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Characterization of Some Italian Ornamental Thyme by Their Aroma

Alessandra Bertoli[a], Szilvia Sárosi[b], Jenő Bernáth[b] and Luisa Pistelli[a]

[a]*Dipartimento di Scienza Farmaceutiche, Università di Pisa, Via Bonanno 33, I-56126 Pisa, Italy*

[b]*Department of Medicinal and Aromatic Plants, Faculty of Horticultural Sciences, Corvinus University of Budapest, 29-43 Villányi út, H-1118 Budapest, Hungary*

bertoli@farm.unipi.it

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The aromatic profiles of five commercial thyme cultivars (*T. vulgaris* 'Silver Poise', *T. vulgaris* 'Erectus', *T. vulgaris* 'Faustini', *T.* × *citriodorus* 'Anderson's Gold', and *T.* × *citriodorus* 'Silver Queen'), cultivated in Italy, were defined both by their static headspaces (HS) and essential oils (EOs). In addition, a botanical garden sample of *T. vulgaris* was considered as reference material to evaluate the morphological and phytochemical differences from the selected market samples. Extractions of the volatile constituents of the different plant material were carried out by SPME (static headspace, HS) and hydrodistillation (HD) processes. GC-MS analysis provided the separation and identification of approximately 70 components in the HS samples and 50 in the hydrodistilled essential oils, accounting for more than 95% of the total. The typical main constituents of *T. vulgaris* were detected in all the EO samples, although qualitative and quantitative differences were found among the selected ornamental *Thymus* varieties. Thymol (50-55%) was the marker constituent for the three *T. vulgaris* cultivars, while geraniol (61-67%) characterized the essential oils of the two *T.* × *citriodorus* varieties. In all the analyzed essential oils, non-oxygenated (16-79%) and oxygenated (5-26%) monoterpenes were the typical volatile constituents. Specific target compounds (thymol, geraniol and 6-methyl-5-hepten-2-one) were selected to characterize the five thyme cultivars that are considered at present only as ornamental plants. A comparative evaluation of their EO quality was carried out in order to propose them as alternative sources of Italian raw plant material for industrial production.

Keywords: *Thymus vulgaris* L., cultivar, essential oil, static headspace, SPME, hydrodistillation, GC-MS.

Thymus vulgaris L. (Lamiaceae) is a well-known natural food preservative and medicinal plant native to the Mediterranean region. [1a] The beneficial effects of this plant, mainly its antioxidant properties, have been described [1b,1c]. Besides these uses, *Thymus* is also commercialized worldwide as an important source of ornamental plants, owing to its great variability. With respect to the essential oil composition, at least six different chemotypes have been described for wild thyme [1d], with thymol, carvacrol, geraniol, linalool, terpinene-4-ol and 4-thujanol as the main components. Later, a new chemotype was found in the Spanish flora containing a great amount of 1,8-cineole [1e]. Spain, Morocco, Albania and Hungary are important producers of garden thyme, with most being exported to the USA (1000 t/year) [1f].

However, garden thyme is native to Italy, where there are ideal ecological conditions for its cultivation, with an average production of 100-600 t, drug yield, per year. In the case of *T. x citriodorus* "Silver Queen" (TC5), the Liguria Region is one of the main Italian suppliers and produces generally 1.5 million pots per year.

Over-collection of natural populations, and annual changes in drug yields and quality of garden thyme made it necessary to initiate a program of selection on this species [2a]. Two different populations of *T. vulgaris* were utilized for producing varieties: a less frost-sensitive one from Germany and a natural population from the northern border of the natural habitat of garden thyme in Valle d'Aosta (north Italy). In 1994, the first officially registered variety was 'Varico I'. This was homogeneous (having exclusively female individuals) and the three-year-old plants contained an average EO level of 3.9 %, with a high content of thymol (at least 50 %) [2b]. These results were acquired during the selection work and there is no evidence that the same yields are obtained in different ecological circumstances. Due to the high price of hybrid varieties, as well as the long process of the hybrid breeding work, it was thought desirable to evaluate the production and EO quality of those cultivars that have been selected only for ornamental purposes. Ornamental *Thymus* cultivars are usually selected by clone selection, a process that is less expensive than hybrid breeding.

