Supporting Information

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

Kristina Ložienė[1], Aušra Šipailienė[2], Ramutė Maždžierienė[2], Juozas Labokas[1], Petras Rimantas Venskutonis[2*]

[1]Institute of Botany, Nature Research Centre, Žaliųjų Ežerų g. 49, LT-08406, Vilnius, Lithuania

[2]Department of Food Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas, LT-50254, Lithuania

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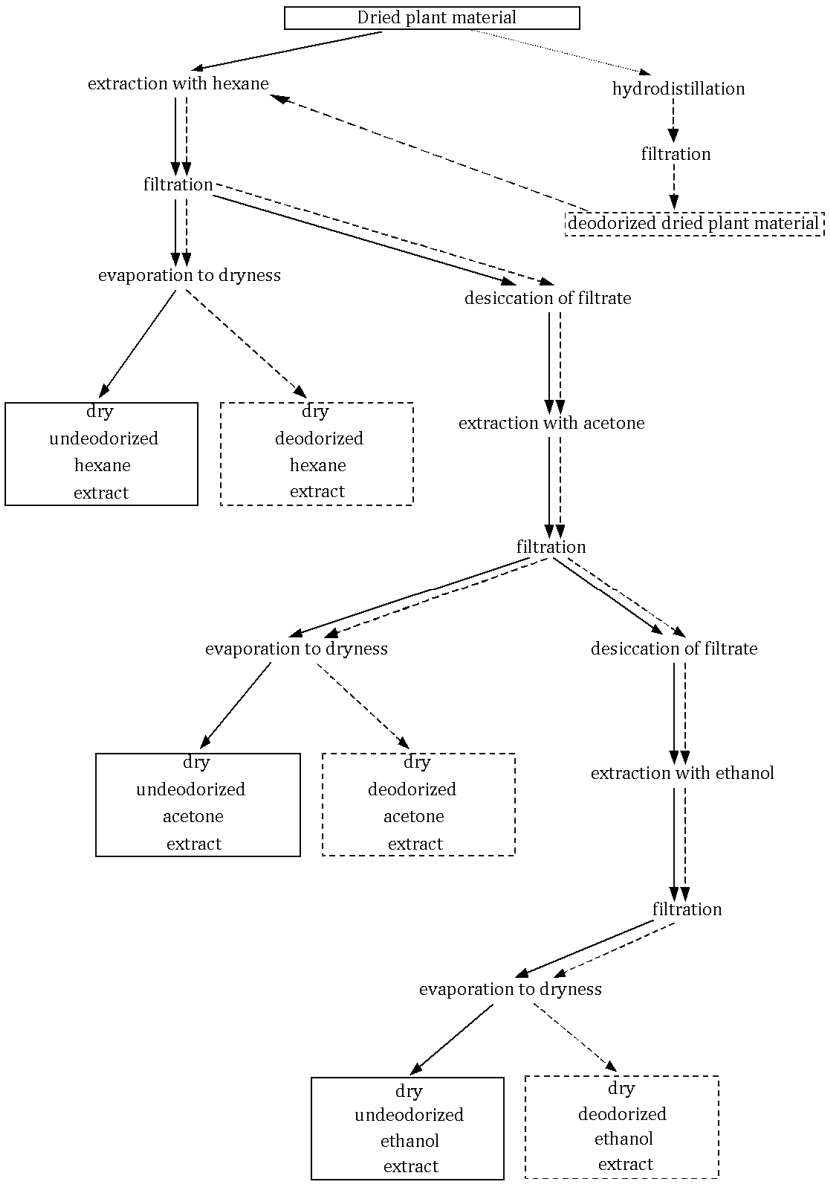

S1: Extraction scheme for the preparation of extracts

S2 : Analysis methods

Preparation of Plant Extracts : Deodorization was performed by hydrodistillation of volatile oil from the ground material in a European Pharmacopoeia apparatus during two hours. All extractions were carried out at a room temperature for two hours; plant material and solvent ratio was 1 to 17.5 (m/v). The solids after the deodorization and each extraction were separated from the liquid by filtration and dried at 50–60°C temperature in an oven. The extracts were concentrated in a rotary vacuum dryer Büchi (Flawil, Switzerland) at the temperature not higher than 50°C; dry extracts were stored in a refrigerator until further analysis.

DPPH Radical Scavenging Assay: The solution of $DPPH^{\bullet}$ (6×10^{-5} M) was prepared daily, before measurements on a UV/visible light spectrophotometer Spectronic Genesys 8 (Rochester, USA) at 515 nm. Three ml of this solution were mixed with 77 µL extract solution in 1 cm path length disposable

microcuvette (Greiner Labortech, Alpher a/d Rijn, The Netherlands). The decreasing absorbance was read during 15 min reaction time at 10 s intervals until the absorbance reached plateau. Simultaneously the absorption of a blank sample containing the same amount of methanol and $DPPH^{\bullet}$ solution was prepared and measured daily. The measurements were performed in triplicate. The RSA was calculated by the following formula: I = ([(AB–AA)/AB]/100, where I – $DPPH^{\bullet}$ inhibition, %; AB – absorption of blank sample (t = 0 min); AA – absorption of extract solution (t = 16 min).

