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EVALUATION OF CHEMICAL AND MEDICINAL VALUE OF THYME (THYMUS VULGARIS L.) AND LAVENDER (LAVANDULA ANGUSTIFOLIA MILL.) ESSENTIAL OILS WITH FIELDWORK, CHROMATOGRAPHIC AND BIOLOGICAL ASSAYS

Final report

General comments:
We have published our results in 10 pieces of original articles in OA (Open Access) international journals. The sum IF of these articles is 42.02. The sum independent citations is 136. The Covid-period caused some delay in laboratory experiments, some of our cooperative partners' lab were closed. Later we could manage these problems. There was one limitation during our studies: we had no preliminary information about the plant material, which could be collected from the cultivation fields and the yield of the essential oil during distillation. Therefore, in some cases the amount of distilled thyme or lavender oils was not enough for all of the experimental assays (for GC, microbiology and cell line studies). Therefore, we included other economically important essential oils e.g. immortelle, in our studies. Main co-operative partners were: Institute of Pharmacognosy (Semmelweis University, Budapest); University of Messina (Italy); Department of Medical Microbiology and Immunology (University of Pécs); University of Szeged; Department of Pharmaceutical Biology (University of Pécs).

The aims of our research were:

- (1) Isolation of the essential oils from the cultivated thyme (*Thymus vulgaris* L.) and lavender (*Lavandula angustifolia* Mill.) medicinal plants
- (2) Determination of the chemical composition of the essential oils
- (3) Microbiological investigation of the essential oils against microorganisms
- (4) Investigation of the anti-inflammatory effects of the essential oils and their main components with LPS-induced cell line studies *in vitro*

(1) Isolation of the essential oils from the cultivated thyme and lavender medicinal plants

The essential oils were isolated from *Thymus vulgaris* L. and *Lavandula angustifolia* Mill. collected during three different blooming periods (at the beginning of blooming period, in full bloom, and at the end of blooming period) between 2019-2021. Both the fresh and dried plant materials were pulverized and the essential oils were prepared with hydro-distillation in glass device based on Hungarian Pharmacopoeia VIII. edition. In 2019 and 2020 we could collect thyme materials in Szigetvár, Baranya county (5000 m^2), but in 2021 this place was sold and there was a new place in Bőszénfa (Somogy county). Lavender was collected in Bolhó (5000 m^2). The collection times can be find in Table 1. The collection of plant materials was influenced by the owners of the cultivation fields.

Table 1. The collection times of plant materials

Thyme	at the beginning of flowering period	in full bloom	at the end of flowering period
Year			
2019	25 May	05 June	12 June
2020	26 May	09 June	17 June
2021	no collection	17 June	29 June
Lavender			
Year			
2019	20 June	no collection	18 July
2020	26 June	07 July	16 July
2021	05 July	15 July	23 July

We experienced that it worth using fresh plant materials for distillation. During drying process (at room temperature, 22 C°) the essential oil content could decrease. We could collect different amount of plant materials from the cultivation fields. The yields of the oils (in ml) can be seen in Table 2.

Table 2. The essential oil yields (in ml) of the thyme and lavender fresh and dried plant materials

Thyme	at the beginning of flowering period		in full bloom		at the end of flowering period	
Year	fresh	dried	fresh	dried	fresh	dried
2019	1.8 kg-8.4 ml	963 g-7.5 ml	1.4 kg-6.3 ml	964 g-6 ml	764 g-3.9 ml	1,4 kg-7.5 ml
2020	2.3 kg-13.9 ml	2.1 kg-17.2 ml	1.1 kg-5.5 ml	303 g-2.7 ml	958 g-3.5 ml	367 g-2 ml
2021	no collection		391 g-2.1 ml	139.2 g-1.8 ml	510 g-1.7 ml	286 g-2.7 ml
Lavender						
Year						
2019	751 g-10.9 ml	470 g-8.4 ml	no collection		1 kg-10.2 ml	1.3 kg-15.3 ml
2020	1.2 kg-8.8 ml	689 g-7.1 ml	862 g-6.2 ml	816 g-9.3 ml	817 g-9.7 ml	256 g-4.3 ml
2021	581 g-6.6 ml	250 g-4.3 ml	379 g-8.1 ml	135 g-4.2 ml	404 g-8.8 ml	306 g-8.9 ml

(2) Determination of the chemical composition of the essential oils

The essential oil samples were analyzed by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (FID). We have published the results from 2019 year. Here we include the GC-MS analysis of essential oil samples distillated in 2020 and 2021. In the thyme essential oil thymol, in the lavender oil linalool were the main compounds.

Table 3. Quantification of essential oil (distillated in 2020) components identified in *Lavandula angustifolia* oils by GC-FID % area (quantitative values are average of three repetitions). Relative amounts of the 85 identified components in the six lavender oil samples are reported for polar and non-polar capillary column. Lavandula 1: fresh material, at the beginning of blooming, Lavandula 2: fresh material, in full bloom, Lavandula 3: fresh material, et the end of blooming, Lavandula 4: dried material, at the beginning of blooming, Lavandula 5: dried material, in full bloom, Lavandula 6: dried material, et the end of blooming

Compounds	**Lavandula 1**		**Lavandula 2**		**Lavandula 3**		**Lavandula 4**		**Lavandula 5**		**Lavandula 6**	
	Apolar (%)	**Polar (%)**	**Apolar (%)**	**Polar (%)**	**Apolar (%)**	**Polar (%)**	**Apolar (%)**	**Polar (%)**	**Apolar (%)**	**Polar (%)**	**Apolar (%)**	**Polar (%)**
Hexanol <n->	0.21	0.21	0.25	0.26	0.32	0.33	0.17	0.17	0.19	0.20	0.29	0.29
Tricyclene	0.02	0.02	0.01	0.01	0.01	0.01	0.03	0.03	0.02	0.02	0.02	0.02
Thujene <alpha->	0.18	0.16	0.23	0.22	0.19	0.18	0.15	0.14	0.21	0.20	0.13	0.12
Pinene <alpha->	0.32	0.27	0.36	0.32	0.30	0.26	0.30	0.27	0.32	0.29	0.23	0.21
Camphene	0.27	0.25	0.18	0.16	0.17	0.15	0.38	0.35	0.22	0.19	0.24	0.22
Sabinene	0.10	0.08	0.07	0.05	0.07	0.05	0.07	0.06	0.06	0.05	0.04	0.04
Pinene <beta->	0.32 *	0.11	0.31 *	0.07	0.39 *	0.05	0.37 *	0.10	0.30*	0.05	0.41 *	0.04
Vinyl amyl carbinol		0.21		0.24		0.33		0.27		0.25		0.37
Octan-3-one	0.34	0.33	0.71	0.70	0.76	0.76	0.35	0.35	0.72	0.72	0.91	0.89

