Comparison of the Chemical Composition of Essential Oils Isolated from Two Thyme (Thymus vulgaris L.) Cultivars

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Abstract

In the studies conducted in 2016-2017, the chemical composition of the essential oils isolated from the herb of two thyme (Thymus vulgaris L.) cultivars was investigated by gas chromatography-mass spectrometry (GC-MS). The GC-MS analyses of the volatile oils of T. vulgaris L. cv. ‘English Winter’ and cv. ‘Summer Thyme de Provence’ led to the identification of 80 and 73 constituents, respectively. The main components found in the oil of ‘English Winter’ cultivar were thymol (44.97 and 38.06% in 2016 and 2017, respectively), p-cymene (7.61 and 10.34%), γ-terpinene (7.08 and 6.66%) and carvacrol (5.11 and 8.27%). Similarly, thymol (36.82 and 37.32%), p-cymene (15.73 and 11.20%), γ-terpinene (5.34 and 11.09%) and carvacrol (6.50 and 5.35%) dominated in the essential oil of ‘Summer Thyme de Provence’ cultivar. However, the higher content of thymol was noted for cultivar ‘English Winter’, while cultivar ‘Summer Thyme de Provence’ was richer in p-cymene.

Keywords: chemotypes; essential oil content; garden thyme; hydrodistillation; linalool

Introduction

Thyme (Thymus vulgaris L.), an aromatic perennial subshrub belonging to the Lamiaceae family, is commonly known for its therapeutic properties since ancient times. The whole plant as well as its different extracts is used in medicine, cosmetic and food industry (Sharafzadeh et al., 2010). Thymi herba has expectorant, antitussive, antibroncholytic, antispasmodic, carminative and diuretic properties (Marzec et al., 2010; Al-Asmari et al., 2019). Therefore the consumption of thyme extracts is recommended all over the world (Sharafzadeh et al., 2010). The aerial parts of Thymus are also used as a condiment in meat industry (to flavor meats such as rabbit, boar and lamb) and in the production of herb wine and liqueurs (Cornara et al., 2000; Marzec et al., 2010).

The beneficial properties of thyme are a consequence of the presence of thymol and carvacrol in its essential oil (Agili, 2014). These major phenolic compounds have antioxidant, antimicrobial and antifungal properties (Alizadeh, 2013). Anti-worm, anti-ptic and antispasmodic activities of thyme volatile oil have been also well documented (Marino et al., 1999). Thymii aetheroleum has been reported to be in world’s top ten of essential oils used as food additive (Marzec et al., 2010; Agili, 2014). It is mainly applied in the production of candies, herbal teas and ice tea (Carlen et al., 2010).

Chemical composition of thyme essential oil is well known. Thymol, γ-terpinene, p-cymene, carvacrol, linalool, α-pinene, borneol, 1,8-cineole, geraniol, caryophyllene as well as limonen, terpinen-4-ol and trans-sabinene hydrate are reported to be its main constituents (Marzec et al., 2010; Sharafzadeh et al., 2010; Satyal et al., 2016). Based on the predominance of particular monoterpenoids in the volatile oil six different chemotypes of T. vulgaris have been identified (Thompson et al., 2003; Torras et al., 2007; Chizzola et al., 2008): phenolic chemotypes (thymol and carvacrol) and non-phenolic chemotypes (geraniol, α-terpinene, linalool and trans-thujan-4-ol/terpinen-4-ol). Interestingly, in Spanish thyme another chemotype has been detected: 1,8-cineole (Guillen and Manzanos, 1998). Moreover, T. vulgaris collected in France represented the linalool chemotype, while geraniol chemotype has been described as characteristic for thyme in Serbia (Satyal et al., 2016). The most valuable for industrial purposes is Thymus vulgaris which contains from 20 to 80% of thymol (Marzec et al., 2010).

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The availability of various chemotypes and the production of new cultivars have resulted in a great variation in the essential oil composition and aroma among thyme species. Because the processing industry needs high quality raw material, it is important for thyme producers to have high quality cultivars with a good level of homogeneity (Carlen et al., 2010).

Nowadays, seeds of Thymus vulgaris L. are offered for sale by many companies. Most of the cultivars are not chemically characterized.