ABTS Radical Cation Decolourisation Assay: Stock solution of ABTS (2 mM) was prepared by dissolving in 50 mL of phosphate buffered saline (PBS) obtained by dissolving 8.18 g NaCl, 0.27 g KH_2PO_4, 1.42 g Na_2HPO_4 and 0.15 g KCl in 1 l of ultra pure water. If the pH was lower than 7.4, it was adjusted with NaOH. Ultra pure water was used to prepare 70 mM solution of $K_2S_2O_8$. $ABTS^{\bullet+}$ radical cation was produced by reacting 50 mL of ABTS stock solution with 200 µL of $K_2S_2O_8$ solution and allowing the mixture to stand in the dark at room temperature for 15–16 hours before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the assessment of extracts, the $ABTS^{\bullet+}$ solution was diluted with PBS to obtain the absorbance of 0.800±0.030 at 734 nm. Three ml of $ABTS^{\bullet+}$ solution were mixed with 30 µl ethanol solution of leaf extract in 1 cm path length microcuvette (Greiner Labortech, Alpher a/d Rijn, Netherlands). The absorbance was read at ambient temperature exactly after 1, 4, 6 and 10 min. PBS solution was used as a blank sample. All determinations were performed in triplicate. The percentage decrease of the absorbance at 734 nm was calculated by the following formula: I = ([(AB–AA)/AB]/100, where I – $ABTS^{\bullet+}$ inhibition, %; AB – absorption of blank sample (t = 0 min); AA – absorption of extract solution (t = 10 min).

Evaluation of Antimicrobial Activity: The antimicrobial properties were evaluated by the agar well diffusion method. For the preparation of bacteria cell suspension bacteria were grown 18 h at 37 ºC on the inclined stiff media (Oxoid, CM325). After washing the cell suspensions were adjusted according to McFarland No 0.5 standard and mixed in a Vortex mixer. The suspension of bacteria cells was poured into the dissolved and cooled to 47 ºC stiff media (Oxoid, CM325) for the determination of the total bacteria count, and mixed one more time to obtain even distribution of the cells. Ten mL of each bacteria culture were pipetted into a 90 mm diameter Petri plates. After the setting 6 wells (8 mm diameter each) were punched in the agar and filled with 50 and 10 (l of 15% ethanolic solutions of *T.* × *oblongifolius* extracts. The plates were incubated at 37 ºC during 24 h and the antimicrobial effect was assessed by the diameter of clear zones developed around wells. When such zones were not observed it was accepted that the extracts do not possess antimicrobial effect.

Sample	Solvent	Material	Amount, µL	1	2	3	4	5	6	7
TO1	A	U	50 µL	2.7±0.07	0.0	0.0	0.0	1.3±0.07	1.5±0.0	0.0
			10 µL	1.3±0.0	0.0	0.0	0.0	0.0	0.0	0.0
		D	50 µL	1.5±0.0	0.0	0.0	0.0	1.5±0.07	1.5±0.07	0.0
			10 µL	0.0	0.0	0.0	0.0	0.0	1.1±0.14	0.0
	E	U	50 µL	2.0±0.0	1.8±0.0	1.2±0.0	1.1±0.07	2.1±0.14	2.0±0.0	0.0
			10 µL	1.7±0.21	0.0	0.0	0.0	1.0±0.0	0.0	0.0
		D	50 µL	1.8±0.28	0.0	0.0	0.0	1.5±0.07	1.3±0.14	0.0
			10 µL	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TO2	A	U	50 µL	2.0±0.0	1.4±0.07	0.0	0.0	1.8±0.0	1.9±0.14	0.0
			10 µL	1.5±0.07	0.0	0.0	0.0	1.2±0.0	1.6±0.047	0.0
		D	50 µL	1.9±0.07	0.0	0.0	0.0	1.7±0.21	1.8±0.0	0.0
			10 µL	1.2±0.07	0.0	0.0	0.0	1.5±0.07	1.4±0.0	0.0
	E	U	50 µL	2.5±0.0	1.5±0.0	1.2±0.0	1.6±0.07	2.0±0.0	1.8±0.07	1.1±0.0
			10 µL	1.5±0.0	0.0	0.0	0.0	0.0	0.0	0.0
		D	50 µL	1.6±0.07	01.2±0.07	0.0	0.0	1.2±0.0	1.2±0.0	0.0
			10 µL	1.2±0.0	0.0	0.0	0.0	0.0	0.0	0.0

S3: Antibacterial activity of *Thymus* × *oblongifolius* extracts

A – acetone, E – ethanol ; U – undeodorized, D – deodorized ; 1 – *Bacillus cereus*, 2 – *Micrococcus luteus*, 3 – *Esherichia coli*, 4 – *Salmonella typhimurium*, 5 – *Staphylococcus epidermidis*, 6 – *Staphylococcus aureus*, 7 – *Enterobacter aerogenes*