Myrcene	2.57	2.38 *A	2.53	2.49 *A	2.49	2.60 *A	2.51	2.68 *A	2.42	2.70 *A	2.20	2.44 *A
Butanoate <butyl->	0.04	0.06	0.09	0.11	0.06	0.08	0.05	0.07	0.10	0.12	0.10	0.11
Octan-3-ol	0.05	0.06	0.11	0.12	0.13	0.15	0.06	0.06	0.11	0.12	0.15	0.15
Phellandrene <alpha->	0.07	2.38 *A	0.08	2.49 *A	0.07	2.60 *A	0.06	2.68 *A	0.06	2.70 *A	0.05	2.44 *A
Carene <delta-3->	0.09	0.10	0.06	0.07	0.04	0.05	0.05	0.06	0.03	0.04	0.01	0.02
Acetate <hexyl->	0.11	0.10	0.20	0.21	0.14	0.13	0.14	0.15	0.28	0.28	0.27	0.26
Terpinene <alpha->	0.11	0.07	0.11	0.08	0.11	0.08	0.09	0.06	0.10	0.07	0.08	0.06
Cymene <ortho->	0.07	0.05	0.04	0.03	0.05	0.03	0.11	0.09	0.06	0.05	0.08	0.07
Cymene <para->	0.54	0.49	0.65	0.60	0.62	0.58	0.66	0.62	0.70	0.66	0.91	0.88
Limonene	1.11	1.01	0.92	0.84	0.84	0.78	1.04	1.01	0.81	0.79	0.74	0.72
Phellandrene <beta->	0.19	0.46 *B	0.19	0.38 *B	0.15	0.35 *B	0.11	0.65 *B	0.12	0.37 *B	0.10	0.41 *B
Eucalyptol	4.12 *	0.46 *B	3.35 *	0.38 *B	2.86 *	0.35 *B	3.41 *	0.65 *B	2.89 *	0.37 *B	1.65 *	0.41 *B
Ocimene <(Z)-, beta->		3.66		3.05		2.60		2.90		2.66		1.38
Ocimene <(E)-, beta->	1.44	1.26	1.57	1.45	1.47	1.42	1.29	1.32	1.41	1.46	0.94	1.01
Terpinene <gamma->	0.10	0.10	0.14	0.14	0.13	0.12	0.08	0.10	0.12	0.13	0.08	0.12
Sabinene hydrate <cis->	0.51 *	nd	0.49 *	nd	0.58 *	nd	0.78 *	nd	0.65 *	nd	1.53 *	nd
Linalool oxide <cis->		0.38		0.36		0.39		0.71		0.55		1.43
Terpinolene	0.15	0.13	0.18	0.17	0.15	0.15	0.13	0.15	0.14	0.16	0.12	0.12
Linalool oxide <trans->	0.32	0.31	0.28	0.29	0.32	0.31	0.59	0.60	0.47	0.47	1.26	1.23
Sabinene hydrate <trans->	31.20 *	0.12	34.25 *	0.13	33.17 *	0.19	30.14 *	0.08	32.35 *	0.11	33.75 *	0.13
Linalool		31.83		35.07		34.14		31.02		33.23		34.66
Propionate <hexyl->		0.01		0.02		0.01		0.02		0.02		0.03
Octene <3-acetoxy->	0.45	0.45	0.62	0.61	0.72	0.72	0.52	0.51	0.67	0.66	0.91	0.91
Acetate <3-octyl->	0.06	0.04	0.08	0.07	0.09	0.08	0.08	0.05	0.11	0.08	0.12	0.10
Menth-2-en-1-ol <cis-, para->	0.04	0.04	0.03	0.03	0.04	0.04	0.07	0.06	0.05	0.04	0.06	0.05
Ocim-(4E,6Z)-ene <allo->	0.56	0.49	0.46	0.40	0.39	0.33	0.43	0.37	0.39	0.34	0.21	0.17
Myroxide <(E)->	0.04	0.04	0.02	0.02	0.02	0.03	0.04	0.04	0.03	0.03	0.06	0.08
Pinocarveol <trans->	0.04	0.06	0.03	0.03	0.03	0.03	0.03	0.05	0.03	0.04	0.03	0.04
Ocimene <neo-allo->	nd	0.03	nd	0.02	nd	0.02	nd	0.02	nd	0.02	nd	0.02
Isobutyrate <hexyl->	0.04	0.05	0.05	0.06	0.05	0.05	0.05	0.06	0.05	0.06	0.06	0.07
Camphor	0.21	0.22	0.17	0.18	0.25	0.25	0.46	0.45	0.28	0.27	0.49	0.45
Nerol oxide	0.02	nd	0.04	nd	0.04	nd	0.01	nd	0.01	nd	0.03	nd
Lavandulol	0.64	0.64	0.70	0.70	0.81	0.82	0.57	0.55	0.60	0.60	0.78	0.80
Borneol	1.54	6.90 *C	1.23	6.76 *C	1.32	6.13 *C	2.22	7.69 *C	1.47	6.55 *C	1.82	7.23 *C
Terpinen-4-ol	8.63	8.36	13.26	13.14	13.28	13.18	8.11	7.89	12.85	12.76	11.38	11.29

Cryptone	0.69 *	0.59	0.30 *	0.24	0.31 *	0.22	0.91 *	0.64	0.37 *	0.23	0.47 *	0.25
Cymen-8-ol <para->		0.12		0.06		0.10		0.24		0.12		0.20
Butyrate <hexyl->	0.21	0.22	0.47	0.40	0.43	0.37	0.26	0.27	0.50	0.42	0.58	0.50
Terpineol <alpha->	5.43	6.90 *C	5.61	6.76 *C	4.83	6.13 *C	5.43	7.69 *C	5.12	6.55 *C	5.40	7.23 *C
Carveol <trans->	0.05	0.05	0.03	0.02	0.04	0.03	0.09	0.07	0.04	0.03	0.03	0.04
Nerol	0.93	1.04	0.95	1.06	0.83	0.93	0.95	1.04	0.86	0.97	0.95	1.02
Neral	0.06	0.07	0.04	0.04	0.04	0.05	0.05	0.06	0.04	0.05	0.06	0.05
Cuminaldehyde	0.17	0.17	0.07	0.07	0.08	0.08	0.23	0.24	0.10	0.11	0.14	0.17
Carvone	0.08	0.24 *D	0.04	0.17 *D	0.04	0.17 *D	0.12	0.29 *D	0.06	0.19 *D	0.09	0.28 *D
Linalyl acetate	14.89	15.64 *E	12.12	12.26 *E	14.04	13.82 *E	14.97	14.39 *E	14.06	13.11 *E	11.76	10.87 *E
Geraniol	2.48	2.84	2.63	2.97	2.26	2.62	2.49	3.03	2.36	2.75	2.53	2.99
Geranial	0.14	0.24 *D	0.11	0.17 *D	0.11	0.17 *D	0.12	0.29 *D	0.11	0.19 *D	0.13	0.28 *D
Phellandral	0.03	1.66 *F	0.02	1.60 *F	0.02	1.58 *F	0.06	1.88 *F	0.02	1.62 *F	0.03	1.65 *F
Lavandulyl acetate	2.19	2.10	1.79	1.88	1.61	1.69	2.31	2.44	1.84	1.92	1.69	1.74
Bornyl acetate	0.13	0.14	0.09	0.10	0.09	0.11	0.17	0.19	0.10	0.12	0.13	0.15
Cymen-7-ol <ortho>	0.11	0.11	0.08	0.08	0.08	0.06	0.15	0.15	0.07	0.08	0.10	0.09
Tiglate <hexyl->	0.04	0.05	0.06	0.05	0.06	0.06	0.05	0.06	0.06	0.06	0.07	0.06
Terpinyl acetate <alpha->	0.10	6.90 *C	0.09	6.76 *C	0.09	6.13 *C	0.10	7.69 *C	0.09	6.55 *C	0.10	7.23 *C
Geranyl acetate <cis->	1.58	1.66 *F	1.54	1.60 *F	1.50	1.58 *F	1.69	1.88 *F	1.50	1.62 *F	1.52	1.65 *F
Geranyl acetate <trans->	3.07	2.91	3.01	2.93	2.97	2.93	3.31	3.34	2.95	2.95	3.00	3.03
Sesquithujene <7-epi->	0.06	0.05	0.03	0.03	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.04
Funebrene <alpha->	0.03	15.64 *E	0.01	12.26 *E	0.02	13.82 *E	0.03	14.39 *E	0.02	13.11 *E	0.02	10.87 *E
Bergamotene <alpha-, cis->	0.03	0.04	0.01	0.02	0.02	0.04	0.02	0.05	0.02	0.04	0.02	0.04
Santalene <alpha->	0.39	0.40	0.17	0.18	0.21	0.23	0.37	0.40	0.29	0.29	0.27	0.32
Caryophyllene <(E)->	2.15	2.13	1.37	1.34	1.48	1.46	1.55	1.56	1.70	1.71	1.22	1.24
Bergamotene <alpha-, trans->	0.11	0.11	0.05	0.06	0.05	0.06	0.08	0.09	0.06	0.07	0.06	0.07
Santalene <epi-, beta->	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.04	0.02	0.02	0.02	0.03
Farnesene <(E)-, beta->	2.07	2.11	1.34	1.36	1.67	1.68	1.43	1.49	1.93	1.94	1.47	1.52
Humulene <alpha->	0.14 *	0.07	0.09 *	0.04	0.09 *	0.04	0.11 *	0.05	0.10 *	0.04	0.09 *	0.04
Sesquisabinene		0.05		0.02		0.03		0.04		0.03		0.03
Muurola-4(14),5-diene <cis->	0.03	nd	0.02	nd	0.02	nd	0.02	nd	0.02	nd	0.02	nd
Germacrene D	0.09	0.10	0.06	0.07	0.07	0.08	0.06	0.07	0.07	0.11	0.05	0.07
Bergamotene <beta-, trans->	0.04	0.06	0.02	0.04	0.02	0.04	0.03	0.06	0.02	0.04	0.02	0.05

