay. Butylhydroxyanisole (BHA) was used as positive control. All spectrophotometric measurements were performed on ABLE-JASCO V550 UV-Vis spectrophotometer (Jasco, Japan).

DPPH radical scavenging assay

DPPH radical scavenging activity was assessed according to the method described by Mighri et al. [6, 8]. A volume of 1 mL of each EO dilution in

methanol (290 µg/mL - 4640 µg/mL) was mixed with 1 mL of DPPH methanolic solution (0.04%, w/v). The mixtures were vortexed and kept for 30 min at room temperature, in the dark. Then, the absorbance was measured at 517 nm. The percentage of DPPH radical scavenging was calculated using the equation:

$$100 \times [(A_0 - A_t) / A_0],$$

where A_0 is the absorbance of the blank and A_t is the absorbance in the presence of essential oil or positive control.

ABTS radical cation scavenging assay

The assay was performed as previously described by Re et al. [14]. ABTS radical cation (ABTS^{•+}) was generated by mixing together equal volumes of ABTS aqueous solution (7 mM) with potassium persulfate (2.45 mM). The mixture was left in the dark at room temperature for 12-16 h before use. Prior to testing, ABTS^{•+} solution, was diluted with methanol to an absorbance of 0.7 ± 0.02 at 734 nm. 0.02 mL of each methanolic dilution of EO (concentration ranging from 29 mg/mL to 464 mg/mL) was mixed with 1.98 mL ABTS^{•+} solution. The absorbance at 734 nm was measured after 6 min of reaction. ABTS^{•+} scavenging activity (%) was calculated using the formula: $100 \times [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}]$, where A_{control} is the absorbance of the control and A_{sample} is the absorbance in the presence of EO/positive control.

Reducing Power Assay

The assay was carried out using Oyaizu method [12]. 500 µL of each EO methanolic dilution (0.52-4.16 mg/mL) were mixed with 0.2 M sodium phosphate buffer (1.2 mL; pH 6.6) and 1% potassium ferricyanide (1.5 mL). The mixture was incubated at 50°C for 20 min. After cooling, 10% trichloroacetic acid (1.25 mL) was added to the mixture which was then centrifuged for 10 min at 3000 rpm. The upper layer of the solution (1.25 mL) was mixed with ultrapure water and with 0.1% (w/v) ferric chloride (0.25 mL). After 10 min, the absorbance was measured at 700 nm. An increased absorbance of the mixture indicates a good reducing power.

Statistical analysis

All antioxidant assays were performed in triplicate and the results are expressed as mean $\pm$ SD. The IC₅₀ value (µg/mL), which is the concentration of essential oil/positive control that reduces 50% of the free-radical concentration, was calculated through linear interpolation between values above and below 50% activity. In reducing power assay, IC₅₀ value represents the concentration of essential oil/positive control that leads to an absorbance of 0.5.

Results and Discussion*Chemical composition of essential oil*

The hydrodistillation of *S. montana* aerial parts gave a dark yellow oil with warm, peppery flavor. The yield of *S. montana* EO was 1.98% (19.8

mL/kg dried aerial parts). Twenty one compounds accounting for 90.79% of the total oil composition were identified (Table I).

Table IChemical composition of *S. montana* essential oil

Peak	Compound	RI ^a	RI ^b	%
1	α -thujene	923	923	0.23
2	α -pinene	930	932	0.67
3	β -myrcene	987	990	0.67
4	<i>para</i> -cymene	1024	1024	10.97
5	limonene	1027	1027	0.12
6	γ -terpinene	1056	1058	3.70
7	linalool	1097	1099	0.82
8	terpinen-4-ol	1175	1177	1.03
9	thymol methylether	1232	1235	1.57
10	carvone	1242	1243	0.66
11	geraniol	1257	1258	0.85
12	thymol	1290	1286	0.61
13	carvacrol	1307	1306	63.40
14	β -bourbonene	1383	1384	1.43
15	aromadendrene	1438	1441	1.43
16	α -humulene	1450	1450	0.30
17	germacrene D	1478	1478	0.35
18	β -bisabolene	1506	1508	0.48
19	γ -cadinene	1511	1513	1.01
20	δ -cadinene	1520	1524	0.23
21	caryophyllene oxide	1580	1581	0.26
Group of constituents				
Monoterpene hydrocarbons		5.39		
Oxygenated monoterpenes		3.36		
Aromatic compounds		76.55		
Sesquiterpenes		5.49		
Total identified		90.79		

^aRetention indices relative to C8–C20 n-alkanes calculated on HP-5MS capillary column; ^bretention indices reported in literature.

