Composition and Antioxidant Capacity of Essential Oils Obtained from *Thymus vulgaris, Thymus pannonicus* and *Satureja montana* Grown in Western Romania

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The study describes the phytochemical profile along with the antioxidant properties of the essential oils (EOs) from Satureja montana, Thymus vulgaris and Thymus pannonicus, grown in western Romania. The chemical composition of the EOs was evaluated by GC-MS. The phytochemical profiles indicated that the T. vulgaris and S. montana EOs belong to the carvacrol chemotype, while the T. pannonicus EO belongs to the thymol/p-cymene chemotype. The DPPH radical-scavenging method was used to test the antioxidant activity. T. pannonicus EO showed the lowest antioxidant capacity (1009.83 μg mL^{-1} of DPPH inhibition), followed by the S. montana EO (861.09 μg mL^{-1}) and T. vulgaris EO (783.87 μg mL^{-1}). According to the data known to us so far, the antioxidant activity of the T. pannonicus EO has not been reported in other experiments. The results confirm the potential of using EOs as natural antioxidants, an alternative to the use of synthetic additives.

Keywords: Thyme, Winter savory, Essential oil, GC-MS analysis, IC50

The oxidation of lipids in foods is responsible for the formation of off-flavours and undesirable chemical compounds which may be detrimental to health [1]. In the food industry various synthetic antioxidants are used to prevent lipid oxidation, such as BHA, BHT, propyl gallate. However, because of possible health risks and toxicity the use of these food additives has been restricted [2]. Due to negative consumer perceptions of synthetic additives, there have been great efforts to find safe and potent natural antioxidants [3, 4]. Various sources have been investigated, including EOs. Some authors report that EOs have properties comparable to, and sometimes better than those of synthetic antioxidants [3-7].

Lamiaceae or *Labiatae*, also called as the mint family, contains about 236 genera and 6900 to 7200 species [8]. *Thymus* and *Satureja* genera *(Lamiaceae* family*)* include numerous wild species and cultivated plants. In Romania, the *Thymus* genus contains one species cultivated as an aromatic plant (*Thymus vulgaris*) and other 18 wild species [9]. The genus *Satureja* is represented in Romanian flora by one species cultivated as an aromatic plant (*Satureja hortensis*) and other 5 wild species (including *S. montana*) [10].

Within the *Thymus* and *Satureja* genera many species present different intraspecific chemotypes; the chemical composition of the essential oils is variable in relation to the stage of development of the plant, the harvesting time, and the field environment conditions [11, 12]. For the essential oils isolated from *Thymus vulgaris* six chemotypes have been reported: geraniol, linalool, γ-terpineol, carvacrol, thymol and trans-thujan-4-ol/terpinen-4-ol [13]; for *Thymus pannonicus* to date the thymol/*p*-cymene and geranial/neral chemotypes have been reported [14]; while for *Satureja montana* four chemotypes have been reported: thymol, carvacrol, linalool, *p*-cymene and α-terpinene [12].

A number of studies has demonstrated the antioxidant properties of *T. vulgaris* and *S. montana* [3-5, 15]. Unfortunately there are very few quantitative data (IC_{50}) regarding the antioxidant properties of EOs obtained from *T. vulgaris* and *S. montana* grown in Romania [9, 16]. Also according to our knowledge to date, the antioxidant activity of the *T. pannonicus* EO has not been reported in other experiments.

The aim of this study was (i) to determine the chemical composition of essential oils (EOs) from *T. vulgaris*, *T. pannonicus* and *S. montana* grown in Romania, and (ii) to determine and compare the functional antioxidant properties of these EOs tested along with 4 most used food antioxidants (ascorbic acid, α-tocopherol, propyl gallate, BHA).

Experimental part

Plant material

The aerial parts of *S. montana*, *T. vulgaris* and *T. pannonicus* were collected from the experimental lots of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" at Timisoara in June-July 2012. Harvesting was done manually at the time of the plants' maximum flowering stage. The plant material was dried under natural conditions (spaces away from sunlight, cool, naturally ventilated) and stored in double paper bags at temperatures of 3-5°C. Voucher specimens were collected from each species that were identified and deposited in the herbarium of the Department of Agricultural Technologies, Faculty of Agronomy, Banat's