Germacrene A	0.02	nd	0.01	nd	0.01	nd	0.01	nd	0.01	nd	0.01	nd
Bisabolene <beta->	0.03	1.66 *F	0.02	1.60 *F	0.02	1.58 *F	0.02	1.88 *F	0.02	1.62 *F	0.02	1.65 *F
Cadinene <gamma->	0.58	0.56	0.25	0.24	0.29	0.27	0.48	0.46	0.31	0.29	0.32	0.30
Calamenene <trans->	0.04	0.03	0.02	0.02	0.02	0.02	0.04	0.03	0.02	0.01	0.02	0.02
Nerolidol <(E)->	0.04	0.02	0.03	0.02	0.04	0.02	0.03	0.02	0.04	0.02	0.05	0.02
Caryophyllene oxide	0.65	0.64	0.39	0.38	0.55	0.52	1.29	1.24	0.63	0.60	1.29	1.22
Cubenol <1-,10-di-epi->	0.13	0.13	0.08	0.08	0.09	0.09	0.17	0.15	0.09	0.08	0.10	0.09
Naphth-1-ol <1,2,3,4,4a,7,8,8a-octahydro-, 4-isopropyl-, 1,6-dimethyl->	1.83	1.87	1.09	1.12	1.16	1.19	1.77	1.83	0.98	1.01	0.93	0.94
Not identified	2.32	2.12	1.82	1.38	2.05	1.41	3.26	2.24	1.94	2.27	3.01	2.25

nd: not detected
*: coelution
*A: coelution between Myrcene and Phellandrene <alpha-> on Supelcowax-10 column
*B: coelution between Phellandrene <beta-> and Eucalyptol on Supelcowax-10 column
*C: coelution between Borneol, Terpineol <alpha-> and Terpinyl acetate <alpha-> on Supelcowax-10 column
*D: coelution between Carvone and Geranial on Supelcowax-10 column
*E: coelution between Linalyl acetate and Funebrene <alpha-> on Supelcowax-10 column
*F: coelution between Phellandral, Geranyl acetate <cis-> and Bisabolene <beta-> on Supelcowax-10 column

Table 4. Identity of the volatile fraction contained in *Thyme* EOs distillated in 2020. Abbreviations: LRI exp: experimental LRI; LRI ref: reference LRI; MS Sim %: database spectral similarity. The volatile compounds are expressed in % values (average of three replicated injections). T.1: fresh material, at the beginning of blooming, T.2: fresh material, in full bloom, T.3: fresh material, et the end of blooming, T.4: dried material, at the beginning of blooming, T.5: dried material, in full bloom, T.6: dried material, et the end of blooming

Compounds	**MS Sim %**	**LRI exp**	**LRI ref**	**T.1**	**T.2**	**T.3**	**T.4**	**T.5**	**T.6**
				% (n=3)	**% (n=3)**	**% (n=3)**	**% (n=3)**	**% (n=3)**	**% (n=3)**
Tricyclene	94	922	923	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
α-Thujene	98	925	927	0.99 ± 0.00	1.09 ± 0.00	0.99 ± 0.00	0.46 ± 0.00	0.21 ± 0.00	0.37 ± 0.00
α-Pinene	97	933	933	0.61 ± 0.00	0.63 ± 0.00	0.69 ± 0.00	0.50 ± 0.00	0.37 ± 0.00	0.55 ± 0.00
Camphene	97	949	953	0.39 ± 0.00	0.39 ± 0.00	0.50 ± 0.00	0.33 ± 0.00	0.25 ± 0.00	0.42 ± 0.00
Sabinene	95	972	972	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
4-Penten-1-ol, propanoate	94	974	974	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
β-Pinene	92	977	978	0.20 ± 0.00	0.20 ± 0.00	0.21 ± 0.00	0.15 ± 0.00	0.12 ± 0.00	0.15 ± 0.00
Vinyl amyl carbinol	95	979	978	0.27 ± 0.01	0.34 ± 0.00	0.42 ± 0.00	0.36 ± 0.00	0.34 ± 0.00	0.51 ± 0.01
Octan-3-one	93	984	986	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.03 ± 0.02

Myrcene	96	988	991	1.45 ± 0.02	1.34 ± 0.01	1.28 ± 0.01	1.17 ± 0.02	0.71 ± 0.02	0.84 ± 0.01
Octan-3-ol	96	997	999	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.00
α-Phellandrene	96	1006	1007	0.15 ± 0.00	0.12 ± 0.00	0.10 ± 0.00	0.13 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
δ-3-Carene	96	1009	1009	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
α-Terpinene	98	1017	1018	1.40 ± 0.01	1.09 ± 0.00	0.82 ± 0.00	1.15 ± 0.01	0.74 ± 0.01	0.79 ± 0.00
p-Cymene	96	1025	1025	12.89 ± 0.03	17.44 ± 0.01	20.64 ± 0.04	12.46 ± 0.08	15.02 ± 0.09	22.78 ± 0.08
Limonene	96	1029	1030	0.29 ± 0.00	0.30 ± 0.00	0.34 ± 0.00	0.27 ± 0.00	0.22 ± 0.00	0.31 ± 0.00
β-Phellandrene	94	1030	1031	0.07 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.11 ± 0.02
Eucalyptol	97	1032	1032	0.62 ± 0.01	0.70 ± 0.01	0.75 ± 0.01	0.50 ± 0.01	0.67 ± 0.01	0.90 ± 0.02
(Z)-, β-Ocimene	90	1034	1035	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
(E)-, β-Ocimene	95	1045	1046	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
γ-Terpinene	95	1058	1058	15.18 ± 0.03	7.38 ± 0.00	6.01 ± 0.01	13.67 ± 0.07	5.06 ± 0.03	5.49 ± 0.02
Butanoic acid, 3-methylbut-2-enyl ester	90	1063	1068	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
(Z)-Sabinene hydrate	93	1070	1069	0.26 ± 0.00	0.33 ± 0.00	0.59 ± 0.00	0.21 ± 0.00	0.31 ± 0.00	0.21 ± 0.00
Terpinolene	96	1086	1086	0.09 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.11 ± 0.00
p-Cymenene	94	1091	1093	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.05 ± 0.00
Linalool	97	1099	1101	1.46 ± 0.01	1.56 ± 0.00	2.15 ± 0.01	1.69 ± 0.00	1.67 ± 0.01	2.17 ± 0.00
(E)-Sabinene hydrate	94	1102	1099	0.11 ± 0.00	0.13 ± 0.00	0.18 ± 0.00	0.10 ± 0.00	0.14 ± 0.01	0.11 ± 0.01
3-Methyl-, 3-butenyl-3-methyl-butyrate	90	1110	1114	tr	tr	0.02 ± 0.00	tr	0.01 ± 0.00	0.02 ± 0.00
(Z)-, *p*-Menth-2-en-1-ol	96	1126	1124	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
Camphor	97	1149	1149	0.27 ± 0.00	0.25 ± 0.00	0.36 ± 0.00	0.26 ± 0.00	0.29 ± 0.00	0.33 ± 0.00
Borneol	98	1173	1173	0.48 ± 0.01	0.44 ± 0.00	0.66 ± 0.00	0.49 ± 0.00	0.51 ± 0.00	0.85 ± 0.00
Terpinen-4-ol	92	1182	1184	0.66 ± 0.00	0.75 ± 0.00	0.63 ± 0.00	0.67 ± 0.00	0.67 ± 0.00	0.87 ± 0.00
Hex-(3Z)-enyl-Butyrate	92	1184	1187	0.01 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.04 ± 0.00
p-Cymen-8-ol	93	1189	1189	0.02 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.08 ± 0.00