The main components were carvacrol (63.40%), *para*-cymene (10.97%) and γ -terpinene (3.70%). Aromatic compounds were predominant (76.55%) followed by monoterpenes (hydrocarbons and oxygenated derivatives) (8.75%).

A literature survey reveals that the essential oil content in *S. montana* plants varies depending on the geographic region. Winter savoury plants from Albania contain from 0.22 to 1.60% essential oil while those from Montenegro contain between 1.1 and 1.9%. For the Croatian varieties, the essential oil content ranged from 1.2 to 2.2%. Also, for Polish *S. montana* there has been reported a content of 2.27%. French winter savoury contains 1.56% essential oil while the plants harvested in Italy contain only 0.59% [18]. In our study, the content of *S. montana* EO was higher compared to most cited data.

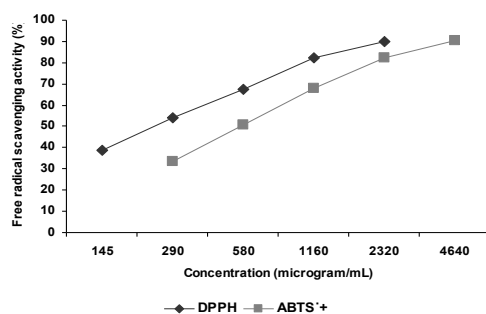
The chemical composition of EOs from *S. montana* plants shows an important variability according to various factors (climate, age of plants, vegetative cycle stage, location) [2]. Depending on the prevalence of phenols or monoterpene alcohols two main chemotypes can be described, namely A and B, each of them including several subtypes such as A (thymol), A (carvacrol), B (linalool), B (geraniol) [13]. Due to high levels of phenols (mainly carvacrol), Romanian *S. montana* EO belongs to chemotype A (carvacrol) as well as EOs from former Yugoslavia, Italy [4, 5] or Portugal [15].

In vitro antioxidant activity

The antioxidant activity of *S. montana* EO was assessed by free radicals (DPPH and ABTS^{•+}) scavenging assays and also by the reducing power assay. *S. montana* EO exhibited a concentration-dependent antioxidant activity. It possessed a high reducing capacity, and it was more effective as DPPH free radical scavenger than as ABTS^{•+} inhibitor (Table II). At the concentration of 2320 μ g/mL, *S. montana* EO caused a 90% inhibition of the DPPH radical. A similar inhibition was noticed in ABTS^{•+} assay for the concentration of 4640 μ g/mL (Figure 1). The maximum reduction of ferric complex (absorbance 0.89 at 700 nm) was obtained for a concentration of 225.26 μ g/mL.

Table IIAntioxidant activity of *S. montana* essential oil

Essential oil / positive control	IC ₅₀ (μ g/mL)		
	DPPH assay	ABTS ^{•+} assay	Reducing power assay
<i>S. montana</i> EO	243.80 $\pm$ 4.93	560.86 $\pm$ 23.15	64.35 $\pm$ 0.00
BHA	3.6 $\pm$ 0.07	1.81 $\pm$ 0.05	4.32 $\pm$ 0.04

**Figure 1.**Free radical scavenging activity of *S. montana* EO

Although, *S. montana* EO was less active than the positive control (BHA), it showed a better radical scavenging activity as compared to the data reported in literature. For the essential oils from Bosnia and Herzegovina, Cavar et al (2008) have reported the IC₅₀ values of 5490 $\pm$ 260 μ g/mL and 18900 $\pm$ 190 μ g/mL, respectively in the DPPH assay [2]. In the same assay, Serrano et al. (2011) found an IC₅₀ of 508.45 $\pm$ 5.8 μ g/mL for the Portuguese EO [15] while Miladi et al (2013) reported an IC₅₀ of 410.5 $\pm$ 4.27 μ g/mL for EO from French winter savoury [9].

The antioxidant potential of Romanian winter savoury essential oil is due to the high content of phenolic constituents (76.55%) and also to the low levels of sesquiterpenes, compounds considered to be weak antioxidants [2].

Conclusions

These findings suggest that the essential oil obtained from Romanian *Satureja montana* belongs to the phenolic chemotype (carvacrol). It exhibits a remarkable antioxidant activity with potential applicability in the protection of susceptible matrices from free radical-mediated oxidative stress, including ionizing radiation-induced oxidative damage.

