applied sciences

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Chemical Composition and Antioxidant Activities of Three Bulgarian Garden Thyme Essential Oils

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Abstract: Garden thyme (*Thymus vulgaris* L.) is an annual herbaceous plant of the family *Lamiaceae*. It can be found both in the wild and as a cultivated plant in Bulgaria. This study is aimed at assessing the garden thyme essential oil composition and antioxidant activities, which were collected from three different areas in Bulgaria. The essential oils were obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry (GC-MS). The main compounds in the garden thyme essential oils (over 2%) were as follows: thymol (42.88–53.55%), p-cymene (14.25–25.51%), γ-terpinene (6.58–15.51%), borneol (2.75–3.57%), carvacrol (2.00–3.02%), β-linalool (2.07–2.31%), *cis*-sabinene hydrate (4.05%), eucalyptol (1.08–3.65%), α-terpinene (1.01–3.24%), carvacrol methyl ether (1.18–3.02%), and thymol methyl ether (2.26–3.16%). The oils were mainly composed of oxygenated phenyl propanoids, and all the essential oils belonged to the chemotype thymol. Antioxidant activities were measured by DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assays. All the samples exhibited antioxidant activity relative to the DPPH radical (from 143.20 mM TE/mL to 165.91 mM TE/mL) and by the ABTS method (from 121.67 mM TE/mL to 127.62 mM TE/mL). These garden thyme essential oils could be used as natural antioxidants for food and as nutraceuticals.

Keywords: garden thyme; essential oil; chemical composition; antioxidant activity

Citation: Dobreva, K.; Dimov, M.; Valev, T.; Iliev, I.; Damyanova, S.; Oprea, O.B.; Stoyanova, A. Chemical Composition and Antioxidant Activities of Three Bulgarian Garden Thyme Essential Oils. *Appl. Sci.* **2024**, *14*, 10261. https://doi.org/10.3390/app142210261

Academic Editor: Ramona Iseppi

Received: 15 October 2024
Revised: 4 November 2024
Accepted: 4 November 2024
Published: 7 November 2024

1. Introduction

Thyme is a herbaceous, perennial plant of the *Lamiaceae* family, known since ancient times for its medicinal properties. About 350 species have been identified in the genus *Thymus*, which differ in habit, growth, development, amount, and composition of biologically active substances—essential oil, polyphenolic acids, flavonoids, etc. Garden thyme (*T. vulgaris* L.) and common thyme (*T. serpyllum* L.) are industrially important for obtaining essential oils and extracts. Different chemotypes are known from these two species, which contain in their essential oil various aromatic substances, for example, the phenols thymol and carvacrol, the hydrocarbons p-cymene and γ-terpinene, as well as many other components in different proportions [1,2].

Garden thyme (*T. vulgaris*) originates from the Mediterranean part of Europe and Africa. It is grown in countries located in Southern and Northern Europe, North Africa, and North America [1,2]. The main component is its essential oil, which in dry plant material ranges in quantities of 0.32–4.90% [1–9].

Wild or cultivated plants, depending on the region of growth, have a different chemical composition, which is determined mainly by soil and climatic factors [2,10–13].

Satyal et al. [10] examined the composition of four essential oils of the species *T. vulgaris* for different chemotypes, and found the following: linalool chemotype (linalool, 76.2%; linalyl acetate, 14.3%); geraniol chemotype (geraniol, 59.8%; geranyl acetate, 16.7%); sabinene hydrate chemotype (cis-sabinene hydrate, 30.8%; trans-sabinene hydrate, 5.0%); and thymol chemotype (thymol, 47.1%; p-cymene, 20.1%).

Mancini et al. [11] determined the chemical composition, and antimicrobial and antioxidant activity of five essential oils of the species *T. vulgaris*, collected from different areas of Italy. They found that phenolic components significantly prevailed over the rest in all the studied oils. The most common compounds were thymol (46.2–67.5%), carvacrol (5.7–7.3%), and caryophyllene oxide (1.7–7.3%).

A predominantly aromatic substance in the composition of the essential oil of garden thyme is the phenol thymol (18.11–55.44%), which is responsible for the typical spicy smell. Thymol is usually accompanied by compounds related to its biosynthesis, its isomer carvacrol (1.00–18.31%), and p-cymene (6.61–25.20%) and γ-terpinene (2.23–10.00%). From the group of monoterpenes, linalool (3.72–4.66%), and in smaller quantities (0.5–1.5%), borneol, camphor, limonene, myrcene, β-pinene, trans-sabine hydrate, α-terpineol, and terpinene-4-ol were found in the composition of the oil. Sesquiterpenes are poorly represented in garden thyme essential oil (β-caryophyllene 5.38–8.47%) [1,2].

