Supplementary Material

Thymus × *citriodorus:* an Emerging Aromatic and Medicinal Hybrid Plant with Relevant Bioactive Potential

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Table S6 Antioxidant activity of *Thymus* × *citriodorus* volatile oils (VO) and extracts evaluated by different methods including: reducing Power (RP), DPPH radical-scavenging activity (DPPH), β-carotene bleaching inhibition (β-carotene), lipid peroxidation inhibition (TBARS), ABTS radical cation scavenging ($ABTS^{\bullet+}$), hydroxyl radicals scavenging assay (•OH) and nitric oxide radical (NO•) scavenging activity

Table S7 Nematicidal potential of *Thymus* × *citriodorus* volatile oils (VO) and extracts

Table 1 Source, processing features, and chemical characterization of the *Thymus* × *citriodorus* volatile oils (VO) analyzed by GC-MS. The major compound in each VO is represented in **bold**, and <u>underlined</u> if its relative abundance was superior to 50%

Origin	**Reported variety**	**Plant parts**	**Harvesting stage (period)**	**Isolation details**	**Yield**	**Major components[b] (%)**	**Ref.**
Portugal	*T. citriodorus*	ND	ND	Steam distillation[a]	ND	**Geraniol (27.5),** 1,8-cineole (16.3), thymol (9.2), geranial (4.2), *p*-cymene (3.7), neral (3.4)	(Oliveira et al. 2022)
Lithuania	*T. citriodorus*	Leaves and flowers	(June 2020)	Hydrodistillation (Clevenger) of dried plants for 2 h[a]	Variable due to fertilization (% of dry weight): ~ 0.5 – 0.6	Variations dependent on the fertilizers (liquid cattle dung *versus* hummus): **Geraniol (23.78 *vs* 26.27);** nerol (14.73 *vs* 14.20); geranial (11.97 *vs* 13.04); neral (11.27 *vs* 10.63); germacrene D-4-ol (6.66 *vs* 8.74)	(Vaičiulytė et al. 2022)
Lithuania	*T. citriodorus*	Leaves and flowers	(June 2021)	Hydrodistillation (Clevenger) of dried plants for 2 h[a]	Variable due to fertilization (% of dry weight): ~ 0.2 – 0.35	Variations dependent on the fertilizers (liquid cattle dung *versus* hummus): **Geraniol (23.60 *vs* 20.55);** nerol (15.73 *vs* 12.29); geranial (13.47 *vs* 12.59); neral (11.27 *vs* 10.63); germacrene D-4-ol (6.66 *vs* 8.74)	(Vaičiulytė et al. 2022)
Lithuania	*T. citriodorus*	Leaf inflorescences	Full flowering (June 2015, 2016 and 2017)	Hydrodistillation (Clevenger) of dried plant for 2 h[a]	0.5-0.7 mL / 100 g	Average (2015-2017): **Geraniol (26.53),** nerol (16.55), geranial (13.49), neral (12.90)	(Ložienė et al. 2021)
Greece	*T. citriodorus*	Aerial parts	Flowering	Hydrodistillation (Clevenger) of dried plant for 3 h. 100 g of plant / 1 L of distilled water	5 mL / 100 g of dry weight	**Geraniol (47.08),** geranial (7.94), neral (7.12), geranyl acetate (4.05)	(Ntalli et al. 2020a)

Austria	*T. citriodorus*	ND	Flowering	ND	1.08% of dry weight	**Geraniol (83.34),** neral (6.84), geranial (2.92)	(Malankina et al. 2019)
Russia	*T. citriodorus* var. variegata	ND	Flowering	ND	ND	**Thymol (53.46),** isobornyl propionate (12.92), carvacrol (6.05), α-terpineol (3.44)	(Malankina et al. 2019)
Lithuania	*T. citriodorus*	Leaves and inflorescences (free from stems)	Flowering (June 2015)	Hydrodistillation according to European Pharmacopeia 6th Edition with 19 distillation times[b]. 30 g / 0.5 L of water	Variable across distillation times Lowest: 0.28% (*m/m*) at 5 min; Highest: 0.75% (*m/m*) at 150 min	Average from different distillation times: **Geraniol (36.6)**, geranial (17.5), nerol (11.5), neral (10.2)	(Jurevičiūtė et al. 2019)
Republic of Moldova	*T. citriodorus* "aureus"	Aerial parts	Full flowering (July 2015)	Hydrodistillation (modified Clevenger) of powered plant for 3 h. 100 g / 1 L of double distilled water	1.31 mL of VO / 100 g of dry weight	**Geraniol (60.31),** geranial (9.26), neral (7.15), thymol (3.45)	(Aprotosoaie et al. 2019)
Republic of Moldova	*T. citriodorus*	Aerial parts	Full flowering (July 2015)	Hydrodistillation (modified Clevenger) of powered plant for 3 h. 100 g / 1 L of double distilled water	1.61 mL of VO / 100 g of dry weight	**Lavandulol (54.27),** geranial (12.25), neral (9.00), nerol (3.88)	(Aprotosoaie et al. 2019)
Turkey	*T. citriodorus*	Aerial parts	Pre-flowering, flowering and post-flowering (May to June 2012)	Hydrodistillation (Clevenger) of dried plant (20 g) for 3 h	2.0% (*m/m*) pre-flowering; 1.9% (*m/m*) flowering; 1.3% (*m/m*) post-flowering	Variations from pre-flowering to post-flowering stages: **Terpinolene (61.20 - 59.0),** α-terpineol (22.84 – 29.56), linalool (3.47 – 1.35), bornyl acetate (1.34 –1.94), borneol (1.11 – 1.24)	(Toncer et al. 2017)
Turkey	*T. citriodorus*	ND	(June 2015)	Hydrodistillation (Clevenger) of dried plant (100 g) for 4 h	1.4% (*m/m*) of dry weight	**Geraniol (38.53),** neral (29.20), nerol (5.61), L-borneol (4.94), 3-octanone (2.66)	(Duman and Özcan 2017)

