SHORT REPORT

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Composition of Essential Oil, Radical Scavenging and Antibacterial Properties of Interspecific Hybrid *Thymus* × *oblongifolius* Opiz

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Abstract: Radical scavenging and antibacterial properties of thyme extracts isolated from the two different *Thymus* × *oblongifolius* Opiz samples (TO1 and TO2) were studied. The oil of the TO1 was constituted mainly of hydrocarbon terpenes (83.4%), while the oil of the TO2 was composed mainly of oxygenated compounds with α-terpenyl acetate (37.9%) being the major one. The extracts isolated with hexane, acetone and ethanol from the whole and deodorized herb were tested for their radical scavenging capacity (RSC) in the model reaction systems containing stable radical $DPPH^{\bullet}$ and cation radical $ABTS^{\bullet+}$. The extracts isolated with ethanol from undeodorized herb possessed the strongest RSC, while hexane extracts were not active. Undeodorized acetone extracts of the TO1 possessed higher RSC than those of the TO2, while undeodorized ethanol extracts of the TO2 were stronger radical scavengers than similar extracts of the TO1. The antibacterial activity of the extracts tested by using seven food pathogenic species depended on the species, extract concentration and extract type. *Bacillus cereus*, *Staphylococcus aureus* and *St. epidermidis* were more sensitive to plant extracts than *Micrococcus luteus*, *Escherichia coli*, *Salmonella typhimurium* and *Enterobacter aerogenes*.

Keywords: Interspecific hybrid *Thymus* × *oblongifolius* Opiz; radical scavenging capacity; DPPH; ABTS; antibacterial activity.

1. Plant Source

T. × *oblongifolius* Opiz (*T. pulegioides* L. × *T. serpyllum* L.) is the interspecific hybrid of natural origin growing wild in Lithuania [1]. In terms of chemical composition, the essential oils of *T.* × *oblongifolius* are intermediate products if compared with those of parental species [2].

Two *T.* × *oblongifolius* samples (TO1 and TO2) were collected from the experimental field collection of the Institute of Botany, Vilnius, Lithuania. The aerial parts were harvested at the flowering phase and air-dried at room temperature. The voucher specimens are deposited in the Herbarium of the Institute of Botany (BILAS, Vilnius, Lithuania); they were labeled 68708 and 68695 for the samples TO1 and TO2, respectively.

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2. Previous Studies

During the last few decades the aromatic and spice plants have become a subject for a search of natural antioxidants and antibacterial agents [3, 4]. The essential oils and extracts isolated from various species of the genus *Thymus* were reported to possess high antioxidant activity and antibacterial properties [5,6]. Therefore, the essential oils and extracts of thyme may be considered as a promising source for natural preservative ingredients to be used in food and other applications [7].

In our recent study the essential oil composition of 17 samples of *T.* × *oblongifolius* was analyzed and wide chemodiversity was established [8]. Two selected samples of the studied *T.* × *oblongifolius* were moved from natural habitats into the experimental field collection. These samples were used for further studies with the aim to examine the composition of their essential oils, as well as antioxidant and antibacterial properties of plant extracts isolated by using the solvents of different polarity.

3. Present Study

Isolation of Essential Oils and Preparation of Extracts: The essential oils of aerial parts of the studied *T.* × *oblongifolius* samples were isolated by the hydrodistillation in a European Pharmacopoeia apparatus during two hours. The extracts were prepared from the whole dried plant material and deodorized part, the residue obtained after hydrodistillation. Three solvents of different polarity, namely n-hexane, acetone and ethanol, were used for the isolation of the active components from the whole and deodorized *T.* × *oblongifolius* herb. The extraction procedure is described in S2 and summarized in S1.