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Constituent	RI	*Thymus vulgaris* (%)	*Thymus pannonicus* (%)	*Satureja montana* (%)	Identification
alpha-Pinene	934	0.90	0.75	0.90	1,2
Camphene	945	-	0.40	0.30	1,2
beta-Pinene	977	0.30	0.25	0.30	1,2
3-Octanone	981	-	3.65	-	1,2
beta-Myrcene	984	1.50	1.60	2.10	1,2
Carene (δ-2)	1001	0.35	0.35	2.30	1,2
alpha-Phellandrene	1004	1.05	1.35	1.65	1,2
alpha-Terpinene	1014	1.05	2.05	1.05	1,2
para-Cymene	1022	**19.2**	**12.0**	**8.30**	1,2,3
1,8-Cineole (Eucalyptol)	1025	0.55	1.00	1.02	1,2
gamma-Terpinene	1051	4.20	**13.9**	**9.40**	1,2,3
Linalool	1094	3.20	0.30	0.65	1,2
Camphor	1148	0.80	0.05	0.50	1,2
Borneol	1167	1.05	0.70	0.65	1,2
Terpinen-4-ol	1175	0.60	-	0.05	1,2
Thymol methyl ether	1235	-	3.75	-	1,2
Carvacrol methyl ether	1246	-	3.45	-	1,2
Thymol	1277	7.60	**34.9**	-	1,2,3
Carvacrol	1297	**44.6**	2.30	**60.9**	1,2,3
Caryophyllene	1409	3.85	3.95	3.15	1,2
Caryophyllene oxide	1596	-	4.65	-	1,2
Total		**91.70**	**92.25**	**94.02**	

1 = retention index; 2 = comparison with MS database; 3 = co-injection with authentic compounds.
-, not detected.

Table 1
CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM ROMANIAN *T. VULGARIS, T. PANNONICUS AND S. MONTANA*

University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" at Timisoara (VSNH.BUASTM-99, VSNH.BUASTM-101 and VSNH.BUASTM-118).

Isolation of essential oils

The EOs were extracted by steam distillation according to the method previously described by Craveiro et al., 1976 [17]. To prevent the formation of artefacts due to overheating that can occur during the isolation of EOs, a water-cooled oil receiver was used. The EOs were separated by decantation, then dried on anhydrous sodium sulfate (Sigma-Aldrich, Germany) and stored prior to analysis in hermetically sealed vials at 4°C.

Free radical scavenging activity (DPPH assay)

The radical scavenging activity was determined by the DPPH assay, as described by Brand-Williams [1]. Briefly, 3 mL of methanolic stock solutions of each tested EO (1 mg/mL) were prepared, and then diluted to different concentrations (0.01-0.5 mg/mL). 0.5 mL of each diluted sample were mixed with 5 mL methanolic solution of DPPH 0.06 mM. The samples were mixed at 800 rpm using a Thermomixer comfort (Eppendorf, Germany) and kept in the dark for 15 min. The same procedure was repeated for ascorbic acid, α-tocopherol, propyl gallate and butylated hydroxyanisole (BHA) (Sigma-Aldrich Chemie GmbH), used as positive controls. Absorbance measurements were read spectrophotometrically at 517 nm using a Cecil UV/VIS spectrophotometer (Model CE 7200, Milton, England). As negative control was used a methanolic solution of DPPH 0.06 mM and methanol (99.8%) as blank.

The inhibition of the DPPH radical in percent (I%) was calculated according to the following equation:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \cdot 100$$

where: A_{blank} is the absorbance of the control, and A_{sample} is absorbance of the test sample.

IC_{50} was obtained using the BioDataFit 1.02 software (Chang Broscience Inc, Castro Valley, CA, USA). Each test was performed in triplicate.

Gas chromatography-mass spectroscopy (GC-MS)

The qualitative analysis of EOs samples was performed on a gas-chromatograph coupled with a quadrupole mass spectrometer model CLARUS 500 - PERKIN ELMER. To obtain the mass spectra was used electron impact (EI) with an electron energy of 70 eV. The sample components were identified by the comparison of the obtained mass spectra with mass spectra in the NIST spectra library, by comparing the retention times with retention times of standards purchased from Sigma-Aldrich Chemie GmbH and by comparing the retention times (calculated from the retention times of alkanes C_9-C_{16}) with the values cited in literature[18].

To determine the concentrations of the sample components was used a gas-chromatograph model CLARUS 500 – PERKIN ELMER, equipped with a flame-ionization detector (FID) and a Perkin Elmer Elite-1701 (14% Cyanopropylphenyl) 15-m capillary column, 0.53 mm i.d. and 1.00 µm thickness of the stationary-phase layer. The temperature of the detector (FID) was 250 °C, of the injector 70-260 °C with a temperature gradient of 5 °C/min, and of the oven 60-250 °C with a temperature gradient of 5 °C/min, the carrier gas being helium 6 mL/min. From the sample diluted with chloroform in a ratio of 1/50 v/v, 1 µL was injected in the on-column mode (directly into the column).

To determine the concentrations of the major compounds in the samples, the internal standard method was used. The concentrations of the minor compounds were calculated using the method of internal normalization of peak areas without response factor.