Italy	*T. citriodorus*	Above ground tissues	(May 2014)	Steam distillation of fresh plants (≈1 kg)[a]	0.16 mL / 100 g	**Citronellol (40.1),** geranyl acetate (27.5), geranial (5.7), neral (4.4), nerol (3.9)	(Checcucci et al. 2017)
Italy	*T. citriodorus*	ND	ND	Hydrodistillation (Clevenger) of dried plant for 2 h	Variable across drought stress conditions: 0.6 mL – 1 mL/ 100 g of dry weight	Variations across different levels of PEG-6000 induced drought stress[c]: **Geraniol (4.2 – 21.9),** pseudophytol (12.1 – 5.0), carvacrol (0 – 31.7), thymol (0 – 4.4)	(Tátrai et al. 2016)
Portugal	*T. citriodorus*	Leaves and small branches of aerial parts	Autumn	Hydrodistillation (Clevenger) of the dried plant (30 g) for 3 h, according to the European Pharmacopeia 6th edition	1.26% (*m/m*)	<u>**Geraniol (76.1)**</u>**,** geranial (5.28), neral (3.65), caryophyllene (1.57), nerol (1.44)	(Teixeira and Rodrigues 2014)
ND	*T. citriodorus*	Leaves	ND	Steam distillation[a]	ND	**Borneol (28.82),** thymol (14.43), linalool (8.26), α-terpineol (8.23), camphor (5.1)	(Wu et al. 2013)
Italy (accessions with different origins)	*T. citriodorus*	ND	ND	Hydrodistillation for 4 h[a]	ND	Variation depending of the accessions' origin: <u>**Geraniol (62.04 - 27.88),**</u> citral[d] (13.75 – 1.18), *trans*-caryophyllene (5.80 – 2.42), citronellol (9.57 –0.82), linalool (2.00 – 0.11), myrcene (12.00 – 0.13)	(Lisi et al. 2011)
Italy	*T. citriodorus* "anderson's gold"	Aerial parts	Full blooming	Hydrodistillation (Clevenger) of air-dried plant for 2 h according to Italian Pharmacopoeia XI	ND	<u>**Geraniol (61.4)**</u>**,** geranial (14.3), neral (9.1)	(Bertoli et al. 2010)

				edition. 10.0 g - 12.5 g / 2 L distilled water			
Italy	*T. citriodorus* "silver queen"	Aerial parts	Full blooming	Hydrodistillation (Clevenger) of air-dried plant for 2 h according to Italian Pharmacopoeia XI edition. 10.0 g - 12.5 g / 2 L distilled water	ND	**Geraniol (67.1),** geranial (9.7), neral (4.8), 3-octanol (3.4)	(Bertoli et al. 2010)
Iran	*T. citriodorus*	Aerial parts	Pre-flowering, beginning of flowering, full flowering and fruit set (June to July)	Hydrodistillation (Clevenger) of dried plants (90 g) for 4 h according to the European Pharmacopoeia 2nd edition	Before flowering: 1.8% (*m/m*); Beginning of flowering: 2.21% (*m/m*); Full flowering: 1.45% (*m/m*); Fruit set: 1.66% (*m/m*)	Variations from pre-flowering to fruit stages: **geraniol (72.5 - 54.2)**, geranial (3.2 – 11.9), neral (2.2 – 7.1), 3-octanol (2.7 – 2.6), nerol (1.3 – 2.4)	(Omidbaigi et al. 2009)
Hungary	*T. citriodorus*	ND	(July 2002)	Steam distillation of air-dried material for 3 h, according to European Pharmacopoeia 4th edition	0.36% of dry weight	**Geraniol (39.2),** carvacrol (15.4), geranial (9.2), neral (7.1), *p*-cymene (4.5)	(Horváth et al. 2006)
Hungary	*T. citriodorus* 'archer's gold'	ND	(July 2002)	Steam distillation of air-dried material for 3 h, according to European Pharmacopoeia 4th edition	0.29% of the dry weight	**Carvacrol (43.5),** *p*-cymene (21.1), 1,8-cineole (7.5), β-caryophyllene (3.5)	(Horváth et al. 2006)