A factor for the different chemical compositions of essential oils is also the season when the plants are harvested [14,15].

Lemos et al. [15] report that the main components in the essential oil of the species *T. vulgaris* cultivated in Brazil are thymol (38.99–52.92%), p-cymene (14.38–26.58%), and γ-terpinene (10.43–19.09%). Antioxidant activity is determined by the DPPH, ABTS, and FRAP methods. They found that the results varied according to the methods.

Thyme essential oils have prominent antimicrobial [12,13,15–26], antioxidant [12,13,17,21–25,27–36], and other biological properties [13,22,34]. This is due to the phenols thymol and carvacrol contained in their composition [24,37–40].

Due to their characteristic biological properties, thyme essential oils are used in the food industry, medicine, and pharmacy [41–45].

In Bulgaria, common thyme is a very popular plant, which is mainly found in the wild in fields and the lower parts of mountains. It finds exclusive use in folk medicine, as well as for flavoring various food products. Several cultivars of the species *T. vulgaris* have been selected in Bulgaria, and their essential oils have different compositions: cv. "Slava" with the main component citral [46,47], cv. "Kresna" with the main component carvacrol [29,30], and cv. "German Winter" with the main component thymol [48]. It has been found that the amount of essential oil varies between different types of thyme: for the cv. "German Winter" from 0.74 to 0.77% and for cv. "Slava" from 1.02 to 1.08%. The best yield of essential oil in both varieties is established in the mass flowering phase [48].

Today, Bulgarian garden thyme essential oil is widely used in pharmaceuticals, food industry, cosmetics, etc., as a raw material rich in biologically active substances [48].

Common thyme (*Thymus serpyllum* L.) is wildly growing in Bulgaria and is found in the mountainous regions of the country, containing about 36 subspecies with many varieties [49]. The chemical composition and biological activity of an essential oil obtained from wild common thyme was investigated [50], and the possibility of its application in food products was established [51].

The aim of this study is to perform a comparative analysis of the chemical composition by determining the chemotype and antioxidant activity of garden thyme cv. "German Winter" and wild thyme (*T. vulgaris*) from three different regions of Bulgaria, harvested in 2024. After a comparison of their chemical composition and their antioxidant activity, the most suitable essential oil sample will be selected, with which attempts for its inclusion in various food products will be continued in the future.

2. Materials and Methods

2.1. Materials

The subject of this study is the aboveground parts of *T. vulgaris* (grass, leaf, and flower), collected during flowering in May–June 2024 from three different locations in Bulgaria:

- The experimental field of the Institute of Roses, Essential and Medical Cultures, Kazanlak (42°63′48.3″ N 25°38′85.6″ E, altitude 407 m); the thyme is cv. "German Winter"—sample 1. The soils in the area are leached cinnamon forest, developed on old diluvial deposits, structureless with good aeration and water permeability, with acidity pH 5.6 and poorly stocked with nitrogen 20.5 mg/1000 g and phosphorus 4.25 mg/100 g, but well stocked with potassium 21.75 mg/100 g, and humus content 1.8%.
- The town of Yambol, Kurtkaya area (42°29′23.4″ N 26°27′56.1″ E, altitude 114 m)—sample 2. The soil cover is diverse, with chernozem resins, and alluvial-meadow soils, which are very fertile.
- The village of Chargan (42°29′50.51″ N 26°35′46.81″ E, altitude 195 m), which is located in the vicinity of Yambol, near the Tundzha River—sample 3. The area is characterized by very fertile black earth and alluvial-meadow soils, which are very fertile.

The authenticity of the plants was determined by morphological features by a botanist. The raw material is dried in a dry, shady and ventilated place immediately after harvesting for 20 days. It is packed in paper bags and stored in a dark, dry and cool place. Before analyzing, the raw material was cut into pieces with sizes ranging from 0.5 to 1 cm and the moisture content was determined [52].

2.2. Methods

2.2.1. Essential Oil Isolation

The essential oil content was determined by water distillation in a laboratory glass apparatus of the British Pharmacopoeia modified by Balinova and Dyakov [53]. Distillation lasted for 3 h and ended when no increase in the amount of essential oil was reported during two consecutive measurements every 30 min. The resulting essential oils were dehydrated with anhydrous Na_2SO_4 and stored in glass vials at 4–6 °C until analysis. The yields of the essential oils were converted to an absolute dry mass.