Essential oil analysis: The essential oils were diluted in diethyl ether (20 µL in 1 mL) and analyzed with a Fisons 8261 gas chromatograph equipped with flame ionization detector and fused silica capillary column DB-5, 25 m, i.d. 0.32 mm, film thickness 0.5 µm. Helium was used as a carrier gas with a flow rate of 1.6 mL/min; detector's temperature was 260°C, oven temperature was programmed from 40°C to 250°C at the rate of 4°C/min. Split injector was heated at 250°C, split ratio was 15:1. For the identification of the essential oils constituents they were also analyzed on a HP 5890 (II) instrument equipped with a 5971 series mass selective detector in the electron impact ionization mode at 70eV, and the following GC parameters: helium as carrier gas at a flow rate of 2 mL/min; fused silica HP5 MS column (Hewlett Packard, crosslinked 5% phenyl methyl silicone) 30 m length, 0.25 mm id, 0.25 µm film thickness, temperature program from 40 to 250°C increasing at 4°C/min. Split injector was heated at 250°C, split ratio was 1:10. Identification was based mainly on the comparison of retention indices (RIs) [9-10] and mass spectra (NIST/EPA/NIH Mass Spectral Database NBS75K).

DPPH$^{\bullet}$ Radical Scavenging Assay: Radical scavenging capacity (RSC) of thyme extracts against stable DPPH$^{\bullet}$ was determined by a slightly modified DPPH$^{\bullet}$ radical scavenging assay [11]. It is widely used reaction based on the ability of the antioxidant molecule to donate hydrogen to DPPH$^{\bullet}$, which consequently turns into an inactive form. The method is described in S2.

ABTS Radical Cation Decolourisation Assay: ABTS$^{\bullet+}$ radical cation was produced by reacting ABTS with potassium persulfate ($K_2S_2O_8$) [12]. The method is described in S2.

Assessment of antibacterial effect: Seven food spoilage bacteria, including gram-positive, namely *Bacillus cereus* (ATCC 10876), *Micrococcus luteus*, *Staphylococcus aureus* (ATCC 25923), *St. epidermidis* and gram-negative pathogens, namely *Salmonella typhimurium* (ATCC 14028), *Esherichia coli* (ATCC 25922) and *Enterobacter aerogenes* (ATCC 13048) were used in this study. The method is described in S2.

Table 1. Composition of the oil (GC area %) of *Thymus* × *oblongifolius*

Component	RI	Sample	
		TO1	TO2
α-Thujene	926	0.6	0.1
α-Pinene	932	4.0	0.5
Camphene	946	4.7	2.2
Sabinene	972	1.8	0.1
β-Pinene	975	2.9	0.4
1-Octen-3-ol	982	–	10.9
Myrcene	993	12.0	t
α-Phellandrene	1002	0.1	t
α-Terpinene	1016	0.7	0.1
p-Cymene	1028	0.8	0.8
Limonene	1030	–	0.9
(Z)-β-Ocimene	1037	11.3	0.4
(E)-β-Ocimene	1048	1.5	0.1
γ-Terpinene	1056	2.7	0.1
Terpinolene	1087	0.3	0.1
Linalool	1101	3.6	1.6
allo-Ocimene	1128	–	t
Borneol	1166	t	0.1
Dihydrocarvone	1191	–	0.1
α-Terpineol	1198	0.3	–
Thymol methyl ether	1232	0.1	–
Carvacrol methyl ether	1241	0.4	t
Geraniol	1249	–	0.7
Geranyl formate	1298	0.9	–
Thymol	1306	0.1	0.3
Carvacrol	1317	1.7	0.1
α-Elemene	1337	0.9	t
α-Terpinyl acetate	1346	–	37.9
Carvacryl acetate	1370	1.9	–
α-Copaene	1376	1.3	
Geranyl acetate	1384	0.1	–
β-Elemene	1392	0.1	–
β-Caryophyllene	1426	16.2	11.5
β-Gurjunene	1431	1.9	0.7
γ-Elemene	1435	t	t
(E)-iso-Eugenol	1452	0.8	0.6
α-Humulene	1456	0.9	0.4
allo-Aromadendrene	1463	0.7	0.5
γ-Muurolene	1479	0.3	0.1
Germacrene D	1487	14.5	4.1
cis-β-Guaiene	1494	–	2.4
β-Bisabolene	1510	1.9	0.2
γ-Cadinene	1516	0.7	0.3
δ-Cadinene	1525	0.6	2.3
Cadina-1,4-diene	1535	–	0.2
α-Cadinene	1539	t	t
cis-Muurol-5-en-4-β-ol	1543	–	0.1
Germacrene B	1572	t	–
Geranyl butyrate	1574	0.3	t
Spathulenol	1581	–	0.2
Caryophyllene oxide	1588	0.4	1.9
Viridiflorol	1598	–	t
epi-α-Cadinol	1648	–	6.1
Total		**94.0**	**89.1**