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References

1. Aprotosoiaie A.C., Șpac A., Hâncianu M., Miron A., Tănăsescu V.F., Dorneanu V., Stănescu U., The chemical profile of essential oils obtained from fennel fruits (*Foeniculum vulgare* Mill.). *Farmacia*, 2010; 58(1): 46-53.
2. Cavar S., Maksimovic M., Šolic M.E., Jerkovic A., Bešta R., Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. *Food Chem.*, 2008; 111: 648-653.
3. Chizzola R., Volatile oil composition of four populations of *Satureja montana* L. from Southern France. *Acta Hort.*, 2003; 598: 143-147.
4. Fraternali D., Giamperi L., Bucchini A., Ricini D., Epifano F., Genovese S., Curini M., Chemical composition and antifungal activity of the essential oil of *Satureja montana* from central Italy. *Chem. Nat. Comp.*, 2007; 43(5): 622-624.
5. Hassanein H.D., Sailyd-Al A.H., Abdelmohsen M.M., Antioxidant polyphenolic constituents of *S. montana* L. growing in Egypt. *Int. J. Pharm. Pharm. Sci.*, 2014; 6(4): 578-581.
6. Lungu Apetrei C., Spac A., Brebu M., Tuchilus C., Miron A., Composition, and antioxidant and antimicrobial activities of the essential oils of a full-grown *Pinus cembra* L. tree from the Calimani Mountains (Romania). *J. Serb. Chem. Soc.*, 2013; 78(1): 27-37.
7. Michaelakis A., Theotokatos S.A., Koliopoulos G., Chorianopoulos N.G., Essential oils of *Satureja* species: insecticidal effect on *Culex pipiens* larvae (Diptera: Culicidae). *Molecules*, 2007; 12: 2567-2578.
8. Mighri H., Hajlaoui H., Akrouit A., Najjaa H., Neffati M., Antimicrobial and antioxidant activities of *Artemisia herba-alba* essential oil cultivated in Tunisian arid zone. *CRC Chimie*, 2010; 13: 380-386.
9. Miladi H., Ben Slama R., Mili D., Zouari S., Bakhrouf A., Ammar E., Chemical composition and cytotoxic and antioxidant activities of *Satureja montana* L. essential oil and its antibacterial potential against *Salmonella* spp. strains. *Journal of Chemistry*, 2013; Article ID 275698, 9 pages. doi.org/10.1155/2013/275698
10. Milo's M., Radonic A., Bezic N., Dunkic V., Locaties and seasonal variations in the chemical composition of essential oils of *S. montana* L. and *S. cuneifolia* Ten. *Flav. and Fragr. J.*, 2001; 16: 157-160.
11. Oliveira T.L.C., de Araújo Soares R., Mendes Ramos E., das Graças Cardoso M., Alves E., Piccoli R.H., Antimicrobial activity of *Satureja montana* L. essential oil against *Clostridium perfringens* type A inoculated in mortadella-type sausages formulated with different levels of sodium nitrite. *Int. J. Food Microbiol.*, 2011; 144(3): 546-555.
12. Iordache T.A., Vlase L., Istudor V., Gird C.E., Researches regarding obtaining selective extracts with hypoglycemic properties from vegetal indigenous products (*Cichorii herba* and *Fraxini folium*). Note III. Phenolic compounds analysis from *Fraxini folium*. *Farmacia*, 2013; 61(4): 625-631.
13. Prieto J.M., Iacopini P., Cioni P., Chericoni S., *In vitro* activity of the essential oils of *Origanum vulgare*, *Satureja montana* and their main constituents in peroxynitrite-oxidative processes. *Food Chem.*, 2007; 104: 889-895.
14. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C., Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio, Med.*, 1999; 26: 1231-1237.
15. Serrano C., Matos O., Teixeira B., Ramos C., Neng N., Nogueiro J., Nunes M.L., Marques A., Antioxidant and antimicrobial activity of *Satureja montana* extracts. *J. Sci. Food Agric.*, 2011; 91: 1554-1560.
16. Slavskovska V., Jancic R., Bojovic S., Milosavljevic S., Djokovic D., Variability of essential oils of *Satureja montana* L., and *Satureja kitaibelii* Wierzb. ex. Heuff. from the central part of the Balkan Peninsula. *Phytochemistry*, 2001; 57(1): 71-76.
17. Vidic D., Maksimovic M., Cavar S., Šolic M.E., Comparison of essential oil profiles of *Satureja montana* L., and endemic *Satureja visianii* Šilic. *Jeobp*, 2009; 12 (3): 273-281.
18. Wesolowska A., Grzeszczuk M., Jadczać D., Influence of harvest term on the content of carvacrol, p-cymene, γ -terpinene and β -caryophyllene in the essential oil of *Satureja montana*. *Not. Bot. Horti. Agrobi.*, 2014; 42(2): 392-397.