Previous work on the cultivation of *T. vulgaris* varieties has been carried out in Canada and Germany [2c,2d]. However, the chosen cultivars (in the first case, 'Laval-1', 'RH-1', 'RH-2', 'QDLB' and 'Madrid'; in the second, 'Varico I', 'Varico II', 'Krajovy', 'Deutscher Winter', 'Rieger I' and 'Deutscher Winter Junghanns') were selected for flavoring and medicinal purposes. Although Echeverrigaray *et al*. [2e] analyzed commercial thyme cultivars ('Burpee', 'Battle', 'Tropical', 'Blumen', 'SEM', 'ISLA'), their experiments were focused on the potential relationship between chemical and genetic features since the harvesting time and real botanical origin of the commercial cultivars were often unknown.

Very few data on the characterization of the volatile constituents of *Thymus* cultivars have been reported. In 2006, *T. vulgaris*, *Thymus* × *citriodorus* (Pers.) Schreb. and *Thymus* × *citriodorus* 'Archer's gold' were compared by their aroma profile [2f]. The T. × *citriodorus* cultivar had a high level of carvacrol, but its essential oil level was rather low (0.29 g/100g $_{\text{dry wt}}$).

The aim of this study was to define the aromatic profiles of three different ornamental cultivars of *T. vulgaris* and two varieties of *Thymus x citriodorus* (*T. vulgaris* x *T. pulegioides* L.) [3a] by the static headspaces of their fresh plant material and the essential oils extracted by hydrodistillation (HD) of air-dried samples.

A comparative evaluation of the morphological features, EO contents and volatile composition were carried out in order to standardize these thyme cultivars for novel industrial usages. The five ornamental thyme varieties chosen for this study are some of the most requested for their plant structure, leaf and flower colors. All the plant samples were cultivated in Italy and collected at the full blooming stage. The plant origin, morphological features and their EOs yields are summarized in Table 1. The plant height, diameter, fresh and dry weights were determined in order to characterize the five market varieties of thyme (TV1-3, TC4-5) and compare them with a 12-year-old control plant sample (control, TVC), collected at the Botanical Garden (Lucca).

The *T. vulgaris* 'Erectus' (TV2) samples were characterized by a significantly bigger plant diameter (average 22.0 cm), as well as higher fresh and dry weight yields (average 46.9 g and 16.2 g, respectively) than the others. This variety showed fairly similar morphological features to the Botanical Garden

Table 1: Morphological features of the selected garden thyme samples. (mean±standard deviation) and their EOs yields.

Cultivars	Bush diameter (cm)	Plant height (cm)	Fresh Wt (g)	Dry Wt (g)
TVC	70.0	43.1	180.0	82.0
TV1	20.0±1.00	15.7±1.53	31.5±3.66	9.0±1.00
TV2	22.0±1.73	15.0±1.73	46.9±3.81	16.2±1.06
TV3	25.3±2.08	14.7±1.53	30.7±3.91	8.7±1.53
TC4	17.7±2.52	17.0±3.61	27.9±4.11	8.7±1.53
TC5	19.0±1.00	17.0±1.00	38.1±5.55	8.2±0.76

T. vulgaris sample (TVC). The lowest EO contents were recorded for *T. x citriodorus* "Silver Queen" (TC5, 0.49 mg/100g$_{\text{dry wt}}$) and *T. vulgaris* "Silver Poise" (TV1, 0.33 mg/100g$_{\text{dry wt}}$). Only *T. vulgaris* "Erectus" (TV2, 1.45 mg/100g$_{\text{dry wt}}$) and *T. vulgaris* "Faustini" (TV3, 1.48 mg/100g$_{\text{dry wt}}$) satisfied the Pharmacopoeia requirements for *Thymi herba* EO yield [3b,3c]. It is important to point out that the thyme aerial parts were collected at the full blooming period, as recommended by the Official Pharmacopoeia, but ornamental thyme is generally sold at a young age (seven months). On the other hand, the *T. vulgaris* collected from the Botanical Garden of Lucca was an old plant, transplanted 12 years previously from Palmaria Island. However, the *T. vulgaris* 'Erectus' (TV2) and *T. vulgaris* 'Faustini' (TV3) cultivars showed the biggest EO yields, not only in comparison with the other market samples, but also with the botanical garden sample (1.36 mg/100 g $_{\text{dry wt}}$, respectively).