Therefore, the aim of the study conducted in 2016-2017 was to determine the chemical composition of two commercial thyme cultivars: ‘English Winter’ and ‘Summer Thyme de Provence’. To the best of our knowledge, no one has characterized chemically these two cultivars.

Materials and Methods

Plant material

The field experiment was conducted at the Horticultural Experimental Station in Dołuje (northwestern Poland), which belongs to the West Pomeranian University of Technology in Szczecin. The experiment was performed on sandy clay soil characterized by low water-holding capacity. The field was prepared according to agrotechnique proper for thyme cultivation. Mineral fertilization was quantified according to the results of the chemical analysis of the soil samples and supplemented to the recommended for thyme level of 60 g N and 50 g K₂O per 10 m². In the first year, nitrogen fertilizer was applied in two doses: ½ - before sowing and ½ - three weeks after sowing, potassium fertilizer - once (before sowing). In the second and third year of cultivation, 50 g K₂O (per 10 m²) and 100 g P₂O₅ (per 10 m²) were applied on early spring. Nitrogen fertilizer was applied in two doses: ½ - after plant vegetation and ½ - after harvest. During plant growth, standard cultivation treatments were performed, including weeding, soil loosening and watering, whenever necessary.

Commercial seeds purchased from the seed company Chiltern Seeds (United Kingdom) were used in the present study. The cultivars were as follows: ‘English Winter’ and ‘Summer Thyme de Provence’. Seeds were sown on seedbed at the second decade of March 2015. In the same year, the obtained seedlings were planted into the open field in the last decade of May, with spacing of 30 x 20 cm. The plants were grown in experimental plots with an area of 1.44 m² (1.2 x 1.2 m²), in four replications.

For laboratory analyses a herb from two- and three-year old plants was collected during flowering (harvest dates: 9 June 2016 and 10 June 2017 - ‘English Winter’ cultivar and 24 May 2016 and 30 May 2017 - ‘Summer Thyme de Provence’ cultivar). A 100 g samples, from each experimental plot (4 x 100 g), were taken. Subsequently, the samples of each cultivar were combined, mixed and dried in a shady and well ventilated place at room temperature (drying room). The dried plants were cut into small pieces and stored in a dry and cool place until analysis. Representative samples (200 g, each year) were used for essential oil isolation.

The weather conditions (the average values of daily temperature (°C), rainfall (mm), insolation (hours)) during the period of Thymus vulgaris L. cultivation are shown in Table 1.

Table 1. Meteorological data from the period of Thymus vulgaris L. growing in 2016-2017

| Years | Months | Mean daily air temperature (°C) | Total rainfall (mm) | Insolation (h) |
|-------|--------|--------------------------------|--------------------|---------------|
|       | I      | II     | III    | IV     | V     | VI    | VII   | VIII  | IX    | X     | XI    | XII   |
| 2016  | -0.9   | 3.7    | 4.3    | 8.8    | 15.7  | 18.5  | 19.0  | 17.8  | 16.8  | 8.6   | -*    | 3.1   |
| 2017  | 2.5    | -2.1   | 0.5    | 6.8    | 13.5  | 16.8  | 17.2  | 17.9  | 13.3  | 11.0  | 5.7   | 3.4   |
| 2016  | 27.0   | 40.6   | 28.1   | 20.2   | 18.9  | 69.0  | 50.0  | 47.8  | 18.3  | 55.3  | -     | 57.5  |
| 2017  | 68.6   | 5.7    | 35.8   | 42.3   | 99.2  | 118.1 | 182.4 | 45.4  | 31.6  | 65.1  | 74.7  | 34.8  |
| 2016  | 56.4   | 62.3   | 41.7   | 200.4  | 287.8 | 267.7 | 196.4 | 217.8 | 256.0 | 43.1  | -     | 48.5  |
| 2017  | 30.3   | 116.6  | 147.4  | 150.8  | 321.9 | 215.5 | 189.7 | 224.5 | 126.4 | 102.2 | 50.2  | 31.4  |

* Lack of the data
The column temperature was initially kept at 40 °C for 5 min, then increased to 60 °C at a rate of 30 °C min⁻¹, next to 230 °C at a rate of 6 °C min⁻¹ (kept constant for 10 min), and then increased to a final temperature of 280 °C at a rate of 30 °C min⁻¹. The oven was held at this temperature for 5 minutes. Mass spectra were taken at 70 eV with a mass scan range of 40–550 amu. Scan speed: 2.94/s.