Italy	*T. citriodorus*	ND	ND	Steam distillation[a]	ND	**Geraniol (36.4),** geranyl acetate (22.4), nerol (5.31), geranial (4.99), neral (4.89)	(Sacchetti et al. 2005)
Iran	*T. citriodorus*	Leaves	Full flowering (June)	Hydrodistillation (Clevenger) of dried plants (90 g) for 4 h according to the European Pharmacopoeia 2nd edition	1.4% (*m/m*) of dry weight	**Geraniol (54.4),** geranial (13.9), nerol (5.2), 3-octanone (3.3), borneol (3.2)	(Omidbaigi et al. 2005)
Hungary	*T. citriodorus*	Aerial parts	ND	Steam distillation of the air dried material, according to European Pharmacopoeia 4th edition	0.36% of dry weight	**Geraniol (43),** carvacrol (6.1), thymol (0.8)	(Horváth et al. 2004)
Hungary	*T. citriodorus* "archer's gold"	Aerial parts	ND	Steam distillation of the air dried material, according to European Pharmacopoeia 4th edition	0.29% of dry weight	**Carvacrol (55.1),** thymol (0.7)	(Horváth et al. 2004)
Germany	*T. citriodorus*	Aerial parts	(July to October 1992)	Hydrodistillation ('Karlsruher apparatus') of the fresh plant for 4h using n-hexane (2 mL) as solvent[a]	ND	**Geraniol (61.3),** geranial (8.2), neral (5.5), β-caryophyllene (3.7), nerol (2.8)	(Stahl-Biskup and Holthuijzen 1995)

[a] Details of the distillation method were not reported; [b] 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 110, 120 and 150 min; [c] variations due to drought stress induced with PEG-6000 from 0% (control) to 4%; [d] Isomeric distribution is not described; ND – Information not disclosed in the original paper

Table 2 Source, processing and extraction features and chemical characterization of *Thymus* × *citriodorus* extracts

Source	Preparation	Plant parts	Harvesting stage (period)	Extraction method and details	Yield	Analyzed by	Major components	Ref.
Greece	Water extract	ND	ND	*Aqueous extraction under sonication* 5 g of plant powder / 25 mL of water and sonication for 15 min. The obtained extract was filtered through cotton	ND	GC-MS[a]	Traces of thymol	(Ntalli et al. 2020b)
Portugal	Aqueous extract	Upper part of stems and their leaves	Post-blooming, end fructification (October 2014)	*Aqueous decoction* 0.5 g of lyophilized plant / 150 mL of distilled water and boiled with agitation for 20 min. The extract was filtered twice and the volume was reduced by concentration in a rotary evaporator	~9% (*m/m*)	HPLC-DAD/ESI-MS	In mg/g of extract Rosmarinic acid (51.80 ± 1.86), luteolin-(?)-*O*-hexuronide (13.22 ± 0.36), salvianolic acid A (11.9 1± 0.96), chrysoeriol-(?)-*O*-hexoside (7.06 ± 0.65), salvianolic acid K (5.21 ± 1.5)	(Taghouti et al. 2020)
Portugal	Hydroethanolic extract	Upper part of stems and their leaves	Post-blooming, end fructification (October 2014)	*Hydroethanolic extraction* 0.5 g of lyophilized plant material / 50 mL of 80 % ethanol solution. The mixture was agitated for 1 h and centrifuged for 5 min, followed by a second extract of the pellet. The extracts were filtered and then concentrated in a rotary evaporator	~14% (*m/m*)	HPLC-DAD/ESI-MS	In mg/g of extract Rosmarinic acid (86.23 ± 13.88), salvianolic acid I (25.87 ± 3.90), luteolin-(?)-*O*-hexuronide (17.25 ± 3.13) chrysoeriol-(?)-*O*-hexoside (11.96 ± 1.83), salvianolic acid A (8.32 ± 0.62)	(Taghouti et al. 2020)
Greece	Hydrosol	Aerial parts	Flowering	*Condensation water from hydrodistillation* Hydrodistillation (Clevenger) of dried plant for 3 h. 100 g of plant / 1 L of distilled water. The obtained hydrosol was filtered	ND	GC-MS[b]	In percentage (%)[c] Geraniol (44.06), carvacrol (4.39), thymol (2.22)	(Ntalli et al. 2020a)