The headspace profile of the fresh plant material was useful in order to define which type of volatiles characterized the aroma emitted spontaneously. The GC-MS results of EO and HS are summarized in Tables 2 and 3. The HS composition of the fresh samples showed significant qualitative and quantitative differences in comparison with the corresponding EOs, especially in the hydrocarbon and oxygenated monoterpene content (Table 4). Furthermore, the HS profiles of both *T. x citriodorus* varieties were characterized by significant amounts of 6-methyl-5-hepten-2-one (TC4, 45.1±1.89; TC5 15.3±1.56%), a carotenoid-derived volatile, which was present in traces only in the HS profile of *T. vulgaris "*Silver Poise" (TV1 0.68±0.36, Table 2). Lower amounts of 6-methyl-5-hepten-2-one has already been reported for the essential oil of other wild thyme species, such as *T. serpyllum praecox* [4], *T. camphoratus* [5a], *T. zygioides* subsp. *zygioides* [5b], and *T. herba-barona* [6a]. However, in the present study, 6-methyl-5-hepten-2-one was detected in higher amounts only in the headspace of the two *T. x citriodorus* samples and not in their EOs, apart from a very low content in the *T. x citriodorus* Silver Queen' EO (TC5, 0.89 ±0.34 %). Therefore, this is the first time that it has been detected as a main constituent of the static HS of some fresh thyme samples. In addition, 6-methyl-5-hepten-2-one was the target compound in the fresh aroma of the two

Table 2: GC-MS results of the static-headspace analysis (relative composition %) of the selected thyme cultivars.