Solvent delay was 4 min. The total running time for a single sample was about 51 minutes.

The relative percentage of the essential oil constituents was evaluated from the total peak area (TIC) by apparatus software.

Identification of essential oil constituents

Essential oil constituents were identified by comparison of their retention indices (relative to n-alkanes C₇-C₉₀ (Supelco, Bellefonte, PA, USA) on HP-5MS column) with those reported in NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/) and literature (Adams, 2007; Babushok et al., 2011). Further identification was made by comparison of their mass spectra with those stored in the Wiley NBS75KL and NIST/EPA/NIH (2002 and 2014 version) mass spectral libraries, as well as by comparison of their mass spectra with authentic compounds available in our laboratory (thymol, carvacrol, p-cymene and β-pinene, purchased from Sigma-Aldrich and Fluka).

Statistical analysis

The results of the study (Table 2 and 4) were subjected to an analysis of variance which was performed with AWAR software, made by the Department of Agrometeorology and Applied Informatics, Institute of Soil Science and Plant Cultivation in Puławy, Poland (Filipiak or Agrometology and Applied Informatics, Institute of Soil Science and Plant Cultivation in Puławy, Poland (Filipiak and Wilkos, 1995). The means of two years were separated using the Tukey’s Studentized Range Test at p=0.05. The statistical analysis of the results given in Table 4 was conducted for constituents of content greater than 1% of essential oil.

Results and Discussion

The yield of essential oils obtained by hydrodistillation from the dried aerial parts of two thyme cultivars ranged from 0.86 to 1.40% (v/w) (Table 2). Statistical analysis of the results obtained showed significant differences in the content of essential oil in the plant material obtained in 2016 (two-year-old plants) and on average for both years of the study. The higher content of essential oil was noted for 'English Winter' cultivar.

The content of essential oil in dry thyme herb varied from 0.32% (Ozguven and Tansi, 1998) to 4.90% (Carlen et al., 2010). The European Pharmacopoeia 7.0 (2010) prescribes a minimum yield of 1.2%. Our results are generally comparable with cited literature. However, in the regard of pharmacopoeial requirements, only the percentage yield of essential oil obtained from two-year-old plants of 'English Winter' cultivar was found to be in conformity with European Pharmacopoeia standards.

The percentage composition of the obtained essential oils as well as the main classes of the identified constituents is shown in Table 3. The volatile components are listed in the order of their elution from a HP-5 MS column.

In the two Thymus vulgaris L. essential oils, a total of ninety-three different compounds were identified: 80 for 'English Winter' cultivar (99.44% of the total oil) and 73 for 'Summer Thyme de Provence' cultivar (99.38% of the oil).

The main constituents found in the oil of 'English Winter' were thymol (44.97 and 38.06% in 2016 and 2017, respectively), p-cymene (7.61 and 10.34%), γ-terpinene (7.08 and 6.66%) and carvacrol (5.11 and 8.27%). Other components accounted for more than 2% were β-caryophyllene (3.50± and 4.18%), linalool (2.77 and 3.68%), α-terpinene (2.27 and 1.77%), thymol methyl ether (2.62 and 0.29%) and carvacrol methyl ether (2.12 and 1.50%). The volatile oil of 'Summer Thyme de Provence' was also rich in thymol (36.82 and 37.32%), p-cymene (15.73 and 11.20%), γ-terpinene (5.34 and 11.09%) and carvacrol (6.50 and 5.35%). Other abundant constituents were β-caryophyllene (4.06 and 3.75%), linalool (3.09 and 2.46%), borneol (2.23 and 1.22%), caryophyllene oxide (2.06 and 1.33%) and α-terpinene (1.67 and 2.49%)

Generally, the main compounds identified in the oils of two thyme cultivars were the same. Although, the higher concentration of thymol (38.06-44.97%) was observed in the oil isolated from 'English Winter' cultivar, while oil obtained from 'Summer Thyme de Provence' cultivar was richer in p-cymene (11.20-15.73%). Moreover, transgeraniol (0.15-0.15%) was found only in 'English Winter' oil, whereas trace amount of phellandral (0.02-0.06%) we detected only in the oil of 'Summer Thyme de Provence'.