Results and discussions

The yield of EO (%v/w) was 1.31% for *T. vulgaris*, 0.39% for *T. pannonicus* and 1.04% for *S. montana*; the chemical components identified are reported in table 1. The GC-MS analysis identifies 16 compounds in the *T. vulgaris* oil, representing 91.7% of the total EO; carvacrol (44.6%) and *p*-cymene (19.2%) are the major components. In the oil of *S. montana* were identified 16 components representing 94.02% of the total detected constituents, carvacrol

Samples	IC_{50} (μg mL^{-1})	SEE	R^2	SD
Ascorbic Acid	28.56	4.63	0.99	0.14
BHA	9.45	3.94	0.99	0.18
α-Tocopherol	64.90	7.93	0.97	0.21
Propyl gallate	15.97	1.44	0.99	0.19
EO *Satureja montana*	861.09	4.57	0.98	0.16
EO *Thymus vulgaris*	783.87	1.84	0.99	1.84
EO *Thymus pannonicus*	1009.83	1.21	0.99	0.05

Table 2
THE ANTIOXIDANT ACTIVITY OF ROMANIAN *T. VULGARIS*, *T. PANNONICUS* AND *S. MONTANA*

(60.9%) also being the dominant component along with gamma-terpinene (9.4%) and *para*-cymene (8.3%). Twenty compounds were identified in the *T. pannonicus* oil, representing 92.25% of the total EO; the main components were *p*-cymene (12%), thymol (34.9%) and gamma-terpinene (13.9%).

The phytochemical profile indicated that the *T. vulgaris* EO analyzed belongs to the carvacrol chemotype, in contrast with previous results reported in Romania [9, 19]; however our findings were in agreement with the profile reported by Hudaib and Aburjai in Jordan [20]. The high percentage of carvacrol (60.9%) indicated that the *S. montana* EO studied belongs to the carvacrol chemotype, in agreement with results found by Trifan et al. [16], Prieto et al. [21] and Masteliæ and Jerkoviæ [22]. In contrast, other studies reported the *S. montana* EO as belonging to the thymol chemotype [3, 15, 23]. Our study indicated that the *T. pannonicus* EO belongs to the thymol/*p*-cymene chemotype, similarly with results reported previously in Hungary [24] and Slovakia [14]. In contrast, *T. pannonicus* EOs from Serbia belong to the geranial, α-pinene and germacrene-D chemotypes [25, 26]. These differences may be due largely to chemical polymorphism within the *Thymus* and *Satureja* genera, caused by environmental factors and genetic variation due to frequent hybridisation and sexual dimorphism (gynodioecy) [14].

The antioxidant properties of *T. vulgaris*, *T. pannonicus* and *S. montana* EOs were evaluated by the DPPH radical scavenging method (table 2).

All three EOs exhibited a lower antiradical capacity than the positive control used (BHT, BHA, propyl gallate and á-tocopherol); the results show that the *T. pannonicus* EO showed the lowest antioxidant capacity (1009.83 μg mL^{-1} of DPPH inhibition), followed by the *S. montana* EO (861.09 μg mL^{-1}) and *T. vulgaris* EO (783.87 μg mL^{-1}).

The IC_{50} of the *T. vulgaris* EO found in our study is higher than in previous studies [27, 28]. The antioxidant activity recorded can be attributed to the major components of the *T. vulgaris* EO, thymol, carvacrol and gamma-terpinene, known for their antioxidant potential [21, 28-32]; while *p*-cymene, the second major component by percentage, exerts a very low antioxidant activity [31]. The same bioactive constituents are also present in the *T. pannonicus* EO, but are probably found in a proportion insufficient to exert strong antioxidant activity. To our knowledge, no data have been published on the antioxidant activity, using the DPPH method, of the *T. pannonicus* EO. For the *S. montana* EO this study shows a higher antiradical capacity than previously reported by Cavar et al. [23]. In contrast, Serrano et al. [15] reported a lower IC_{50} for the *S. montana* EO. Safaei-Ghomi et al. and Tepe et al. report for carvacrol, the main component of the *S. montana* EO (ca. 61%), a lower IC_{50} than for the whole plant EO [29, 31].

These observations suggest possible antagonistic effects between the chemical constituents of the studied EO. Even the presence in significant proportions of phenolic monoterpenes in the chemical composition of EOs is not synonymous with antioxidant activity [30]. The antioxidant activity is dependent on the synergistic or antagonistic effects of the components, being a resultant of their bioactive properties.

Conclusion

The study reports for the first time data on the antioxidant activity of the *T. pannonicus* EO and complements the existing data in the literature on the biological activity of EOs isolated from *T. vulgaris* and *S. montana*. The antioxidant activity recorded could be due in part to the presence in the chemical composition of compounds like thymol, carvacrol and gamma-terpinene, respectively. The results suggest possible antagonistic effects of the constituents of the EOs studied. The results confirm the potential of using EOs as natural antioxidants with applicability in the food industry, an alternative to the use of synthetic additives. However, for the use of EOs as food additives, new studies are needed that would explain synergistic, antagonistic or additive effects of the EOs constituents, along with a safety assessment in relation to the food consumer.

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