Compound	LRI*[1]	*Thymus vulgaris* (TV)*[2]				*Thymus x citriodorus* (TC)*[2]	
		TVC *[2]	'TV1'	'TV2'	'TV3'	'TC4'	'TC5'
α-Thujene	933	2.5	3.1	3.3	4.8	-	1.3
α-Pinene	942	1.8	6.6	1.9	3.6	0.9	0.9
Camphene	959	2.7	2.9	0.5	2.0	1.9	1.7
Sabinene	979	0.2	-	0.3	0.5	-	0.9
1-Octen-3-ol	984	-	2.2	-	-	0.9	-
ß-Pinene	984	0.5	-	0.6	1.1	-	0.8
6-Methyl-5-hepten-2-one	988	-	0.7	-	-	45.1	15.3
Myrcene	992	2.1	1.5	3.8	1.7	-	-
6-Methly-5-heptene-2-ol	995	-	-	-	-	-	2.4
3-Octanol	999	-	-	-	-	2.3	2.7
Octanal	1005	-	-	-	-	-	0.3
p-Mentha-1(7),8-diene	1008	-	-	-	-	0.3	-
α-Phellandrene	1010	0.1	-	0.2	0.3	-	-
δ3-Carene	1013	0.2	-	0.4	-	-	-
1,4-Cineole	1020	-	-	-	1.0	-	-
α-Terpinene	1022	0.8	0.3	2.8	4.3	-	0.8
p-Cymene	1030	29.2	19.6	40.6	35.6	1.1	3.5
Limonene	1030	-	-	-	-	0.2	1.10
1,8-Cineole	1039	-	2.2	5.3	-	4.6	5.5
(E)-Ocimene	1039	-	0.1		-	-	-
γ-Terpinene	1064	5.7	3.0	21.5	22.5	0.2	4.0
cis-Sabinene hydrate	1075	0.7	1.5	1.6	1.1	0.2	0.5
Terpinolene	1089	0.1	0.1	0.1	0.3	0.2	-
Fenchone	1094	0.5	-	-	-	-	-
Linalool	1101	-	1.0	1.2	1.1	5.0	5.9
trans-Sabinene hydrate	1104	0.2	0.4	0.4	-	-	-
Nonanal	1104	-	0.2	0.1	-	-	-
Camphor	1154	5.0	2.0	0.9	0.4	2.6	0.5
Menthone	1154	0.1	0.4	0.1	-	-	-
Borneol	1177	1.6	0.7	-	0.7	0.5	0.5
Terpinen-4-ol	1177	0.3	0.1	0.2	0.2	-	-
α-Terpineol	1182	0.1	-	-	-	-	-
cis-Dihydrocarvone	1197	0.1	-	-	-	0.1	-
trans-Dihydrocarvone	1200	0.1	-	-	-	-	0.2
Decanal	1206	-	-	-	-	0.3	-
Nerol	1227	-	-	-	-	2.1	4.0
Thymol methyl ether	1233	0.2	6.7	0.1	0.8	1.6	3.5
Carvacrol methyl ether	1242	0.2	5.4	-	2.1	2.1	1.9
Thymoquinone	1254	0.4	0.2	-	0.6	-	-
Geraniol	1254	-	-	-	-	5.3	18.0
Geranial	1274	-	-	-	-	2.2	2.5
Isobornylacetate	1286	0.7	0.3	-	0.4	-	-
Anethole	1289	7.3	10.7	1.5	1.4	2.7	5.0
Thymol	1292	14.1	20.7	2.7	5.4	-	-
Carvacrol	1300	0.7	-	-	0.1	-	-
Methyl geranate	1323	-	-	-	-	0.4	0.9
Isobutyl benzoate	1331	0.8	-	-	-	-	-
Longicyclene	1375	0.7	-	0.2	-	-	-
□- Copaene	1377	-	-	-	0.2	-	-
Geranyl acetate	1381	-	-	-	-	-	0.2
ß-Bourbonene	1384	0.3	0.1	0.1	-	1.9	0.5
ß-Elemene	1390	-	-	-	0.2	-	-
Longifolene	1410	0.7	0.3	0.1	-	0.3	0.2
ß-Caryophyllene	1420	6.9	4.2	6.4	3.5	4.9	5.1
ß-Gurjunene	1420	-	-	0.1	-	0.2	-
trans-α-Bergamotene	1434	1.3	-	-	0.6	0.2	-
α-Humulene	1457	0.2	-	0.2	0.1	0.1	-
Alloaromadendrene	1462	0.3	-	-	-	-	-
ß-Cadinene	1462	-	-	-	-	0.2	-
cis-Muurola-4(14),5-diene	1464	-	-	-	0.2	0.2	0.3
γ-Muurolene	1476	0.2	-	0.1	-	-	-
Germacrene D	1482	1.5	0.2	0.4	1.1	1.1	2.3
Bicyclogermacrene	1496	0.4	-	-	-	-	-
ß-Himachalene	1499	0.2	-	-	-	-	-
α-Farnesene	1503	0.7	-	-	-	-	-
ß-Bisabolene	1506	2.7	-	-	-	4.2	5.2
cis-γ-Cadinene	1511	0.2	-	0.2	0.4	-	-
δ-Cadinene	1519	0.5	-	0.4	-	-	-
ß-Sesquiphellandrene	1525	0.1	-	-	-	-	-
Spathulenol	1578	0.6	-	-	-	-	-
Caryophyllene-oxide	1584	0.6	1.3	0.3	-	0.5	-
□-Cadinol	1645	-	-	-	0.1	-	-
Total		**96.8**	**98.5**	**98.5**	**98.6**	**97.1**	**98.3**

*[1] L.R.I. = linear retention index relative to C_8-C_{23} *n*-alkanes on an HP-5 column. *[2] TVC — *Thymus vulgaris* control sample, TV1 – *T. vulgaris* 'Silver Poise', TV2 – *T. vulgaris* 'Erectus', TV3 – *T. vulgaris* 'Faustini', TC4 – *Thymus* × *citriodorus* 'Anderson's Gold', TC5 – *Thymus* × *citriodorus* 'Silver Queen'.