Portugal	Supercritical fluid extract	Leaves and small branches	Autumn	*Supercritical CO_2 fluid extraction* Compressed CO_2 (40 °C) was added to 30.0 g ± 0.1 g of plant material in an in-house built equipment for 3 h.	0.05% (*m*/*m*)	GC-MS	In percentage (%) Geraniol (66.47 ± 0.03), geranial (8.63 ± 0.06), thymol (7.63 ± 0.09)	(Teixeira and Rodrigues 2014)
Poland	Methanol extract	Fresh plant leaves	ND	*Methanolic extraction* 0.2 g of fresh plant / 30% methanol (with1 %acetic acid and 1% ascorbic acid) for 20 min under sonication with occasional shaking. After centrifugation the supernatant was filtered	ND	UPLC-MS	In mg/100 g of fresh wight Gallic acid (2754.3 ± 2.4), (+) – catechin (1414.1 ± 1.4), kaempferol 3-*O*-rutinoside (718.8 ± 3.9)	(Kolniak-Ostek and Oszmiańsk i 2015; Pasławska et al. 2020)
Poland	Methanol extract	Dried plant Leaves	ND	*Methanolic extraction* 0.2 g of powered dry (by CD, FB, MFB or MV) plant / 30% methanol (with1 % acetic acid and 1% ascorbic acid) for 20 min under sonication with occasional shaking. After centrifugation the supernatant was filtered	ND	UPLC-MS	Variable due to different drying techniques (mg/100 g of dry matter) (+) - Catechin (290.0 ± 0.6 – 1592.3 ± 2.1), *cis*-5-caffeolquinic acid (174.7 ± 0.4 – 3603.3 ± 6.2), B-type procyanidin dimer (85.7 ± 0.7 – 1131.1 ± 0.3), procyanidin C1 (30.3 ± 0.2 – 403.1 ± 4.2)	(Kolniak-Ostek and Oszmiańsk i 2015; Pasławska et al. 2020)
Portugal	Aqueous infusions	Apical leaves	Summer months	*Aqueous infusion* 0.3 g of dry plant material / 100 mL of heated distilled water (85 °C). The infusion was left to stand for 7 min until filtration	ND	HPLC-DAD-ESI/MS	Variable accordingly to lots in µg/mL (standard lot *versus* reserve lot) Rosmarinic acid (78 ± 2 *vs* 66 ± 2), luteolin 7-*O*-glucuronide (39 ± 1 *vs* 41.2 ± 0.9), lithospermic acid A (19.4 ± 0.6 *vs* 18.8 ± 0.7)	(Rita et al. 2018)
Lithuania	Methanol extract	Above-ground parts	From floral budding to	*Methanolic extraction* 0.5 g sieved plant / 10 mL of 100% methanol in an ultrasonic bath for 30	ND	HPLC–UV	Variation from floral bud to end vegetation (µg/g, dry weight):	(Raudone et al. 2017)

			the end of vegetation (May and September 2016)	min. The extracted samples were centrifuged and filtered			Oleonolic acid (1820 ± 29 – 1506 ± 24), ursolic acid (4869 ± 86 – 3965 ± 70)	
Lithuania	Ethanol extract	Above-ground parts	From floral budding to the end of vegetation (May and September 2016)	*Ethanolic extraction* 0.1 g of sieved plant material / 10 mL of 70 % ethanol in an ultrasonic bath for 30 min. The extracted samples were centrifuged and filtered	ND	HPLC–UV	Variation through different phenological phases (µg/g, dry weight): Rosmarinic acid (27.273 ± 170 – 9008 ± 18), caffeic acid (146 ± 1 – 138 ± 1), luteolin-7-rutinoside (581 ± 7 – 72 ± 1), luteolin-7-glucoside (148 ± 2 – 5 ± 0.1)	(Raudone et al. 2017)
Portugal	Ethanol extract	Leaves and stems	ND	*Ethanolic extraction* 5 g of aerial parts (previously defatted 3 times with 150 mL n-hexane for 30 min at room temperature) / 150 mL of ethanol solution (80% (v/v)) at room temperature, for 30 min. The resulting mixture was filtered and the residue was re-extracted for four times.	ND	HPLC/DAD - ESI/MS	In mg/g extract Luteolin-7-*O*-α-glucuronide (12 ± 2), rosmarinic acid (10.4 ± 0.6), apigenin-7-β-*O*-glucuronide (9 ± 2)	(Pereira et al. 2013b)(Pereira et al. 2013a)
Portugal	Hydrolate	ND	ND	*Condensation of water from steam distillation*[e]	ND	GC-MS[d]	Relative amount in volatile part (%) 1,8-Cineole (26.3), linalool (24.3), geraniol (13.9), α-terpineol (11.2) Relative amount in hydrolate (%): 1,8-Cineole (0.0145), linalool (0.0134), geraniol (0.0077), α-terpineol (0.0062)	(Oliveira et al. 2022)