The identification of aromatic compounds in the essential oil and in the extracts was determined by GC/MS analysis. A GC analysis was performed with an Agilent 7890A instrument (Agilent Technologies Inc., Santa Clara, CA, USA) under the following conditions: HP-5 ms column, 30 m × 250 mm × 0.25 µm; temperature 35 °C/3 min, 5 °C/min to 250 °C for 3 min, total 49 min; helium as a carrier at a constant rate of 1 mL/min; and split 30:1. The GC-MS analysis was performed on an Agilent 5975C under the same conditions as the GC analysis. Component identification was based on comparing retention indices with spectral databases [54]. Volatile contents were given as a percentage of the total ion current (TIC).

2.2.2. Antioxidant Activity

DPPH radical scavenging activity: The DPPH radical scavenging activity was evaluated as the garden thyme essential oils (0.15 mL) were added to 2.85 mL freshly prepared 0.1 mM DPPH (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) solution in methanol. The samples were incubated for 15 min at 37 °C in darkness. The reduction in the absorbance at 517 nm was measured by a spectrophotometer in comparison to the blank containing methanol [55]. The radical scavenging activity of the essential oils was expressed as mM Trolox® equivalent (TE) per gram dw.

ABTS assay: ABTS radical was generated by mixing aliquot parts of 7.0 mM 2,2′azinobis(3)-ethylbenzthiazoline-6-sulfonicacid (ABTS, Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) in distilled water and 2.45 mM potassium persulfate (Merck, Darmstadt, Germany) in distilled water. The reaction was performed for 16 h at ambient temperature in darkness and the generated

ABTS radical was stable for several days. Before analysis, 2.0 mL of the generated $ABTS^{\cdot+}$ solution was diluted with methanol at proportions 1:30 (*v*/*v*), so the obtained final absorbance of the working solution was about 1.0 ÷ 1.1 at 734 nm. For the assay, 2.85 mL of this $ABTS^{\cdot+}$ solution was mixed with 0.15 mL of the obtained extracts. After 15 min at 37 °C in darkness the absorbance was measured at 734 nm against methanol [55]. The antioxidant activity was expressed as mM (TE) per gram dw.

2.3. Statistical Analysis

All the experiments were performed in triplicate, with the values in the tables averaged, and represented with their mean and standard deviation (SD). The experimental data were subjected to statistical evaluation using an analysis of variance (ANOVA) with the Statgraphics Centurion XVI Version 16.2.04 software (StatPoint Technologies, Inc., Warrenton, VA, USA). Duncan's multiple range tests were used to determine the difference among the means, and the significance was defined at $p < 0.05$.

3. Results

The plants studied (sample 1, sample 2, and sample 3) showed a low moisture content (6.04%, 4.96%, and 4.29%, respectively), which is reasonable to believe since they can be stored for a longer time.

3.1. Chemical Composition

The chemical composition of the garden thyme essential oils is presented in Table 1 and in Figures 1–3. The data presented in Table 1 show the following:

- In total, 42 components (99.65% of the total composition) have been identified in garden thyme essential oil (sample 1). The main compounds (over 2%) were thymol (53.55%), γ-terpinene (11.13%), p-cymene (14.96%), borneol (2.75%), β-linalool (2.07%), and carvacrol (2.00%).
- In total, 39 components (99.67% of the total composition) have been identified in garden thyme essential oil (sample 2). The main compounds (over 2%) were thymol (44.09%), p-cymene (25.51%), γ-terpinene (6.58%), eucalyptol (3.65%), thymol methyl ether (3.16%), β-linalool (2.31%), and carvacrol (2.33%).
- In total, 38 components (99.57% of the total composition) have been identified in garden thyme essential oil (sample 3). The main compounds (over 2%) were thymol (42.88%), p-cymene (14.25%), γ-terpinene (15.51%), *cis*-sabinene hydrate (4.05%), borneol (3.57%), α-terpinene (3.24%), carvacrol methyl ether (3.02%), β-linalool (0.98%), and thymol methyl ether (2.26%).

The data show that the essential oil of sample 1 (cultivated garden thyme) contains the highest amount of thymol; the oil of sample 2 (wild garden thyme) is richer in p-cymene, carvacrol, and β-linalool; and that of sample 3 of γ-terpinene, cis-sabinene hydrate, and borneol.

The data for the distribution of the identified components by groups of compounds (Figure 4) shows that the garden essential oils are dominated by oxygenated phenyl propanoids (from 49.19 to 58.25%), followed by monoterpene hydrocarbons (from 10.46 to 22.11%), phenyl propanoid hydrocarbons (from 14.31 to 25.59%), and oxygenated monoterpenes (from 8.31 to 11.38%). The amounts of the other compounds (oxygenated aliphatics, monoterpene hydrocarbons, sesquiterpene hydrocarbons, and oxygenated sesqui-terpenes) are much lower.