t = trace (<0.05%)

The content of essential oil in the interspecific *T.* × *oblongifolius* hybrids TO1 and TO2 was 0.3±0.01% and 0.5±0.03% (by dry mass), respectively. The composition of essential oils of TO1 and TO2 is presented in Table 1. In total, 56 compounds were identified by capillary GC and coupled GC/MS. Hydrocarbon monoterpenes (43.4%) and sesquiterpenes (40.0%) where the major chemical classes in TO1. The TO1 was selected in this study as a chemotype accumulating mainly hydrocarbon terpenes. The main essential oil constituents in the TO2 were α-terpenyl acetate (37.9%) followed by β-caryophyllene (11.5 %) and 1-octen-3-ol (10.9%). Thus, the oil of the TO2 was composed mainly of oxygenated compounds, monoterpenes constituting only 5% in the total oil content. The TO2 was selected in this study as a representative producing high amounts of oxygenated compounds.

Volatile compounds present in the essential oil of aromatic plants are responsible for their aroma characteristics, while antioxidant and antimicrobial properties may be associated with their bioactivities and health effects. Therefore, the use of so-called agrorefinery approach, aimed at utilization of raw plant material in the most efficient way, e.g. optimal use of various anatomical parts, volatile and non-volatile fractions, isolation and fractionation of different components with various beneficial properties, etc., seems to be a promising trend in the processing of such plants. Essential oil in aromatic plants very often constitutes only up to 1% in the total mass, therefore the possibilities to explore the plants for other purposes should also be considered. In our study we examined radical scavenging and antimicrobial properties of plant extracts, isolated from the whole dried plant material and the residue which is obtained after distilling the essential oil.

The model reaction systems containing stable radical $DPPH^{\bullet}$ and cation radical $ABTS^{\bullet+}$ (Figure 1) were used to assess radical scavenging capacity (RSC) of *T.* × *oblongifolius* extracts. These methods are simple and widely used for the fast screening of plant antioxidant properties and they provide quite reliable preliminary information on the presence of antioxidatively active constituents in the extracts.

In general either reactions are based on the ability of radicals to accept an electron or hydrogen radical, however, the main difference is that DPPH$^{\bullet}$ can be only dissolved in organic solvents (e.g., methanol), while ABTS$^{\bullet+}$ is soluble in both aqueous and organic media. Therefore, the ABTS$^{\bullet+}$ test can be performed in hydrophilic and lipophylic systems [12]. The extracts were isolated consecutively by using increasing polarity solvents, hexane, acetone and ethanol. In general, the results demonstrated the effect of three factors on the RSC, namely, the polarity of the solvent, the type of free radical used in reaction and plant material treatment before extraction (whole plant or deodorized part).