Oxgenated monoterpenes (58.67-64.01% in 'English Winter' and 52.39-55.17% in 'Summer Thyme de Provence') and monoterpane hydrocarbons (22.41-23.16% in 'English Winter' and 27.45-30.02% in 'Summer Thyme de Provence') were shown to be the main group of constituents in both the oils, whereas oxygenated diterpenes were present in very low amounts (0.05% in 'English Winter' and 0.08% in 'Summer Thyme de Provence') (Table 3).

The statistical analysis of the results presented in Table 4 indicated that the studied essential oils, regardless of the year of cultivation, were characterized by the highest content of thymol - from 36.82 to 44.97%.

| Cultivar                      | 2016  | 2017  | Mean |
|-------------------------------|-------|-------|------|
| 'English Winter'              | 2.29  | 0.51  | 1.40 |
| 'Summer Thyme de Provence'   | 1.08  | 0.64  | 0.86 |
| Mean                          | 1.69  | 0.58  | 1.13 |
| LSDₜₒₜₜₒₜₜ     | 1.17  | n.s.  | 0.257|
Table 3. Percentage composition of the essential oils of two *T. vulgaris* L. cultivars

| No. | Compound† | RI‡ | 2016 | 2017 | 2016 | 2017 |
|-----|-----------|-----|------|------|------|------|
| 1.  | Methyl α-methylbutanoate | 783 | 0.31 | 0.25 | 0.20 | 0.12 |
| 2.  | α-Thujone | 925 | 0.36 | 0.31 | 0.16 | 0.52 |
| 3.  | α-Pinene | 931 | 0.93 | 0.68 | 0.97 | 0.80 |
| 4.  | Camphene | 946 | 0.36 | 0.51 | 0.88 | 0.34 |
| 5.  | β-Pinene | 953 | 0.28 | 0.20 | 0.17 | 0.47 |
| 6.  | 1-Octen-3-ol | 977 | 1.03 | 0.85 | 0.52 | 0.34 |
| 7.  | α-Terpinene | 984 | - | 0.08 | 0.06 | 0.05 |
| 8.  | β-Methyl | 989 | 1.75 | 1.18 | 1.06 | 1.67 |
| 9.  | α-Octane | 1094 | 0.99 | 0.23 | 0.10 | - |
| 10. | α-Pinellene | 1002 | 0.57 | 0.25 | 0.22 | 0.32 |
| 11. | 3-Carene | 1008 | 0.15 | 0.12 | 0.10 | 0.13 |
| 12. | α-Terpinene | 1015 | 2.27 | 1.77 | 1.67 | 2.49 |
| 13. | p-Cymene | 1024 | 7.61 | 10.54 | 15.73 | 11.20 |
| 14. | D-Limonene | 1027 | 0.69 | 0.82 | 0.89 | 0.62 |
| 15. | l-Rosabinol | 1030 | 1.24 | 1.22 | 1.40 | 1.26 |
| 16. | (Z)-β-Oxymene | 1036 | 0.09 | - | - | - |
| 17. | (E)-β-Oxymene | 1047 | 0.09 | - | - | 0.10 |
| 18. | γ-Terpine | 1058 | 7.08 | 6.66 | 5.34 | 11.09 |
| 19. | α-Sabinene hydrate | 1066 | 1.00 | 0.69 | 0.28 | 0.92 |
| 20. | α-Terpinolene | 1077 | 0.38 | 0.32 | 0.26 | 0.30 |
| 21. | Linalool | 1096 | 2.77 | 3.68 | 3.09 | 2.46 |
| 22. | trans-Sabinene hydrate | 1104 | 0.24 | - | - | - |
| 23. | β-Thujone | 1110 | - | 0.05 | - | - |
| 24. | (Z)-γ-Terpinol | 1121 | - | 0.15 | 0.07 | 0.10 |
| 25. | Pinocarvone | 1139 | - | 0.08 | 0.06 | - |
| 26. | Camphor | 1145 | 0.22 | 0.17 | 0.16 | - |