Table 1. Chemical composition of three samples of garden thyme essential oils (% of TIC [a]).

No	RT [b], min	RI [c]	Components	Content		
				Sample 1	Sample 2	Sample 3
1.	6.46	840	trans-2-Hexenal	0.07 ± 0.0 [a]	nd [d]	0.06 ± 0.0 [a]
2.	8.44	917	Tricyclene	0.05 ± 0.0 [a]	0.06 ± 0.0 [a]	0.08 ± 0.0 [a]
3.	8.60	922	β-Thujene	0.68 ± 0.0 [a]	0.59 ± 0.0 [a]	0.81 ± 0.0 [a]
4.	8.81	929	α-Pinene	0.46 ± 0.0 [a]	0.37 ± 0.0 [a]	0.38 ± 0.0 [a]
5.	9.32	945	Camphene	0.59 ± 0.0 [a]	0.34 ± 0.0 [a]	0.46 ± 0.0 [a]
6.	10.10	970	Sabinene	0.08 ± 0.0 [a]	0.07 ± 0.0 [a]	0.15 ± 0.0 [b]
7.	10.22	974	β-Pinene	0.16 ± 0.0 [a]	0.20 ± 0.0 [a]	0.17 ± 0.0 [a]
8.	10.45	981	1-Octen-3-ol	0.35 ± 0.0 [a]	0.52 ± 0.0 [a]	1.01 ± 0.01 [b]
9.	10.58	985	3-Octanone	0.08 ± 0.0 [a]	0.06 ± 0.0 [a]	0.35 ± 0.0 [b]
10.	10.70	988	β-Myrcene	0.89 ± 0.0 [a]	0.70 ± 0.0 [a]	0.54 ± 0.0 [a]
11.	11.01	981	3-Octanol	0.07 ± 0.0 [a]	0.06 ± 0.0 [a]	0.16 ± 0.0 [ab]
12.	11.18	1004	α-Phellandrene	0.15 ± 0.0 [a]	0.09 ± 0.0 [a]	0.17 ± 0.0 [a]
13.	11.25	1006	delta-3-Carene	0.07 ± 0.0 [a]	0.09 ± 0.0 [a]	0.06 ± 0.0 [a]
14.	11.54	1015	α-Terpinene	1.37 ± 0.01 [a]	1.01 ± 0.01 [a]	3.24 ± 0.03 [b]
15.	11.87	1023	p-Cymene	14.96 ± 0.13 [a]	25.51 ± 0.24 [b]	14.25 ± 0.13 [a]
16.	11.96	1027	α-Limonene	0.29 ± 0.0 [a]	0.23 ± 0.0 [a]	0.26 ± 0.0 [a]
17.	12.03	1033	Eucalyptol	1.08 ± 0.01 [a]	3.65 ± 0.03 [b]	1.62 ± 0.01 [ac]
18.	12.54	1035	trans-β-Ocimene	0.04 ± 0.0 [a]	0.01 ± 0.0 [a]	0.04 ± 0.0 [a]
19.	12.95	1057	γ-Terpinene	11.13 ± 0.01 [a]	6.58 ± 0.06 [b]	15.51 ± 0.14 [a]
20.	13.28	1069	cis-Sabinene hydrate	1.36 ± 0.01 [a]	1.82 ± 0.01 [a]	4.05 ± 0.03 [b]
21.	13.72	1083	Terpinolene	0.06 ± 0.0 [a]	0.09 ± 0.0 [a]	0.14 ± 0.0 [b]
22.	14.25	1099	β-Linalool	2.07 ± 0.0 [a]	2.31 ± 0.02 [a]	0.98 ± 0.0 [b]
23.	14.57	1105	trans-Sabinene hydrate	0.16 ± 0.0 [a]	0.19 ± 0.0 [a]	0.14 ± 0.0 [a]
24.	15.86	1145	Camphor	0.08 ± 0.0 [a]	0.03 ± 0.0 [a]	0.03 ± 0.0 [a]
25.	15.95	1150	trans-Verbenol	0.08 ± 0.0 [a]	nd [d]	0.08 ± 0.0 [a]
26.	16.38	1171	Borneol	2.75 ± 0.0 [a]	1.41 ± 0.01 [b]	3.57 ± 0.03 [c]
27.	16.64	1180	Terpinen-4-ol	0.35 ± 0.0 [a]	0.35 ± 0.0 [a]	0.60 ± 0.0 [b]
28.	17.32	1195	α-Terpineol	0.16 ± 0.0 [a]	0.47 ± 0.0 [b]	0.27 ± 0.0 [c]
29.	18.04	1228	Thymol methyl ether	1.03 ± 0.01 [a]	3.16 ± 0.03 [b]	2.26 ± 0.02 [c]
30.	18.31	1237	Carvacrol methyl ether	1.24 ± 0.01 [a]	1.18 ± 0.01 [b]	3.02 ± 0.0 [c]
31.	18.75	1240	Neral	0.09 ± 0.0 [a]	0.08 ± 0.0 [a]	nd
32.	19.74	1270	Geranial	0.06 ± 0.0	nd [d]	nd
33.	20.11	1293	Thymol	53.55 ± 0.50 [a]	44.09 ± 0.43 [b]	42.88 ± 0.40 [b]
34.	20.21	1300	Carvacrol	2.00 ± 0.01 [a]	2.33 ± 0.02 [a]	0.73 ± 0.0 [b]
35.	21.27	1350	Thymyl acetate	0.22 ± 0.0 [a]	0.12 ± 0.0 [b]	0.09 ± 0.0 [b]
36.	23.19	1420	β-Caryophyllene	0.98 ± 0.0 [a]	0.77 ± 0.0 [a]	0.88 ± 0.0 [a]
37.	24.44	1472	Geranyl propanoate	0.05 ± 0.0 [a]	0.05 ± 0.0 [a]	nd
38.	24.73	1480	Germacrene D	0.12 ± 0.0 [a]	0.16 ± 0.0 [a]	0.24 ± 0.0 [b]
39.	25.51	1512	γ-Cadinene	0.07 ± 0.0 [a]	0.09 ± 0.0 [a]	0.08 ± 0.0 [a]
40.	25.63	1517	δ-Cadinene	0.07 ± 0.0 [a]	0.09 ± 0.0 [a]	0.05 ± 0.0 [a]
41.	27.15	1581	Caryophyllene oxide	0.29 ± 0.0 [a]	0.71 ± 0.0 [b]	0.17 ± 0.0 [c]
42.	28.53	1641	tau-Cadinol	0.24 ± 0.0 [a]	0.05 ± 0.0 [b]	nd