Ukrain	Alcoholic extract	ND (var. "silver queen")	(June to October 2017–2021)	*Alcoholic extraction* (1:10)[e]	ND	GC-MS	In percentage (%) Oleic acid (10.35 ± 1.42), caryophyllene oxide (9.62 ± 0.97), matricarin (7.59 ± 0.86), geranial (6.35 ± 0.75), β-bisabolene (6.15 ± 0.75), thymol (5.46 ± 0.70)	(Steshenko and Mazulin 2022)
Japan	Aromatic extract	ND	ND	*Solvent extraction* Overnight extraction with diethyl ether[e]		GC-MS	In percentage (%) Geraniol (50), geranial (5.6), caryophyllene (4)	(Hirai et al. 2022)
USA	Methanolic extract	Edible parts of fresh plant	ND	*Methanolic extraction* 10 g / 0.2 L of methanol at room temperature (twice). The extracts were centrifuged and the supernatants were filtered		HPLC	Variation among plants purchased with 2-3 months interval (mg / g, wet weight): Rosmarinic acid (5.60 – 9.70)[f]	

ND – Information not disclosed in the original paper; [a] To proceed to GC-MS chemical analysis, water extracts and hydrosols were subjected to an extraction with two portions of petroleum ether (25 mL each); [b] submitted to chemical analysis after 1/1 (v/v) fractionation in petroleum ether; [c] Percentage in relation to the volatile fraction isolated from the hydrolate; [d] Prior GC-MS analysis, the sample (100 mL) was added of *n*-dodecane as internal standard and submitted to liquid/liquid partition with *n*-pentane/diethyl oxide [93/7 (v/v)]. The organic fraction was concentrated at low pressure and room temperature and further adjusted to 10 mL with *n*-pentane; [e] Further details were not disclosed; [f] Only rosmarinic acid was quantified. MFB - microwave- assisted fluidized bed; MV - microwave-vacuum; CD - convective drying; FB - fluidized bed

Table S3 Reported relevant activities (in bold) of *Thymus* × *citriodorus* volatile oils (VO) and extracts and the major compounds described for each preparation

Preparation	**Source**	**Main compounds**	**Reported activity**	**Ref.**
VO	Greece	Geraniol, geranial, neral	**Nematicidal activity** (*M. incognita* and *M. javanita)*	(Ntalli et al. 2020a)
Water extracted (filtered)	Greece	ND	**Nematicidal activity** (*M. incognita* and *M. javanita)*	(Ntalli et al. 2020a)
Hydrolate	Greece	Geraniol, carvacrol, thymol	**Nematicidal activity** (*M. incognita* and *M. javanita)*	(Ntalli et al. 2020a)
Water extract (from powder plant)	Greece	Traces of thymol	**Nematicidal activity** (*M. incognita* and *M. javanita)*	(Ntalli et al. 2020b)
Plant powder	Greece	ND	**Bio-fertilizing properties**	(Ntalli et al. 2020a)
VO from *T. citriodorus* “aureus” cultivar	Republic of Moldova	Geraniol, geranial, neral	**Antifungal activity** (*Aspergilus flavus)*; **Antioxidant capacity** (RP, DPPH, $ABTS^{\bullet+}$)	(Aprotosoaie et al. 2019)
VO	Republic of Moldova	Lavandulol, geranial, neral	**Antifungal activity** (*Aspergilus flavus)*; **Antioxidant capacity** (DPPH, $ABTS^{\bullet+}$)	(Aprotosoaie et al. 2019)
VO	Italy	Geraniol, geranyl acetate, nerol	**Antimicrobial activity** (*C. albicans, R. glutinis, S. cerevisiae*, *S. pombe*, *Y. lypolitica*); **Antioxidant capacity** (DPPH, β-carotene)	(Sacchetti et al. 2005)
Ethanol extracts of frozen and dried plant	Slovenia	ND	**Antimicrobial activity** (*S. aureus, L. monocytogenes, C. jejuni*, Verotoxin *E. coli*)	(Abramovič et al. 2018)
Methanol–water extracts of leaves and rhizomes	Poland	ND	**Antibacterial activity** (*Bacillus subtilis)*	(Orłowska et al. 2015)
VO	Portugal	Geraniol, 1,8-cineole, thymol	**Antibacterial activity** (*S. aureus*, *S. epidermidis*, *C. acnes*); **Anti-biofilm activity** (*C. acnes*); **Anti-inflammatory activity** on macrophages cell line (Raw 264.7); **Antioxidant capacity** (DPPH); **Environmental toxicity** on freshwater crustacean *Daphnia magna*	(Oliveira et al. 2022)
Hydrolate	Portugal		**Antibacterial activity** (*S. aureus*, *S. epidermidis*, *C. acnes*); **Anti-biofilm activity** (*C. acnes*); **Anti-inflammatory activity** on macrophages cell line (Raw 264.7); **Antioxidant capacity** (DPPH)	(Oliveira et al. 2022)