| 27. | (+)-2-Isoborneol | 1151 | 0.38 | - | - | - |
| 28. | Bunol | 1166 | 0.99 | 1.78 | 2.33 | 1.22 |
| 29. | Terpinen-4-ol | 1177 | 0.66 | 1.27 | 1.00 | 1.22 |
| 30. | p-Cymen-8-ol | 1186 | 0.78 | 0.12 | 0.13 | 0.14 |
| 31. | α-Terpinen | 1190 | 0.83 | 0.15 | 0.20 | 0.13 |
| 32. | Methyl | 1196 | 0.41 | 0.27 | 0.15 | 0.21 |
| 33. | trans-Dihydrocarvone | 1204 | 0.11 | - | - | - |
| 34. | trans-Carveol | 1216 | - | 0.05 | - | - |
| 35. | Thymol methyl ether | 1234 | 2.62 | 0.29 | 0.17 | 0.14 |
| 36. | Carvone methyl ether | 1243 | 2.32 | 1.50 | 1.65 | 1.61 |
| 37. | Thymoquinone | 1257 | 0.09 | 0.09 | 0.23 | 0.11 |
| 38. | Pulegone | 1277 | - | - | 0.06 | 0.02 |
| 39. | trans-γ-Geraniol | 1283 | 0.15 | 0.16 | - | - |
| 40. | Bornyl acetate | 1284 | 0.02 | 0.22 | 0.20 | 0.18 |
| 41. | Thymol | 1293 | 44.97 | 30.06 | 36.82 | 37.32 |
| 42. | Carvacrol | 1303 | 5.11 | 8.27 | 6.50 | 5.35 |
| 43. | α-Caryophyllene | 1353 | 0.25 | 0.14 | 0.04 | 0.08 |
| 44. | Yangene | 1374 | 0.07 | 0.07 | 0.08 | 0.03 |
| 45. | α-Copaene | 1379 | 0.18 | 0.22 | 0.29 | 0.10 |
| 46. | β-Bourbonene | 1395 | 0.07 | 0.14 | 0.07 | 0.05 |
| 47. | γ-Caryophyllene | 1424 | 3.50 | 4.14 | 4.06 | 3.75 |
| 48. | β-Copaene | 1430 | - | 0.09 | - | 0.06 |
| 49. | Aromadendrene | 1442 | - | - | - | 0.05 |
| 50. | α-Humulene | 1458 | 0.21 | 0.26 | 0.20 | 0.32 |
| 51. | α-Elemene | 1465 | 0.13 | 0.09 | 0.08 | 0.19 |
| 52. | γ-Geraniol | 1472 | 0.35 | - | - | - |
| 53. | γ-Muurolene | 1478 | 0.06 | 0.58 | 0.49 | 0.27 |
| 54. | Germacrene D | 1484 | 0.34 | 0.25 | 0.10 | 0.17 |
| 55. | α-Muurolene | 1498 | 0.19 | 0.22 | 0.18 | 0.16 |
| 56. | Bornylgermacrene | 1503 | 0.22 | 0.21 | 0.24 | 0.24 |
| 57. | β-Sabinene | 1530 | 0.08 | 0.06 | - | - |
| 58. | γ-Cadinene | 1518 | 0.63 | 0.64 | 0.73 | 0.97 |
| 59. | δ-Cadinene | 1526 | 0.84 | 0.87 | 0.91 | 0.78 |
| 60. | α-Cadinene | 1536 | 0.09 | 0.04 | - | - |
| 61. | α-Calassene | 1543 | 0.06 | 0.07 | 0.05 | 0.06 |
| 62. | Germacrene B | 1554 | 0.11 | 0.07 | - | - |
| 63. | β-Calassene | 1562 | - | 0.07 | - | - |
| 64. | Spathulenol | 1581 | - | - | - | 0.11 |
| 65. | Caryophyllene oxide | 1589 | 0.69 | 2.05 | 2.06 | 1.33 |
| 66. | Valeraldehyde | 1595 | - | 0.07 | - | - |
| 67. | Tetradecanal | 1615 | - | 0.07 | 0.06 | 0.04 |
| 68. | Humulene epoxide II | 1619 | 0.13 | 0.15 | 0.13 | 0.33 |
| 69. | γ-Eudesmol | 1626 | 0.41 | 0.16 | 0.25 | - |
| 70. | Isopropylalcohol | 1630 | 0.13 | - | - | - |
However, its content was significantly higher in the oil obtained from 'English Winter' cultivar (the difference was 4.45%).