Table 3: GC-MS results (relative composition %) of the essential oils (EOs) obtained by hydrodistillation from the selected thyme cultivars.

Compound	LRI*[1]	*Thymus vulgaris* (TV)*[2]				*Thymus x citriodorus* (TC)*[2]	
		TVC*[2]	'TV1'	'TV2'	'TV3'	'TC4'	'TC5'
α-Thujene	933	1.1	1.1	1.0	1.0	-	-
α-Pinene	942	0.5	0.6	0.6	0.6	0.1	-
Camphene	959	0.5	0.6	0.2	0.3	0.2	0.1
Sabinene	979	-	-	-	0.1	-	-
1-Octen-3-ol	984	1.2	1.1	0.6	0.7	0.4	0.2
3-Octanone	985	-	0.2	-	-	1.8	-
6-Methyl-5-hepten-2-one	988	-	-	-	-	-	0.9
Myrcene	992	1.5	0.9	1.5	1.4	-	0.1
3-Octanol	999	0.1	0.2	-	-	0.7	3.4
α-Phellandrene	1010	0.1	-	0.2	0.2	-	-
δ3-Carene	1013	0.1	-	0.1	0.1	-	-
α-Terpinene	1022	1.2	0.7	1.4	1.7	-	-
p-Cymene	1030	11.6	12.4	11.4	10.0	0.1	0.2
Limonene	1030	0.3	-	0.3	0.3	-	-
ß-Phellandrene	1037	0.1	0.2	-	-	-	-
1,8 Cineole	1039	0.3	1.2	1.1	1.5	0.4	0.3
γ-Terpinene	1064	8.5	4.4	11.1	6.6	-	0.2
cis-Sabinene hydrate	1075	1.2	-	1.3	1.5	-	-
1-Nonen-3-ol	1084	0.1	1.3	-	-	-	-
Terpinolene	1089	0.1	-	0.1	-	-	-
Linalool	1101	0.7	2.2	5.1	1.9	0.4	0.4
trans-Sabinene hydrate	1104	0.3	0.3	0.2	0.3	-	-
Nonanal	1107	0.1	-	-	-	-	-
Camphor	1154	-	1.1	-	-	0.3	-
Borneol	1177	1.8	1.2	0.5	0.7	0.7	0.6
Terpinene-4-ol	1182	0.7	0.4	0.5	0.7		-
α-Terpineol	1197	0.1	0.2	0.2	0.4	-	0.1
cis-Dihydrocarvone	1200	-	0.1	-	-	-	-
Nerol	1227	-	-	-	-	1.8	1.2
Thymol methyl ether	1233	0.1	2.1	-	1.0	0.3	0.4
Carvacrol methyl ether	1242	-	3.5	-	1.4	-	-
Neral	1245	-	-	-	-	9.1	4.8
Thymoquinone	1254	-	-	-	0.7	-	-
Geraniol	1254	-	-	0.8	-	61.4	67.1
Geranial	1274	-	-	0.2	-	14.3	9.7
Isobornyl acetate	1286	-	0.1	-	-	-	-
Thymol	1292	55.1	56.8	50.4	54.4	0.2	1.5
Carvacrol	1300	3.9	1.8	4.1	3.4	-	-
Methyl geranate	1323	-	-	-	-	0.1	0.1
α-Terpinil acetate	1349	-	-	-	0.4	-	-
Eugenol	1356	-	-	-	0.1	-	-
Geranyl acetate	1381	-	-	-	-	0.2	0.9
ß-Bourbonene	1384	-	-	-	-	0.2	0.1
ß-Caryophyllene	1420	2.0	-	2.6	3.9	1.8	2.4
α-Humulene	1457	-	-	0.1	-	-	0.2
γ-Muurolene	1476	-	-	0.1	0.2	-	-
Germacrene D	1482	0.6	0.1	0.3	1.2	0.5	1.1
Bicyclogermacrene	1496	0.6	-	-	-	-	-
ß-Bisabolene	1506	0.9	-	-	-	1.7	2.6
cis-γ-Cadinene	1511	-	-	0.1	0.3	-	-
δ-Cadinene	1519	0.1	-	0.2	0.3	-	-
Spathulenol	1578	0.1	-	0.1	0.1	-	0.1
Caryophyllene oxide	1584	0.3	0.6	0.4	0.2	0.4	0.1
tau-Cadinol	1600	-	-	-	0.7	-	-
Total		**96.5**	**95.2**	**98.6**	**97.2**	**95.7**	**99.4**