α-Terpineol	97	1197	1195	0.12 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.13 ± 0.00	0.16 ± 0.00	0.21 ± 0.01
(Z)-, Dihydro-carvone	94	1200	1198	0.03 ± 0.00	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.01	0.07 ± 0.01
n-Decanal	95	1206	1208	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	nd	nd
Thymol methyl ether	94	1230	1229	0.19 ± 0.00	0.50 ± 0.00	0.62 ± 0.00	0.57 ± 0.00	0.43 ± 0.00	0.53 ± 0.00
Carvacryl methyl ether	96	1239	1239	0.27 ± 0.00	0.36 ± 0.00	0.37 ± 0.00	0.35 ± 0.00	0.32 ± 0.00	0.32 ± 0.00
Neral	96	1242	1238	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Carvone	95	1249	1246	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Geranial	97	1274	1268	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Thymol	94	1294	1293	55.81 ± 0.05	57.10 ± 0.03	54.21 ± 0.03	56.39 ± 0.18	62.46 ± 0.16	52.33 ± 0.11
Carvacrol	94	1302	1300	2.30 ± 0.05	2.92 ± 0.02	2.90 ± 0.04	2.98 ± 0.01	3.48 ± 0.02	3.11 ± 0.01
Thymol acetate	93	1345	1348	0.04 ± 0.00	0.02 ± 0.00	nd	0.03 ± 0.00	0.01 ± 0.00	nd
Eugenol	95	1354	1357	0.05 ± 0.00	0.09 ± 0.00	0.12 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.00
α-Ylangene	92	1371	1371	nd	nd	nd	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Isobornyl propionate *	93	1376	1377	0.04 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	0.10 ± 0.00
α-Copaene *	88	1377	1375						
β-Bourbonene	95	1385	1382	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.06 ± 0.00
(Z)-Jasmone	93	1394	1394	nd	0.01 ± 0.00	0.01 ± 0.00	tr	0.01 ± 0.00	tr
(E)-Caryophyllene	97	1421	1424	1.56 ± 0.00	2.05 ± 0.00	1.92 ± 0.00	2.15 ± 0.01	2.50 ± 0.01	2.44 ± 0.01
β-Copaene	94	1431	1433	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Aromadendrene	94	1441	1438	nd	nd	nd	0.04 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
α-Humulene	97	1457	1454	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
(Z)-Muurola-4(14),5-diene	94	1464	1466	tr	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	tr
Geranyl propanoate	97	1468	1471	0.09 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.06 ± 0.00
Cadina-1(6),4-diene	91	1473	1474	nd	nd	nd	0.01 ± 0.00	0.02 ± 0.00	tr
γ-Muurolene	92	1476	1478	0.05 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.12 ± 0.00

α-Amorphene	90	1481	1482	nd	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Germacrene D	95	1482	1480	0.11 ± 0.00	0.07 ± 0.00	0.04 ± 0.00	nd	nd	nd
β-Selinen	94	1491	1492	tr	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
γ-Amorphene	87	1494	1490	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
α-Selinene	89	1497	1501	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
α-Muurolene	93	1500	1497	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00
δ-Amorphene	89	1505	1506	nd	nd	nd	0.02 ± 0.00	0.01 ± 0.00	tr
γ-Cadinene	95	1515	1512	0.06 ± 0.00	0.12 ± 0.00	0.10 ± 0.00	0.16 ± 0.00	0.24 ± 0.00	0.17 ± 0.00
δ-Cadinene	94	1520	1518	0.11 ± 0.00	0.13 ± 0.00	0.11 ± 0.00	0.26 ± 0.00	0.27 ± 0.00	0.22 ± 0.00
(E)-Calamenene	90	1522	1527	0.02 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.07 ± 0.00	0.06 ± 0.00
(E)-Cadina-1,4-diene	93	1534	1536	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
α-Cadinene	95	1539	1538	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
α-Calacorene	91	1543	1544	nd	nd	nd	tr	0.01 ± 0.00	0.01 ± 0.00
Geranyl butyrate	97	1554	1559	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Caryophyllene oxide	93	1585	1587	0.27 ± 0.00	0.37 ± 0.00	0.47 ± 0.01	0.52 ± 0.00	0.70 ± 0.01	0.70 ± 0.00
Humulene epoxide II	89	1613	1613	tr	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
1-,10-di-*epi*-Cubenol	89	1618	1614	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.01 ± 0.00
epi-γ-Eudesmol	95	1626	1624	0.05 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
1,2,3,4,4a,7,8,8a-Octahydro-, 4-isopropyl-, 1,6-dimethyl-naphth-1-ol	94	1645	1641	0.06 ± 0.00	0.17 ± 0.00	0.12 ± 0.00	0.07 ± 0.00	0.17 ± 0.00	0.05 ± 0.00
Cadin-4-en-10-ol	95	1658	1659	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00

nd: not detected
tr: trace level
*: indicates a coelution on SLB-5ms column

Table 5. Identity of the terpenes and terpenoids in lavender EOs distillated in 2021. MS Match represents MS similarity match; LRI exp: experimental LRI; LRI ref: reference LRI. Quantitative results are expressed in % values (average of three replicated injections). RSD is relative standard deviation. Lavender 1: fresh material, at the beginning of blooming, Lavender 2: fresh material, in full bloom,

Lavender 3: fresh material, et the end of blooming, Lavender 4: dried material, at the beginning of blooming, Lavender 5: dried material, in full bloom, Lavender 6: dried material, et the end of blooming