On the other hand, as comparing the results from two years of the study, regardless of thyme cultivar, a significantly higher content of thymol was found in the oil isolated from two-year-old plants as compared with three-year-old plants - the difference was 3.205%.

The other components found in significant amount in the investigated oils were: p-cymene, γ-terpinene, carvacrol and β-caryophyllene. It was proved that the essential oil obtained from 'Summer Thyme de Provence' cultivar contained more p-cymene and γ-terpinene, while there was no statistical differentiation in the content of carvacrol and β-caryophyllene in the essential oils of investigated cultivars. However, when comparing the content of these compounds in dependence on the year of cultivation, significantly higher percentage of p-cymene was noted in the oil extracted from two-year-old plants, while the oil isolated from three-year-old plants was richer in γ-terpinene and carvacrol.

### Table 4. Statistical analysis of the content of main constituents of essential oils of two Thymus vulgaris L. cultivars

| Cultivar (A) | 'Summer Thyme de Provence' | 'English Winter' | Mean for the year of cultivation | LSD0.05 |
|--------------|----------------------------|------------------|---------------------------------|---------|
|              | 2016                      | 2017             | 2016                            | 2017    | 2016 | 2017 | A | B | A/B |
| 1-Octen-3-ol | 0.52±0.15                 | 0.34±0.04        | 0.43                            | 1.03±0.065 | 0.85±0.045 | 0.94               | 0.775             | 0.595             | 0.115             | 0.355             | n.s.         |
| E-Mycene     | 1.06±0.075                | 1.67±0.010       | 1.36                            | 1.75±0.075 | 1.10±0.045  | 1.465              | 1.405             | 1.425             | 0.102             | n.s.         | 0.144       |
| γ-Terpinene  | 1.67±0.008                | 2.49±0.11        | 2.08                            | 2.12±0.09  | 1.77±0.04   | 1.92               | 1.97              | 1.93              | n.s.             | 0.113         | 0.16       |
| p-Cymene     | 15.73±1.20                | 11.20±0.48       | 13.46                           | 7.61±0.189 | 10.34±0.295 | 9.75               | 11.67             | 10.77             | 0.890             | 0.890         | 1.259       |
| Eucalyptol   | 1.48±0.16                 | 1.26±0.095       | 1.37                            | 1.24±0.05  | 1.22±0.10   | 1.23               | 1.36              | 1.24              | 0.153             | n.s.         | n.s.        |
| β-Terpinene  | 5.34±0.27                 | 11.09±0.75       | 8.23                            | 7.08±0.12  | 6.66±0.16   | 6.87               | 6.21              | 5.87              | 0.544             | 0.544         | 0.770       |
| α-Sabinene Hydrocarbons | 0.28±0.015 | 0.92±0.06 | 0.60                            | 1.00±0.011 | 0.89±0.10   | 0.95               | 0.64              | 0.61              | 0.081             | 0.081         | 0.115       |
| Linalool     | 1.69±0.17                 | 2.46±0.17        | 2.77                            | 2.77±0.02  | 3.08±0.17   | 3.23               | 3.29              | 3.23              | 0.279             | n.s.         | 0.394       |
| Bornyl      | 2.3±0.13                  | 1.22±0.010       | 1.72                            | 0.99±0.11  | 1.78±0.05   | 1.385              | 1.61              | 1.50              | 0.121             | n.s.         | 0.17       |
| Terpinen-4-ol | 1.00±0.02               | 1.22±0.004       | 1.11                            | 0.66±0.03  | 1.27±0.01   | 0.965              | 0.83              | 1.245             | 0.036             | 0.036       | 0.05       |
| Thymol methyl ether | 0.17±0.006 | 0.14±0.02 | 0.155                           | 2.62±0.19  | 0.29±0.08   | 1.455              | 1.395             | 0.215             | 0.138             | 0.138       | 0.195       |
| Carvacrol methyl ether | 1.45±0.09 | 1.61±0.09 | 1.63                            | 2.12±0.17  | 1.50±0.02   | 1.81               | 1.805             | 1.555             | 0.140             | 0.140       | 0.191       |
| Thymol     | 36.82±0.06               | 37.32±0.41       | 37.97                           | 44.97±0.39 | 38.06±0.23  | 41.52              | 40.89             | 37.69             | 0.812             | 0.812        | 1.140       |
| Carvacrol   | 6.50±0.00                | 5.35±0.62        | 5.95                            | 5.18±0.09  | 8.27±0.77   | 6.69               | 5.805             | 6.81              | n.s.             | 0.772         | 1.092       |
| β-Caryophyllene | 4.06±0.36     | 3.75±0.12     | 3.95                            | 5.90±0.06  | 4.18±0.17   | 3.44               | 3.74              | 3.965             | n.s.             | n.s.         | 0.395       |
| Caryophyllene methyl | 2.60±0.08   | 1.55±0.14 | 1.70                            | 0.60±0.06  | 2.05±0.26   | 1.37               | 1.375             | 1.49              | 0.204             | 0.204       | 0.208       |
| α-Cadinol   | 1.24±0.06                | 2.48±0.21        | 1.86                            | 0.97±0.06  | 1.45±0.17   | 1.21               | 1.10              | 1.96              | 0.188             | 0.188       | 0.266       |
| Limonene acid | 1.28±0.13       | 0.75±0.13     | 0.94                            | 0.71±0.02  | 0.73±0.075  | 0.55               | 0.79              | 0.74              | 0.153             | n.s.         | 0.108       |