*[1]LRI = retention index relative to C_8-C_{21} *n*-alkanes on an HP-5 column; *[2] TVC — *Thymus vulgaris* control sample, TV1 – *T. vulgaris* 'Silver Poise', TV2 – *T. vulgaris* 'Erectus', TV3 – *T. vulgaris* 'Faustini', TC4 – *Thymus* × *citriodorus* 'Anderson's Gold', TC5 – *Thymus* × *citriodorus* 'Silver Queen'.

Table 4: The different terpenic classes (% composition) in the HSs and EOs of the selected thyme cultivars.

	Monoterpenes				Sesquiterpenes			
	Non-oxygenated		Oxygenated		Non-oxygenated		Oxygenated	
Cultivars*[1]	HS	EO	HS	EO	HS	EO	HS	EO
TVC	45.6	25.4	34.0	66.1	25.6	12.8	2.9	0.2
TV1	36.9	21.1	55.4	74.9	4.8	0.1	1.2	0.6
TV2	75.7	14.7	14.4	65.2	8.1	3.5	0.3	0.4
TV3	79.0	22.4	15.9	69.1	6.3	6.3	-	0.2
TC4	4.6	0.4	78.9	92.1	13.3	4.2	0.5	0.4
TC5	15.2	0.6	54.7	68.4	13.5	6.4	-	0.1

*[1]TVC — *Thymus vulgaris*. control sample, TV1 – *T. vulgaris* 'Silver Poise', TV2 – *T. vulgaris* 'Erectus', TV3 – *T. vulgaris* 'Faustini', TC4 – *Thymus* × *citriodorus* 'Anderson's Gold', TC5 – *Thymus* × *citriodorus* 'Silver Queen'.

T. x citriodorus samples, which were the only varieties with colored leaves (bright golden TC4, cream-variegated TC5). According to the literature data, some *Thymus* species (*T. pulegioides* L. and T. *glabrescens*

Willd.) can be characterized by a typical lemon scent that is mainly due to the high level of geraniol in the essential oil. The accumulation of this component is influenced by genetic and ecological circumstances [6b,6c]. *Thymus* × *citriodorus* is also lemon-scented because in the EO the main compounds are geraniol (*trans*-citral), neral (*cis*-citral) and geranyl acetate [2f,6d,6e]. Taking into account the EO composition, the *T. x citriodorus* cultivars were specialized in the production of geraniol-type EOs (TC4, 61.4±1.4%; TC5, 67.4±1.3%). On the contrary, thymol was the target monoterpene in the EOs of *T. vulgaris*: 56.7± 1.90% in *T. vulgaris* "Silver Poise" (TV1), 50.4± 1.76% in *T. vulgaris* "Erectus" (TV2), and 54.4± 1.89% in *T. vulgaris* "Faustini" (TV3). Significant amounts of thymol were found also in the Botanical garden *T. vulgaris* control sample (55.1±0.98 %).

In the thymol-enriched EO samples (TV1, TV2, TV3), the oxygenated monoterpenes (65-75%) were predominant in comparison with the hydrocarbon monoterpenes (15-22%), while the corresponding HS fingerprints showed higher amounts of *p*-cymene (20-41%) and γ-terpinene (3-23%), well-known precursors of thymol. This was confirmed also in the EO of the control sample (TVC) (Tables 2-4). It is generally reported that, for fresh samples, the non-oxygenated compounds were higher than in the essential oils obtained from the dried plant material, due to the drying process.