VO from *T. citriodorus* “silver queen” cultivar	Ukrain	ND	**Antimicrobial activity** (*S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*)	(Steshenko et al. 2021)
Hydroethanolic extract	Portugal	Rosmarinic acid, salvianolic acid I, luteolin-(?)-*O*-hexuronide	**Anti-proliferative activity** on human colon adenocarcinoma cell line (Caco-2); **Antioxidant capacity** ($ABTS^{•+}$, •OH, NO•)	(Taghouti et al. 2020)
Aqueous decoction	Portugal	Rosmarinic acid, luteolin-(?)-*O*-hexuronide, salvianolic acid A	**Anti-proliferative activity** on human colon adenocarcinoma cell line (Caco-2); **Antioxidant capacity** ($ABTS^{•+}$, •OH, NO•)	(Taghouti et al. 2020)
Aqueous infusions	Portugal	Rosmarinic acid, luteolin 7-*O*-glucuronide, lithospermic acid A	**Antioxidant capacity** (RP, DPPH, β-carotene)	(Rita et al. 2018)
Ethanolic extract	Portugal	Luteolin-7-*O*-α-glucuronide, rosmarinic acid, apigenin-7-β-*O*-glucuronide	**Cytoprotective activity** on human hepatoblastoma cell line (HepG2); **Antioxidant capacity** (RP, DPPH, ROS)	(Pereira et al. 2013a)
VO	ND	Borneol, thymol, linalool, α-terpineol	**Antiproliferative activity** on human hepatoblastoma cell line (HepG2)	(Wu et al. 2013)
Extract[a]	ND	ND	**Anti-aging properties** by stimulation of Emilin-1 (normal human skin fibroblasts cell line)	(Fitoussi et al. 2011)
Methanolic extract	Palestina	ND	**Pesticidal (antifungal) properties** (*Ustilago tritici* and *Ustilago nuda*)	(Karsou and Samara 2021)
Ethanolic extract	Turkey	ND	**Antibacterial activity** (*S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli*)	(Kerem et al. 2023)
Hexane extract	Turkey	ND	**Antibacterial activity** (*S. aureus*)	(Kerem et al. 2023)
Chloroformic extract	Turkey	ND	**Antibacterial activity** (*S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli)*	(Kerem et al. 2023)

[a]Details on the type of extract were not available; ND – information not disclosed in the original work; RP – reducing Power; DPPH – 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity; β-carotene – β-carotene bleaching inhibition; ABTS•+ – ABTS radical cation scavenging; •OH – hydroxyl radicals scavenging assay; NO• – nitric oxide radical scavenging activity; ROS – Reactive oxygen species

Table S4 Reported antimicrobial activity of *Thymus* × *citriodorus* volatile oils (VO) and extracts