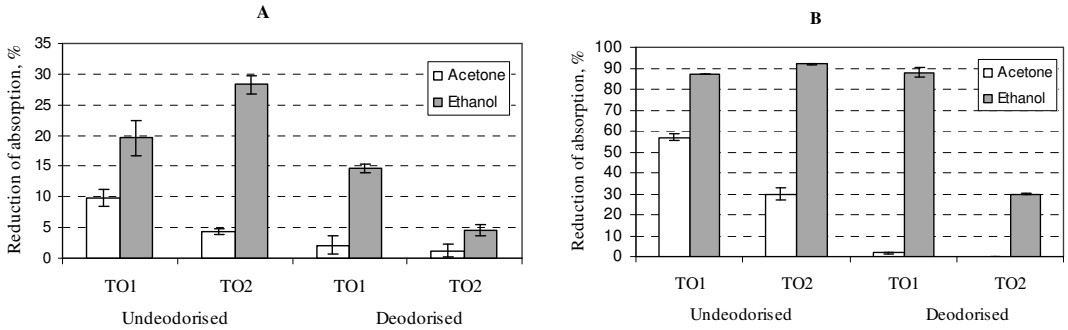

Figure 1. Radical scavenging capacity of *Thymus* × *oblongifolius* extracts
A – ABTS$^{\bullet+}$, B – DPPH$^{\bullet}$, TO1 and TO2 – the samples of *Thymus* × *oblongifolius*

It may be observed that the extracts isolated by using higher polarity solvent ethanol possessed stronger RSC as compared with the extracts isolated with lower polarity solvent acetone. Hexane extracts did not scavenge free radicals. It indicates that antioxidatively active compounds in *T.* × *oblongifolius* are mainly polar phytochemicals, most likely as in many other *Lamiaceae* species belonging to the classes of phenolic acids, flavonoids and their derivatives. Comparing two investigated samples it may be observed that acetone extracts of the TO1 possessed higher RSC than TO2, while in case of undeodorized ethanol extracts; on the contrary, the TO2 was stronger radical scavenger than TO1. However, in case of deodorized ethanol extracts the TO1 possessed stronger RSC than TO2. These preliminary findings indicate that the composition of non-volatile fractions of *T.* × *oblongifolius* might be also rather different as well as the composition of essential oils.

In general, the extracts isolated from the whole plant material were stronger radical scavengers than deodorized extracts in both reaction systems, particularly in case of acetone extracts used in DPPH$^{\bullet}$ reaction. However, the RSC of ethanol extracts of deodorized TO1 in DPPH$^{\bullet}$ reaction system was similar to that of undeodorized ethanol extract. It was reported that deodorized plant extracts may be stronger antioxidants than undeodorized ones in oils [13]. The preliminary results on RSA indicate that further studies on the possibilities of the application of agrorefinery processing of *T.* × *oblongifolius* should be focused on determination and distribution of individual antioxidants in various plant extracts.

Essential oils of various *Thymus* species exhibit distinctive antibacterial activity mainly due to the presence of phenolic compounds, thymol and carvacrol. Antimicrobial activity of other essential oil components including those, which were found in the studied *T.* × *oblongifolius* hybrids, were also reported as antimicrobial agents. For instance, myrcene which is one of the major constituents in the sample TO1 was active against food pathogen *St. aureus* and contributed to the overall antibacterial activity of the tea tree oil [14]. The antimicrobial effects of nonvolatile components of thyme have been less studied. Therefore, in our study the effects of *T.* × *oblongifolius* extracts were determined by using selected food pathogens listed in S3. It may be observed that the extracts possessed some inhibiting effect which depended on bacteria species, extract concentration and extract type. *B. cereus*, *St. epidermidis* and *St. aureus* were affected almost by all extracts, particularly applied at higher 50 µL concentration, while such species as *M. luteus*, *E. coli*, *S. typhimurium* and *E. aerogenes* were resistant to the applied extracts. Undeodorized extracts in most cases were slightly stronger antimicrobial agents. However, in general the differences were not significant. It may be explained by the presence of antimicrobial essential oil components which are present in undeodorized extracts. However, due to

a low content of essential oil in plant materials, these components may occur in the extracts at rather low concentrations and their effects are not sufficiently pronounced. Some preliminary correlations between the RSC and antimicrobial effects may be observed, e.g. in case of $DPPH^{\bullet}$ and most sensitive *B. cereus*.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/RNP

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