Several studies have been focused on the variability in mono- and sesquiterpenes, hydrocarbon and oxygenated volatiles, during the plant drying process, after storage and by applying different extraction methods [6f,7a-7d]. Therefore, the significant qualitative and quantitative differences found in this study between the EO constituents obtained from air-dried plant material and the HS volatiles of the fresh samples could be the result of the different status of the plant material used in the study. Furthermore, the HS technique largely depends on many factors related to the coating fiber, but also on the volatility and the location of aroma in the plant structures. It is well known that the correlation between the HP-SPME and the conventional distilled EO profiles cannot be proven in every case [7e]. This finding was confirmed also for the different *T. vulgaris* varieties analyzed in the present study. In fact, the static SPME method used on the fresh plant material showed a lesser recovery of thymol than the hydrodistillation method used for the dried thyme samples (Table 2-3). In conclusion, the GC-MS screening of the static headspace and the essential oils allowed us to discriminate the five commercial ornamental thyme into two different groups: thymol type (TV1, TV1, and TV3) and geraniol type (TC4, TC5). Considering the availability of the selected plant material, which is already produced by standardized agronomic protocols to supply the ornamental plant market, the two *T. x citriodorus* varieties could represent a potential source of geraniol (TC4, TC5 more than 60%), and the other *T. vulgaris* cultivars of thymol (TV1,TV2, and TV3 more than 50%) for industrial purposes.

Experimental

Plant material: The analysed *T. vulgaris* 'Silver Poise' (TV1) (silver leaves with cream edges and purple undersides, tinged red stems), *T. vulgaris* 'Erectus' (TV2) (upright growing, white and narrow flowers, grayish- green leaves), *T. vulgaris* 'Faustini' (TV3) (light or deep green small leaves, fragrant, white and lilac-rose flowers), *Thymus* cultivars – *T.* × *citriodorus* 'Anderson's Gold' (TC4) (dwarf carpeting variety, evergreen bright golden leaves with strong, aromatic smell), and *T. x citriodorus* 'Silver Queen' (TC5) (citrus aroma, cream-variegated leaves, pale-pink flowers) were kindly supplied by the Centro Regionale di Sperimentazione e Assistenza Agricola (Albenga, Italia). All the cultivars were propagated by vegetative shoots in the previous winter and were 5 months old at the time of collection. Three plants were examined in each case to analyze the morphological characteristics, while average samples were used for essential oil analysis. The foliage of the plants was collected during their full flowering period (May 2007). Aliquots of the fresh plant material (3.0-5.7 g) were sampled for the static headspaces and extracted by SPME (PDMS fiber, 100 μm, Supelco). Air-dried plant samples (10.0-12.5g) were hydrodistilled (2 h, 2 L water distilled, flow 2.0 mL/min) using a Clevenger apparatus described in the Italian Pharmacopoeia F.U.I.XI Ed. The EO contents (mg/100 g plant material) are summarized in Table 1. GC-FID analyses of the essential oils were accomplished using a HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m x 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60°C for 10 min, ramp of 5°C/min up to 220°C; injector and detector temperatures 250° C; carrier gas nitrogen (2 mL/min); detector dual FID; split ratio 1:30; injection volume 1 μL (10%, *n*-hexane).

Identification of the essential oil constituents was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by means of their Linear Retention Indices (L.R.I.) relative to a series of *n*-hydrocarbons (C_9-C_{23}). The GC/EI-MS analyses were performed on a Varian CP-3800 gas chromatograph equipped with a HP DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector.

Analytical conditions: injector and transfer line temperatures 220 and 240°C, respectively; oven temperature programmed from 60°C to 240°C, at 3°C/min; carrier gas helium at 1 mL/min; injection volume 1 μL (10% *n*-hexane solution); split ratio 1:30; scan time 1s; mass range m/z 35-400; 70 eV. The identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to a series of *n*-hydrocarbons (C_9-C_{23}) and on computer matching against two commercial data baseS (NIST 98, ADAMS) and literature [7f-7h], as well as an experimental home-made library of MS built up from pure substances and known oils.

Statistical analysis: All measured and derived data were analyzed by one-way ANOVA using Statistica 6 program.

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