Preparation	Source	Antimicrobial activity	Ref.
VO from *T. citriodorus* “aureus”	Republic of Moldova	***Aspergilus flavus* MUCL 19006** MIC: 0.5 μL/mL; MLC: 1 μL/mL	(Aprotosoaie et al. 2019)
VO	Republic of Moldova	***Aspergilus flavus* MUCL 19006** MIC: 1 μL/mL; MLC: 1 μL/mL	(Aprotosoaie et al. 2019)
VO	Italy	***Candida albicans* ATCC 48274 -** MIC[a]: 0.06 mg/mL ***Rhodotorula glutinis* ATCC 16740 -** MIC[a]: 0.09 mg/mL ***Saccharomyces cerevisiae* ATCC 60232 -** MIC[a]: 0.06 mg/mL ***Schizosaccharomyces pombe* ATCC 2365 -** MIC[a]: 0.06 mg/mL ***Yarrowia lipolytica* ATCC 16617 -** MIC[a]: 0.03 mg/mL	(Sacchetti et al. 2005)
Ethanol extracts of frozen and dried plant	Slovenia	MIC (mg CAE / mL growth media) ***Staphylococcus aureus*** Frozen: 621 ± 217; Dried: 490 ± 172 ***Listeria monocytogenes*** Frozen: 1064 ± 0; Dried: 1190 ± 413 ***Campylobacter jejuni*** Frozen: 288 ± 252; Dried: 306 ± 286 ***Verotoxin Escherichia coli*** Frozen: 3900 ± 868; Dried: 6158 ± 1371	(Abramovič et al. 2018)
Methanol–water extracts of leaves and rhizomes	Poland	Inhibition zone (cm^2) [b] ***Bacillus subtilis* ATCC 6633** Leaves: 1.44 (±0.07); Rhizomes: 0.78 (±0.06)	(Orłowska et al. 2015)
VO	Portugal	***Staphylococcus aureus* ATCC 6538** MIC: 0.125% (v/v); MLC: 0.250% (v/v) ***Staphylococcus epidermidis* DSM 28764** MIC: 0.06% (v/v); MLC: 0.250% (v/v)	(Oliveira et al. 2022)

		***Cutibacterium acnes* DSM 1897** MIC: 0.06% (v/v); MLC: 0.125% (v/v)	
Hydrolate	Portugal	***Staphylococcus aureus* ATCC 6538** MIC: > 50% (v/v); MLC: > 50% (v/v) ***Staphylococcus epidermidis* DSM 28764** MIC: > 50% (v/v); MLC: > 50% (v/v) ***Cutibacterium acnes* DSM 1897** MIC: 50% (v/v); MLC: > 50% (v/v)	(Oliveira et al. 2022)
VO from *T. citriodorus* 'silver queen'	Ukraine	Growth inhibition zones (mm) ***Staphylococcus aureus* ATCC 25923** – 14.60 ± 1.5 ***Escherichia coli* ATCC 259220** – 19.60 ± 1.8 ***Pseudomonas aeruginosa* ATCC 90270** – 0 (no activity) ***Candida albicans* ATCC 885/6530** – 29.30 ± 2.8	(Steshenko et al. 2021)
Ethanolic extract	Turkey	Growth inhibition zones (mm) depend on applied volume (15 µL, 20 µL or 25 µL, respectively) ***Enterococcus faecalis* ATCC 29212** – 18; 22; 14.5 ***Staphylococcus aureus* ATCC 29213** – 45; 43; 32.5 ***Pseudomonas aeruginosa* ATCC 2753 –** 9; 11.5; 9 ***Escherichia coli* ATCC 25922** – 3.5; 4; 9	(Kerem et al. 2023)
Hexane extract	Turkey	Growth inhibition zones (mm) depend on applied volume (15 µL, 20 µL or 25 µL, respectively) ***Staphylococcus aureus* ATCC 29213** – 5.5; 17; 8.5	(Kerem et al. 2023)
Chloroformic extract	Turkey	Growth inhibition zones (mm) depend on applied volume (15 µL, 20 µL or 25 µL, respectively) ***Enterococcus faecalis* ATCC 29212** – 17; 19; 20.5 ***Staphylococcus aureus* ATCC 29213** – 30; 50; 16.5 ***Pseudomonas aeruginosa* ATCC 2753 –** 0; 4; 0 ***Escherichia coli* ATCC 25922** – 0; 0; 3.5	(Kerem et al. 2023)

MIC – Minimum inhibitory concentration; MLC – Minimum lethal concentration; [a] MIC was considered as the lowest concentration of each VO showing a clear zone of inhibition by standard discs diffusion technique; [b] by Dot-blot Test

Table S5 Bioactive properties of *Thymus* × *citriodorus* volatile oils (VO) and extracts regarding their antiproliferative and anti-inflammatory activities

Preparation	Source	Anti-proliferative / cytotoxic activity (IC_{50})	Anti-inflammatory activity (EC_{50})	Ref.
Hydroethanolic extract	Portugal	**Caco-2 cells** 24h: 128.2 µg/mL ± 5.75 µg/mL 48h: 114.6 µg/mL ± 4.38 µg/mL **HepG2 cells** 24h: > 500 µg/mL 48h: > 500 µg/mL		(Taghouti et al. 2020)
Aqueous decoction	Portugal	**Caco-2 cells** 24h: 223.70 µg/mL ± 8.38 µg/mL 48h: 159.40 µg/mL ± 16.85 µg/mL **HepG2 cells** 24h: > 500 µg/mL 48h: > 500 µg/mL		(Taghouti et al. 2020)
VO	Portugal	**Raw 264.7 cells** 24h: 0.01% (v/v)	**NO production (Raw 264.7 cells)** EC_{50}: 0.0019% (v/v)	(Oliveira et al. 2022)
Hydrolate	Portugal	**Raw 264.7 cells** 24h: 16.90% (v/v)	**NO production (Raw 264.7 cells)** EC_{50}: 2.76% (v/v)	(Oliveira et al. 2022)