n.s. – not significant
± standard deviation (n = 3)
Thymus vulgaris L. is probably one of the most studied Thymus species from the agronomic point of view due to its wide utilization for industrial purposes (Trindade et al., 2018). Thymol, carvacrol and linalool are considered as the important chemical constituents of thyme herb (Syamasundar et al., 2008). However, the content of thymol in essential oil determines the quality of thyme (Stahl-Biskup and Sace, 2002).

The chemical composition of thyme’s essential oil depends on the several factors, such as environment of growing, development stage (Hudaib and Aburjai, 2007) and chemotypes (Thompson et al., 2003). Literature indicated that thymol, p-cymene, γ-terpinene, carvacrol, linalool and β-caryophyllene are usually reported as the main constituents of thyme essential oil (Thompson et al., 2003; Raal et al., 2005; Hudaib and Aburjai, 2007; Chizzola et al., 2008; Boruga et al., 2014). According to Trindade et al. (2018), thymol chemotype is the most frequent in Thymus vulgaris L.

Our results are in agreement with some other studies (Ozcan and Chalchat, 2004; Zawislak, 2007; Porte et al., 2008; Anzlovar et al., 2014; Boruga et al., 2014; Duscova et al., 2016; Satyal et al., 2016), where thymol, p-cymene, γ-terpinene and carvacrol were reported to be the major thyme oil components. The essential oil obtained from fresh leaves of T. vulgaris from Brazil (Porte et al., 2008) contained thymol (44.7%), p-cymene (18.6%) and γ-terpinene (16.5%) as the main components. In the oils isolated from two varieties of thyme: ‘Krajojy’ and ‘Winter’ cultivated in Czech Republic (Duscova et al., 2016), thymol (52.13-61.92%), α-cymene (11.39-24.86%) and γ-terpinene (4.61-13.84%) were found as the most abundant constituents. Volatile oil isolated from plants collected in Richerenches, France (Satyal et al., 2016) was rich in thymol (47.06%) and p-cymene (20.07%) with lesser quantities of linalool (5.00%) and carvacrol (3.24%). Thymol (47.59%), γ-terpinene (30.90%) and p-cymene (8.11%) dominated in the essential oil obtained from T. vulgaris cultivated in Romania (Boruga et al., 2014). The high content of thymol (68.91%) in volatile oil was found by Anzlovar et al. (2014) in T. vulgaris cv. ‘Deutscher winter’ cultivated in Slovenia.