Compounds	MS Match	LRI exp	LRI ref	Lavender 1		Lavender 2		Lavender 3		Lavender 4		Lavender 5		Lavender 6	
				%	RDS	%	RDS	%	RDS	%	RDS	%	RDS	%	RDS
Tricyclene	902	923	924	0.03	3.70	0.02	10.07	0.02	7.37	0.04	2.71	0.04	4.76	0.03	2.01
α-Thujene	911	924	927	0.14	1.45	0.16	3.52	0.22	0.26	0.06	3.70	0.12	2.20	0.13	1.21
α-Pinene	979	932	933	0.33	1.71	0.32	3.02	0.40	0.66	0.24	2.68	0.32	3.36	0.31	2.43
Camphene	903	949	950	0.40	1.52	0.32	2.94	0.28	0.42	0.58	1.89	0.56	2.70	0.42	1.80
Sabinene	935	971	972	0.05	5.19	0.03	5.80	0.02	9.62	0.06	3.17	0.04	4.66	0.02	13.48
β-Pinene	884	977	978	0.15	3.16	0.10	10.05	0.09	0.65	0.04	1.53	0.05	4.99	0.05	10.19
Vinyl amyl carbinol	977	980	978	0.58	3.69	0.51	1.47	0.40	1.20	0.43	0.69	0.45	0.47	0.38	2.82
Octan-3-one	964	984	986	0.49	2.77	0.61	4.18	0.83	0.64	0.66	0.61	0.79	0.74	1.03	1.47
Myrcene	982	988	991	1.00	2.29	0.84	2.03	0.71	0.77	0.61	1.40	0.49	4.24	0.51	1.85
butyl-Butanoate	958	995	999	0.07	3.53	0.08	3.65	0.11	0.93	0.11	2.28	0.13	2.78	0.13	3.17
Octan-3-ol	846	999	999	*nd*	-	0.05	6.52	0.09	3.47	0.04	7.97	0.07	3.53	0.12	3.40
α-Phellandrene	857	1006	1006	0.02	6.66	0.01	4.56	0.01	0.00	0.06	4.55	0.04	10.73	0.02	3.01
δ-3-Carene	986	1009	1009	0.28	1.58	0.21	3.05	0.21	0.55	0.20	0.59	0.21	0.84	0.20	3.57
hexyl-Acetate	912	1012	1012	0.43	1.65	0.32	2.97	0.31	0.49	0.29	0.52	0.31	0.75	0.31	0.75
o-Cymene	821	1019	1024	0.19	0.82	0.15	3.16	0.06	2.57	0.24	0.88	0.20	1.04	0.12	4.08
p-Cymene	972	1024	1025	0.74	1.26	0.81	1.99	0.75	0.63	1.04	1.02	1.11	1.35	1.07	1.59
Limonene	986	1028	1030	1.45	1.54	1.02	3.89	0.73	0.57	0.60	1.06	0.61	1.23	0.48	2.00
Eucalyptol	984	1033	1032	0.38	0.52	0.43	0.61	0.33	0.76	0.62	0.66	0.71	0.89	0.54	1.22
(Z)-. β-Ocimene	860	1034	1035	0.32	3.10	0.20	6.54	0.10	1.11	0.07	1.76	0.05	4.63	0.06	1.04
(E)-. β-Ocimene	910	1044	1046	0.12	2.96	0.10	2.88	0.10	1.19	0.10	4.95	0.09	10.07	0.10	1.80
(Z)-Linalool oxide	902	1071	1069	0.70	0.52	0.83	2.02	1.02	0.54	3.56	0.22	2.68	0.18	2.89	0.18
(E)-Linalool oxide	920	1088	1086	0.56	0.45	0.62	2.42	0.72	0.28	2.62	0.32	1.96	0.19	2.12	0.19
Linalool	947	1102	1101	31.02	0.38	33.96	0.46	36.33	0.39	24.00	0.20	27.45	0.15	29.23	0.21
3-acetoxy-Octene	972	1107	1109	1.48	0.86	1.17	1.16	1.01	0.15	2.30	0.47	1.58	0.47	1.31	0.54
3-octyl-Acetate	979	1119	1120	0.08	1.30	0.07	2.29	0.07	2.26	0.11	2.13	0.09	1.11	0.10	0.57
hexyl-Isobutyrate	945	1146	1150	0.06	0.00	0.06	4.34	0.06	2.74	0.09	8.06	0.08	1.23	0.07	2.05
Camphor	942	1148	1149	0.37	1.08	0.43	1.10	0.29	1.30	0.84	1.41	0.84	0.36	0.66	0.32

Nerol oxide	882	1153	1152	*nd*	-	*nd*	-	*nd*	-	0.12	0.59	0.06	0.91	0.06	0.90
Lavandulol	955	1165	1165	0.20	8.49	0.50	2.54	0.33	1.54	0.15	1.95	0.23	1.73	0.27	2.04
(Z)-Linalool oxide (pyranoid)	865	1171	1169	*nd*	-	*nd*	-	0.10	2.42	0.18	7.18	0.15	0.68	0.16	1.29
Borneol	930	1173	1173	*nd*	-	2.20	1.40	1.35	0.53	2.83	0.48	2.75	0.76	2.16	1.19
(E)-Linalool oxide (pyranoid)	843	1176	1174	*nd*	-	*nd*	-	0.07	4.11	0.10	10.65	0.09	1.54	0.13	12.53
Terpinen-4-ol	868	1182	1184	6.44	0.65	9.56	0.19	10.68	0.19	4.90	0.16	7.59	0.18	8.26	0.14
Cryptone	881	1189	1187	1.46	0.99	1.15	2.10	0.73	1.17	1.69	0.25	1.32	0.40	0.93	0.63
hexyl-Butyrate	955	1191	1195	0.28	2.17	0.43	3.26	0.49	0.90	0.41	2.93	0.55	0.69	0.62	0.40
α-Terpineol	973	1197	1195	5.54	0.26	4.85	0.60	4.31	0.24	4.24	0.62	3.63	0.48	2.99	0.45
Verbenone	890	1213	1208	0.06	3.29	*tr*	-	*tr*	-	0.26	3.45	0.19	1.60	0.12	1.71
(E)-Carveol	916	1221	1223	0.13	10.58	0.13	9.38	0.12	1.43	0.14	2.65	0.12	0.94	0.14	7.95
Nerol	892	1227	1229	0.53	8.31	0.54	4.25	0.48	1.88	0.54	0.74	0.45	0.78	0.36	1.44
Neral	885	1240	1238	0.04	9.63	0.02	7.44	0.02	2.84	0.04	5.46	0.02	2.59	0.02	6.93
Cuminaldehyde	945	1243	1243	0.20	4.50	0.05	10.10	0.03	6.03	0.08	8.32	0.03	3.03	0.03	11.25
Carvone	943	1246	1246	0.13	3.51	0.12	9.59	0.05	6.67	0.26	6.60	0.19	1.53	0.11	5.87
Linalyl acetate	973	1250	1250	23.66	0.32	20.58	0.27	22.13	0.13	25.96	0.20	24.10	0.12	26.22	0.11
Geraniol	957	1252	1255	0.34	5.91	0.14	0.50	0.09	9.46	0.10	9.30	0.11	2.05	0.07	6.76
Geranial	946	1269	1268	0.15	11.96	0.10	4.02	0.10	0.98	0.08	0.73	0.08	1.51	0.06	0.91
Lavandulyl acetate	872	1283	1284	2.30	0.55	2.23	2.36	2.47	0.39	2.17	0.39	2.38	0.25	2.23	0.17
Bornyl acetate	923	1284	1285	0.21	1.43	0.18	5.45	0.13	0.45	0.38	3.16	0.28	0.20	0.20	2.01
Geranyl formate	830	1297	1300	0.13	2.56	0.07	4.81	0.08	10.56	0.10	1.94	0.08	0.74	0.07	2.78
hexyl-Tiglate	921	1328	1329	0.06	9.09	0.05	2.13	0.06	10.31	0.08	2.97	0.09	2.22	0.07	1.57
Neryl acetate	977	1358	1361	1.57	0.41	1.36	0.67	1.19	0.13	1.16	0.37	1.04	0.91	0.85	0.45
Geranyl acetate	977	1377	1380	3.45	0.33	2.85	0.11	2.26	0.12	2.62	0.43	2.22	0.86	1.68	0.67
α-. (E)-Bergamotene	838	1412	1416	0.05	14.38	0.13	2.24	0.17	1.02	0.06	1.72	0.12	0.93	0.13	0.86
α-Santalene	874	1418	1418	0.31	0.95	0.23	1.34	0.16	1.85	0.31	0.50	0.25	0.79	0.23	0.66
(E)-Caryophyllene	889	1420	1424	0.45	1.61	0.44	3.29	0.31	1.12	0.08	2.47	0.12	0.83	0.17	0.35
Coumarin	963	1439	1438	0.08	2.09	0.07	8.21	0.03	2.09	0.05	9.48	0.04	3.79	0.07	0.00