Table S6 Antioxidant activity of *Thymus* × *citriodorus* volatile oils (VO) and extracts evaluated by different methods including: reducing Power (RP), DPPH radical-scavenging activity (DPPH), β-carotene bleaching inhibition (β-carotene), lipid peroxidation inhibition (TBARS), ABTS radical cation scavenging (ABTS•+), hydroxyl radicals scavenging assay (•OH) and nitric oxide radical (NO•) scavenging activity

Preparation	**Source**	**RP**	**DPPH**	**β-carotene**	**TBARS**	**ABTS•+**	**•OH**	**NO•**	**Ref.**
Aqueous extract	Portugal					1.21 ± 0.07 mmol Trolox eq. / g extract Or 0.11 ± 0.03 07 mmol Trolox eq. / g dry plant	(% inhibition) +EDTA: 37.97 ± 1.12% -EDTA: 30.59 ± 2.08%	(% inhibition) 41.15 ± 3.64%	(Taghouti et al. 2020)
Hydroethanolic extract	Portugal					1.52 ± 0.21 mmol Trolox eq. / g extract Or 0.22 ± 0.05 mmol Trolox eq. / g dry plant	ND	ND	(Taghouti et al. 2020)
Aqueous infusions	Portugal	EC_{50} (mg/mL) Standard: 0.228 ± 0.003; Reserve: 0.393 ± 0.002	EC_{50} (mg/mL) Standard: 0.34 ± 0.01; Reserve: 0.32 ± 0.02	EC_{50} (mg/mL) Standard: 1.01 ± 0.06; Reserve: 0.73 ± 0.03	EC_{50} (mg/mL) Standard: 0.118 ± 0.001; Reserve: 0.117 ± 0.001				(Rita et al. 2018)
VO	Republic of Moldova		EC_{50} (mg/mL) 34.281 ± 0.653			EC_{50} (mg/mL) 0.522 ± 0.011			(Aprotosoaie et al. 2019)
VO from *T. citriodorus* “aureus"	Republic of Moldova	EC_{50} (mg/mL) 3.057 ± 0.151	EC_{50} (mg/mL) 1.565 ± 0.045			EC_{50} (mg/mL) 0.014 ± 0.001			(Aprotosoaie et al. 2019)
Ethanolic extract	Portugal	EC_{50} (mg/mL) 0.0882 ± 0.0008	EC_{50} (mg/mL) 0.0117 ± 0.0015						(Pereira et al. 2013)

VO	Italy	25 % compared with 1 mM trolox (28.2 ± 0.20%)	50 % compared with BHA (86.74 ± 0.61%)					(Sacchetti et al. 2005)
VO	Portugal	EC_{50} (% v/v) 0.071 ± 0.002					ND	(Oliveira et al. 2022)
Hydrolate	Portugal	EC_{50} (% v/v) 20.08 ± 1.25					ND	(Oliveira et al. 2022)

ND – Not determined due to low activity

Table S7 Nematicidal potential of *Thymus* × *citriodorus* volatile oils (VO) and extracts

Preparation type	**Source**	**Nematicidal activity**		**Ref.**
		Meloidogyne incognita **EC_{50}**	***Meloidogyne javanica*** **EC_{50}**	
VO	Greece	Day 1: 0.091 ± 0.087% (v/v) Day 2: 0.084 ± 0.079% (v/v)	Day 1: 0.093 ± 0.091% (v/v) Day 2: 0.092 ± 0.090% (v/v)	(Ntalli et al. 2020a)
Water extracted (filtered)	Greece	Day 1: 2.860 ± 2.470% (v/v) Day 2: 8.419 ± 7.137% (v/v)	Day 1: 3.892 ± 3.104% (v/v) Day 2: 6.197 ± 4.401% (v/v)	(Ntalli et al. 2020a)
Hydrolate	Greece	Day 1: 38.950 ± 26.704% (v/v) Day 2: 7.464 ± 5.968% (v/v)	Day 1: > 50% (v/v) Day 2: 4.353 ± 3.703% (v/v)	(Ntalli et al. 2020a)
Water extract (from powder plant)	Greece	Day 1: 35.974 ± 1.066 µL/mL Day 2: ND Day 3: ND	Day 1: 38.057 ± 3.736 µL/mL Day 2: 27.914 ± 2.557 µL/mL Day 3: ND	(Ntalli et al. 2020b)

ND – EC_{50} values were outside the tested concentration range