The other abundant components were p-cymene (13.61%), γ-terpinene (7.60%), ocimene (2.11%) and carvacrol (1.55%). In the essential oil obtained from garden thyme cultivated in south-eastern Poland (Zawislak, 2007), thymol (49.9-61.2%), γ-terpinene (11.3-12.1%), p-cymene (9.1-13.5%) and carvacrol (5.0-6.3%) were the main components. Thymol (46.2%), γ-terpinene (14.1%), p-cymene (9.9%) and linalool (4.0%) dominated in the essential oil obtained from T. vulgaris growing wild in Turkey (Ozcan and Chalchat, 2004), while in the essential oil isolated from leaves of T. vulgaris cultivated in North Yemen (Maqtari et al., 2011) thymol (51.34%), p-cymene (18.35%), carvophyllene (4.26%), α-pinene (2.9%), δ-myrcene (2.50%) and carvacrol (2.03%) were found in significant amounts.

Interestingly, thymol was not detected in the oil of thyme from Morocco (El Hattabi et al., 2016). Moroccan plants contained mainly carvacrol (78.4%) and p-cymene (4.6%) in the essential oil.

The amount of thymol (36.82-44.97%) detected in our oils was lower as compared to the results obtained by Ozcan and Chalchat (2004), Zawislak (2007), Maqtari et al. (2011), Boruga et al. (2014), Anzlovar et al. (2014), Duscova et al. (2016) and Satyal et al. (2016). However, thymol content was found to be in good agreement with the quality standards of the European Pharmacopoeia (EP limits 36.0-55%) for thyme oil. Moreover, our cultivars were richer in carvacrol (5.11-8.27%) in comparison with thyme from France (Satyal et al., 2016), Slovenia (Anzlovar et al., 2014) and North Yemen (Maqtari et al., 2011). Only T. vulgaris from Morocco (El Hattabi et al., 2016) had higher content of carvacrol in the essential oil.

The percentage content of γ-terpinene (5.34-11.09%) in our essential oil samples was lower than those reported by Ozcan and Chalchat (2004), Porte et al. (2008) and Boruga et al. (2014). However, the content of p-cymene (7.61-15.73%) in our oils was higher than those found in thyme oil from Turkey (Ozcan and Chalchat, 2004) and in garden thyme oil from south-eastern Poland (Zawislak, 2007).

Our results differ from those obtained by Torras et al. (2007) who studied the oil composition of T. vulgaris growing wild in Catalonia, in which linalool (60.55%) and α-terpenyl acetate (13.95%) were the main constituents. Similarly, thyme from Southern Italy (De Lisi et al., 2011) was rich in linalool (58.00%), while plants from Croatia (Jukic and Milos, 2005) contained linalool (40.2-43.0%), p-cymene (13.5-15.5%) and 1,4-terpineol (4.3-6.8%) in the essential oil.

The content of linalool in our T. vulgaris cultivars varied from 2.77 to 3.68%, while the content of terpinen-4-ol (1,4-terpineol) did not exceed 1.27%. We not detected α-terpenyl acetate in our oils.

The major constituents of the essential oils isolated from T. vulgaris cultivars: ‘English Winter’ and ‘Summer Thyme de Provence’ were thymol, p-cymene and γ-terpinene, which suggest that the analyzed oils belong to the thymol chemotype.

Conclusions

Both examined Thymus vulgaris L. cultivars belong to the thymol chemotype, with differences in the concentration of p-cymene and γ-terpinene. The higher content of p-cymene and γ-terpinene we noted in ‘Summer Thyme de Provence’ cultivar, while ‘English Winter’ cultivar was richer in thymol. Based on these results we suppose that the isolated essential oils will exhibit good antioxidant and antimicrobial properties. Moreover, both cultivars can find wide application in food industry as flavor ingredients to beverages and confectionary products as well as in cosmetic industry as natural preservatives.

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