(E)-. β-Farnesene	898	1451	1452	0.53	0.66	0.59	1.81	0.45	0.46	0.19	1.12	0.27	0.00	0.36	0.27
γ-Cadinene	883	1513	1512	0.43	0.59	0.41	0.28	0.28	0.35	0.32	0.78	0.32	0.96	0.35	0.59
Caryophyllene oxide	911	1584	1587	1.44	2.58	1.47	0.14	1.14	0.94	2.84	1.14	2.59	0.16	2.25	0.98
Humulene epoxide II	903	1617	1613	0.06	5.72	0.05	3.03	0.05	3.16	0.08	1.27	0.08	1.51	0.06	4.56
1-,10-di-*epi*-Cubenol	896	1620	1614	0.19	2.16	0.17	4.37	0.12	1.80	0.15	2.16	0.15	0.39	0.11	2.86
1.2.3.4.4a.7.8.8a-octahydro-. 4-isopropyl-. 1.6-dimethyl-Naphth-1-ol	911	1644	1641	2.78	0.57	2.53	0.51	1.69	0.14	1.30	0.69	1.33	0.12	1.09	1.19

Table 6. Identity of the terpenes and oxygenated derivatives in thyme EOs distillated in 2021. MS Match represents MS similarity match; LRI exp: experimental LRI; LRI ref: reference LRI. Quantitative results are expressed in % values (average of three replicated injections). Thyme 1: fresh material, in full bloom, Thyme 2: fresh material, at the end of blooming, Thyme 3: dried material, in full bloom, Thyme 4: dried material, at the end of blooming

Compounds	**MS Match**	**LRI exp**	**LRI ref**	**Thyme 1**		**Thyme 2**		**Thyme 3**		**Thyme 4**	
				%	**RDS**	**%**	**RDS**	**%**	**RDS**	**%**	**RDS**
Tricyclene	927	924	927	0.03	6.66	0.03	5.33	0.03	2.19	0.03	3.57
α-Thujene	919	926	927	0.96	2.96	0.83	2.68	0.51	2.70	0.46	2.44
α-Pinene	978	932	933	0.79	2.90	0.85	2.65	0.86	2.94	0.95	2.51
Camphene	900	948	950	0.65	2.60	0.69	2.40	0.64	2.82	0.78	2.11
β-Pinene	904	977	978	0.03	8.27	0.09	1.26	0.06	3.33	0.03	3.33
Vinyl amyl carbinol	980	979	978	0.74	0.21	0.60	0.86	0.65	0.79	0.79	0.19
Octan-3-one	905	985	986	0.05	8.51	0.03	1.88	0.05	3.23	0.06	1.02
Myrcene	983	987	991	1.32	0.92	1.66	1.55	1.14	1.88	0.92	0.60
Octan-3-ol	840	999	999	0.05	10.83	0.03	1.96	0.05	10.66	0.06	6.28
α-Phellandrene	848	1006	1007	0.07	3.79	0.15	1.40	0.08	2.60	0.06	6.30
δ-3-Carene	973	1009	1009	0.08	8.94	0.08	1.36	0.08	2.02	0.08	10.81
α-Terpinene	941	1016	1018	0.61	2.06	1.23	1.62	0.69	2.17	0.56	1.19
p-Cymene	972	1024	1025	24.49	1.19	11.99	1.13	26.97	1.54	28.29	0.79
Limonene	978	1028	1030	0.40	1.26	0.35	0.33	0.39	1.94	0.41	2.03
β-Phellandrene	836	1030	1031	0.11	8.40	0.10	19.52	0.12	8.27	0.12	10.33
Eucalyptol	985	1032	1032	0.86	0.38	0.63	3.38	0.52	1.01	1.04	0.99
<(E)-. β-Ocimene	893	1044	1046	0.01	10.83	0.04	2.64	0.01	7.69	0.01	9.12
γ-Terpinene	957	1057	1058	5.00	1.35	13.94	1.19	5.62	1.65	3.61	0.96
(Z)-Sabinene hydrate	912	1072	1069	0.54	1.44	0.27	0.75	0.22	2.07	0.27	0.64

Terpinolene	956	1085	1086	0.09	2.95	0.11	0.93	0.09	0.67	0.08	0.71
p-Cymenene	907	1090	1093	0.03	2.86	0.02	2.44	0.06	5.22	0.06	2.99
Linalool	914	1099	1101	1.91	0.11	1.39	0.19	1.46	0.54	2.28	0.70
Camphor	971	1148	1149	0.57	0.36	0.40	0.25	0.49	0.42	0.64	0.50
Borneol	926	1173	1173	1.12	0.73	1.00	1.02	0.96	0.37	1.19	0.49
Terpinen-4-ol	903	1181	1184	0.84	0.43	0.67	1.21	0.77	1.04	0.83	1.58
α-Terpineol	969	1197	1195	0.18	0.86	0.14	1.22	0.14	1.20	0.20	0.58
(Z)-, dihydro-Carvone	854	1200	1198	0.06	6.75	0.05	11.26	0.05	1.89	0.06	0.91
Thymol methyl ether	897	1229	1229	1.81	0.06	0.31	0.19	0.32	0.36	0.58	0.43
Carvacryl methyl ether	898	1238	1239	0.83	0.25	0.30	0.39	0.27	0.37	0.27	0.21
Thymol	943	1295	1293	48.92	0.82	56.63	0.73	49.72	1.18	48.31	0.67
Carvacrol	988	1301	1300	2.54	0.72	2.47	0.77	2.94	1.45	3.01	0.86
Thymol acetate	916	1345	1348	0.02	2.59	0.06	5.67	0.01	8.45	0.03	4.00
isobornyl-Propionate	930	1380	1384	0.09	4.12	0.04	1.94	0.04	1.40	0.06	1.61
β-Bourbonene	920	1388	1382	0.04	2.99	0.02	0.00	0.04	1.33	0.04	1.42
(E)-Caryophyllene	903	1419	1424	1.47	0.20	0.95	0.12	1.26	0.20	1.09	0.30
β-Copaene	860	1435	1433	0.02	2.59	0.03	37.67	0.02	5.56	0.07	1.41
α-Humulene	876	1455	1454	0.05	1.96	0.03	3.53	0.04	2.38	0.04	4.09
Geranyl propanoate	948	1467	1471	0.06	3.22	0.08	3.95	0.09	1.26	0.06	1.61
Germacrene D	937	1486	1480	0.06	1.72	0.07	2.08	0.08	1.32	0.07	5.08
γ-Cadinene	871	1513	1512	0.14	0.80	0.12	0.00	0.09	2.32	0.14	1.68
δ-Cadinene	820	1518	1518	0.14	0.74	0.19	0.00	0.15	0.79	0.14	1.83
Caryophyllene oxide	900	1583	1587	0.67	1.09	0.41	0.37	0.88	1.55	0.85	0.24
epi-γ-Eudesmol	864	1630	1624	0.07	2.22	0.05	8.90	0.08	2.28	0.06	8.24
1,2,3,4,4a,7,8,8a-octahydro-, 4-isopropyl-, 1,6-dimethyl-Naphth-1-ol	902	1643	1641	0.29	1.94	0.14	0.80	0.08	3.58	0.21	0.28

(3) Microbiological investigation of the essential oils against microorganisms

The antibacterial activity of the essential oils was investigated with TLC-direct bioautography, broth dilution methods. The anti-biofilm activity of the oils was tested with crystal-violet assay. Scanning electron microscopy was used to visualize the bacterial membrane degradation after essential oil treatment. We could optimized this assay for respiratory tract pathogens: methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *H. parainfluenzae*, *Streptococcus pneumoniae*. Due to the volatility and hydrophobic character of the essential oils it was necessary to optimize our test systems for these materials. Our main findings were published in the following articles:

Experiment 1: Flowering phenopases influence the antibacterial and anti-biofilm effects of *Thymus vulgaris* L. essential oil

Essential oil content of thyme is responsible for its antimicrobial activity, however, it has been reported that the chemical composition of essential oils influences its biological activity. In order to explore flowering phenophases influence on the chemical composition of thyme essential oil and its antibacterial and anti-biofilm activity, plant materials were collected at the beginning of flowering, in full bloom and at the end of flowering periods in 2019. The antibacterial activity was performed by broth microdilution and thin layer chromatography-direct bioautography (TLC-DB) assays and the anti-biofilm effect by crystal violet assay, respectively. Scanning electron microscopy was applied to illustrate the cellular changes of bacterial cells after essential oil treatment. Thymol (52.33–62.46%) was the main component in the thyme essential oils. Thyme oil distilled from fresh plant material and collected at the beginning of flowering period exerted the highest antibacterial and anti-biofilm activity against *Haemophilus influenzae*, *H. parainfluenzae* and *Pseudomonas aeruginosa*. The different flowering periods of *Thymus vulgaris* influence the antibacterial and anti-biofilm activity of its essential oils, therefore, the collection time has to be taken into consideration and not only the full bloom, but the beginning of flowering period may provide biological active thyme essential oil.

Experiment 2: Immortelle (Helichrysum italicum (Roth) G. Don) Essential Oil Showed Antibacterial and Biofilm Inhibitory Activity against Respiratory Tract Pathogens

The biofilm formation of bacteria in different parts of the human body can influence the success of antibiotic therapy. The immortelle EO has been used traditionally as an expectorant; however, there are no studies summarizing its antibacterial effect against respiratory tract bacteria. Our aim was to investigate the antibacterial and biofilm inhibitory activity of immortelle (*Helichrysum italicum*) EO against respiratory tract pathogens. Our results showed that immortelle EO has antibacterial and anti-biofilm effects against respiratory tract bacteria used in this study. *H. parainfluenzae* was the most sensitive to each treatment, however, *P. aeruginosa* was the most resistant bacteria. In conclusion, the studied EO may have a role in the treatment of respiratory tract infections due to their antibacterial and anti-biofilm activity.

Experiment 3: Anti-Haemophilus activity of selected essential oils detected by TLC-Direct Bioautography and biofilm inhibition.

Direct bioautography (DB) combined with thin layer chromatography (TLC) is a screening method for the detection of antimicrobial compounds in plant extracts, for example, in EOs. Due to their lipophilic character, the common microbiological assays (etc. disk diffusion) could not provide reliable results. The aim of this study was the evaluation of antibacterial and anti-biofilm properties of the EO of cinnamon bark, clove, peppermint, thyme, and their main components against Haemophilus influenzae and H. parainfluenzae. Oil in water (O/W) type Pickering nano-emulsions stabilized with silica nanoparticles from each oil were prepared to increase their water-solubility. Samples with Tween80 surfactant and absolute ethanol were also used. Results showed that *H. influenzae* was more sensitive to the EOs than *H. parainfluenzae* (except for cinnamon bark oil). In TLC-DB the ethanolic solutions of thyme oil presented the best activity against *H. influenzae*, while cinnamon oil was the most active against *H. parainfluenzae*. Pickering nano-emulsion of cinnamon oil inhibited the biofilm formation of *H. parainfluenzae* (76.35%) more efficiently than samples with Tween80 surfactant or absolute ethanol. In conclusion, Pickering nano-emulsion of EOs could inhibit the biofilm production effectively.

Experiment 4: Anti-biofilm effect of selected essential oils and main components on mono- and polimicrobic bacterial cultures.

Biofilms are surface-associated microbial communities resistant to sanitizers and antimicrobials. Various interactions that can contribute to increased resistance occur between the populations in biofilms. These relationships are the focus of a range of studies dealing with biofilm-associated infections and food spoilage. The present study investigated the effects of cinnamon, marjoram, and thyme essential oils (EOs) and their main components, i.e., trans-cinnamaldehyde, terpinen-4-ol, and

thymol, respectively, on single- and dual-species biofilms of *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas putida*, and *Staphylococcus aureus*. In dual-species biofilms, *L. monocytogenes* was paired with each of the other three bacteria. Minimum inhibitory concentration (MIC) values for the individual bacteria ranged between 0.25 and 20 mg/mL, and trans-cinnamaldehyde and cinnamon showed the highest growth inhibitory effect. Single-species biofilms of *L. monocytogenes*, *P. putida*, and *S. aureus* were inhibited by the tested EOs and their components at sub-lethal concentrations. Scanning electron microscopy images showed that the three-dimensional structure of mature biofilms embedded in the exopolysaccharide matrix disappeared or was limited to micro-colonies with a simplified structure. In most dual-species biofilms, to eliminate living cells from the matrix, concentrations exceeding the MIC determined for individual bacteria were required.

Experiment 5: Combination of analytical and statistical methods in order to optimize antibacterial activity of clary sage supercritical fluid extracts.

The extraction of clary sage (*Salvia sclarea* L.) using supercritical carbon dioxide (SC-CO2) was systematically studied by using TLC-DB and response surface methodology (RSM). The three parameters temperature, pressure, and cosolvent ratio were optimized for the maximum antibacterial activity of clary sage extracts against *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. The highest inhibition zone was 7.51 mm for P. aeruginosa and 7.57 mm for MRSA. According to RSM analysis, the predicted optimum extraction parameters are 18.6 MPa pressure, 40 °C temperature, and 2% ethanol (EtOH) ratio. The combination of this analytical and statistical method allows saving time, money, and instrument runtime in the optimization of essential oil composition, which is tailored to a specific task and could be useful on any kind of herbs in a wide range of use from perfume manufacturing to the food industry.

Furthermore, 6 of Hungarian pharmacy thesis were prepared by students and 1 thesis is in progress related to thyme and lavender essential oils. We showed these results at different conferences.

Bordás Bence: A kakukkfű illóolaj és antibiotikumok antibakteriális hatásának vizsgálata in vitro checkerboard módszerrel, védés tervezett éve: 2024

Szula Zsófia: Kakukkfű illóolaj értékelése bioautográfiás rendszerben légúti kórokozók ellen, védés éve: 2023

Biskup Dóra: A levendula (Lavandula angustifolia Mill.) illóolaj biofilm képződést gátló hatásának vizsgálata légúti megbetegedést okozó baktériumokkal szemben, védés éve: 2022

Oszkó Csenge: A levendula illóolajának anti-Haemophilus aktivitása bioautográfiás rendszerben tesztelve, védés éve: 2022

Tompa Júlia: A kakukkfű illóolajának biofilm képződést gátló hatása Streptococcus pneumoniae baktériummal szemben, védés éve: 2022

Simonics Dóra: A levendula és a kakukkfű illóolajok in vitro antibakteriális hatásának vizsgálata légúti patogénekkel szemben, védés éve: 2021

Toriz Veronika: A kakukkfű (Thymus vulgaris L.) illóolaj biofilm képződést gátló hatásának vizsgálata légúti megbetegedést okozó baktériumokkal szemben, védés éve: 2020

(4) Investigation of the anti-inflammatory effects of EOs and their main components with LPS-induced cell line studies in vitro

Experiment 1: Anti-inflammatory effect of lavender (*Lavandula angustifolia* Mill.) essential oil prepared during different plant phenophases on THP-1 macrophages.

The anti-infammatory activity of essential oils may depend on the composition and the ratio of the compounds. The constitution of the essential oils extracted from the diferent stages of fowering period varies, which makes it plausible that the collection time of the flowers influences the anti-inflammatory effects. Different types of essential oils reduce inflammation acting similarly by modulating the activity and action of the NFκB signalling pathway, which is the major regulator of the transcription of pro-inflammatory cytokines. The experiments were carried out on THP-1 human monocyte/macrophage cell line as in vitro cell culture model for monitoring the effects of lavender essential oils and the main compound linalool on *P. aeruginosa* LPS stimulated inflammation. The mRNA and protein levels of four pro-inflammatory cytokines, IL-6, IL-1β, IL-8 and TNFα were determined by Real Time PCR and ELISA measurements. The effects of essential oils were compared to the response to two NFκB inhibitors, luteolin and ACHP. Linalool and lavender essential oil extracted from plants at the beginning of flowering period were successful in decreasing pro-inflammatory cytokine production following LPS pretreatment. In case of IL-8 and IL-1β lavender oil showed stronger effect compared to linalool and both of them acted similarly to NFκB inhibitors. Pretreatments with linalool and lavender essential oil/beginning of flowering period prevented pro-inflammatory cytokine production compared to LPS treatment alone. Although lavender essential oil/end of flowering period decreased IL-6, IL-1β and IL-8 mRNA expression in case of LPS pretreatment, it was not capable to reduce cytokine secretion. Based on our results it has been proven that lavender essential oil extracted at the beginning of flowering period is a potent inhibitor of the synthesis of four pro-inflammatory cytokines IL-6, IL-8, IL-β and TNFα of THP-1.

Experiment 2: Three chemotypes of thyme (*Thymus vulgaris* L.) essential oil and their main compounds affect differently the IL-6 and TNFα cytokine secretions of BV-2 microglia by modulating the NF-κB and C/EBPβ signaling pathways.

Neuroinflammation is responsible for several diseases of the central nervous system. Some plant-derived bioactive molecules have been shown to have role in attenuating neuroinflammation by regulating microglia, the immune cells of the CNS. In this study, the anti-inflammatory effect of three chemotypes of thyme essential oil and their main compounds (geraniol, thujanol and linalool) were examined on lipopolysaccharide-induced BV-2 microglia. Three different experimental setups were used, LPS pretreatment, essential oil pretreatment and co-treatments of LPS and essential oils in order to determine whether essential oils are able to prevent inflammation and can decrease it. The concentrations of the secreted tumour necrosis factor α (TNFα) and interleukin-6 (IL-6) proinflammatory cytokines were measured and we analysed by Western blot the activity of the cell signalling pathways, NF-κB and CCAAT-enhancer binding protein β (C/EBPβ) regulating TNFα and IL-6 proinflammatory cytokine expressions in BV-2 cells. Our results showed definite alterations in the effects of essential oil chemotypes and their main compounds at the different experimental setups. Considering the changes of IL-6 and TNFα secretions the best reduction of inflammatory cytokines could be reached by the pretreatment with the essential oils. In addition, the main compounds exerted better effects than essential oil chemotypes in case of LPS pretreatment. At the essential oil pretreatment experiment, the effect of linalool and geraniol was outstanding but there was no major difference between the actions of chemotypes and standards. Main compounds could be seen to have large inhibitory effects on certain cell signalling components related to the activation of the expression of proinflammatory cytokines. Thyme essential oils are good candidates to use in prevention of neuroinflammation and related neurodegeneration, but the exact ratio of the components has to be selected carefully.

Experiment 3: Anti-inflammatory effects of lavender and eucalyptus essential oils on the *in vitro* cell culture model of bladder pain syndrome using T24 cells.

Interstitial cystitis (IC) has a chronic chemical irritation and inflammation of non-bacterial origin in the bladder wall leading to various severe symptoms. There is evidence that chronic inflammation is significantly associated with abnormal urothelial barrier function, epithelial dysfunction. This is the underlying cause of urothelial apoptosis and sterile inflammation. The anti-inflammatory effects of

lavender and eucalyptus essential oils and their main components (linalool and eucalyptol) were investigated in the T24 human bladder epithelial cell line on TNFα stimulated inflammation, at 3 types of treatment schedule. The mRNA of pro-inflammatory cytokines (IL-1β, IL-6, IL-8) were measured by Real Time PCR. Human IL-8 ELISA measurement was performed as well at 3 types of treatment schedule. The effects of lavender and eucalyptus EOs and their main components were compared to the response to NFκB inhibitor ACHP (2-amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-(4-piperidinyl)-3-pyridinecarbonitrile). There is no significant difference statistically, but measurements show that lavender EOs are more effective than eucalyptus EO. Long time treatment (24 h) of both lavender EO and linalool showed higher effect in decreasing pro-inflammatory cytokine mRNA expression than ACHP inhibitor following TNFα pre-treatment. Moreover, both lavender EOs were found to be significantly more effective in decreasing IL-8 secretion of T24 cells after TNFα pre-treatment compared to the ACHP NFκB-inhibitor. The lavender EOs may be suitable for use as an adjunct to intravesical therapy of IC. Their anti-inflammatory effect could well complement glycosaminoglycan-regenerative therapy in the urinary bladder after appropriate pharmaceutical formulation.

Experiment 4: Antioxidant and Anti-Inflammatory Effects of Thyme (*Thymus vulgaris* L.) Essential Oils Prepared at Different Plant Phenophases on Pseudomonas aeruginosa LPS-Activated THP-1 Macrophages.

Thyme (Thymus vulgaris L.) essential oil (TEO) is widely used as an alternative therapy especially for infections of the upper respiratory tract. TEO possesses antiviral, antibacterial, and antifungal properties. The emerging antibiotic resistance of bacterial strains, including *Pseudomonas aeruginosa*, has prompted the urge to find alternative treatments. In the present study, we examined the anti-inflammatory and antioxidant effects of thymol, the main compound of TEO, and two TEOs prepared at the beginning and at the end of the flowering period that may make these oils promising candidates as complementary or alternative therapies against *P. aeruginosa* infections. The activity measurements of the antioxidant enzymes peroxidase (PX), catalase (CAT), and superoxide dismutase (SOD) as well as the determination of total antioxidant capacity of *P. aeruginosa*-activated THP-1 cells revealed that thymol and both TEOs increased CAT and SOD activity as well as the antioxidant capacity of the THP-1 cells. The measurements of the pro-inflammatory cytokine mRNA expression and secreted protein level of LPS-activated THP-1 cells showed that from the two TEOs, only TEO prepared at the beginning of the flowering period acted as a potent inhibitor of the synthesis of IL-6, IL-8, IL-β, and TNF-α. Our results suggest that not only thymol, but also the synergism or the antagonistic effects of the additional compounds of the essential oils are responsible for the anti-inflammatory activity of TEOs.

Main findings

- The collection time of essential oil plants involved in our study (*Thymus vulgaris* L. and *Lavandula angustifolia* Mill.) influence not only the chemical composition of the oils but their biological (antibacterial and anti-inflammatory) effects.
- The two cultivation fields involved in our study provided thymol-chemotype and linalool-chemotype essential oils.
- In case of both plants the beginning of the flowering period and full blooming period provided the most biologically active essential oils.
- In case of both plants there is possibility to collect plant material for isolation of essential oils not only in full bloom period but at the beginning of flowering period and at the end of flowering period. The warmer summers in Hungary influences positively the essential oil production in these two Mediterranean plants (thyme and lavender).
- We could optimized TLC-DB, broth dilution and anti-biofilm methods for the non-water soluble and volatile plant extracts such as essential oils.
- We have also demonstrated that the nanoemulsion formulation (Pickering emulsion) of the essential oils showed the highest antibacterial activity.

- The results of our studies could provide complex, integrative in vitro experimental data for the ability of thyme and lavender essential oils to inhibit inflammatory processes (e.g. in neuroinflammation, in interstitial cystitis) and to determine their antibacterial property.
- The most efficient oils could serve as the basis for development of a new natural, safety applicable therapeutical product against respiratory tract infection and inflammation.