[a] total ion current; [b] retention time, min; [c] retention index (Kovat's); [d] lower than 0.05% of TIC or not identified; values with different letters in the same row indicate significant differences ($p < 0.05$).

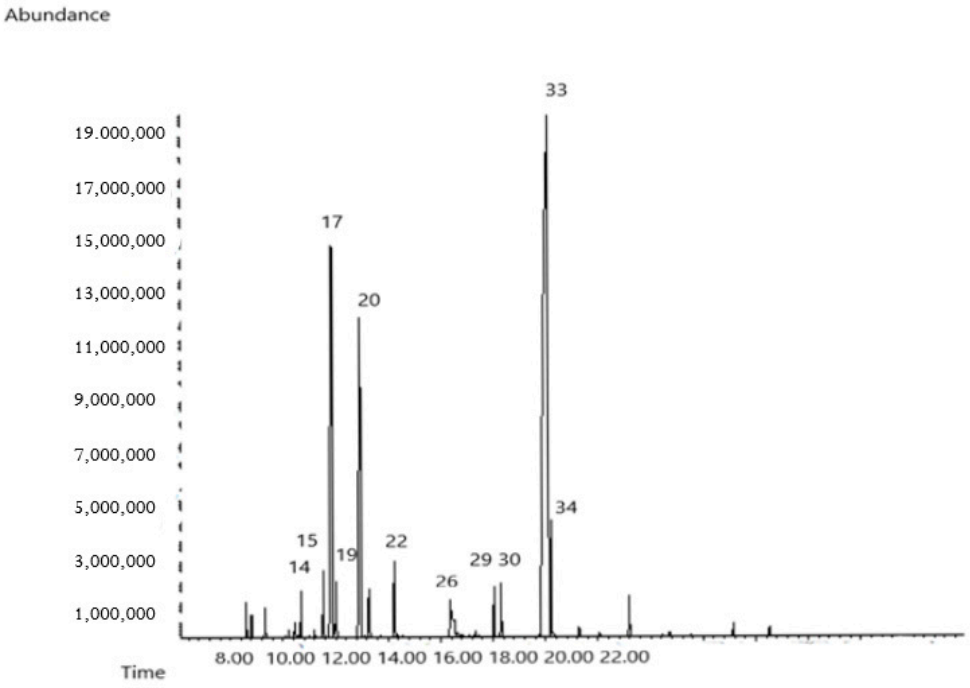


Figure 1. Chromatogram of garden thyme essential oil, sample 1 (peak numbers correspond to those shown in Table 1).

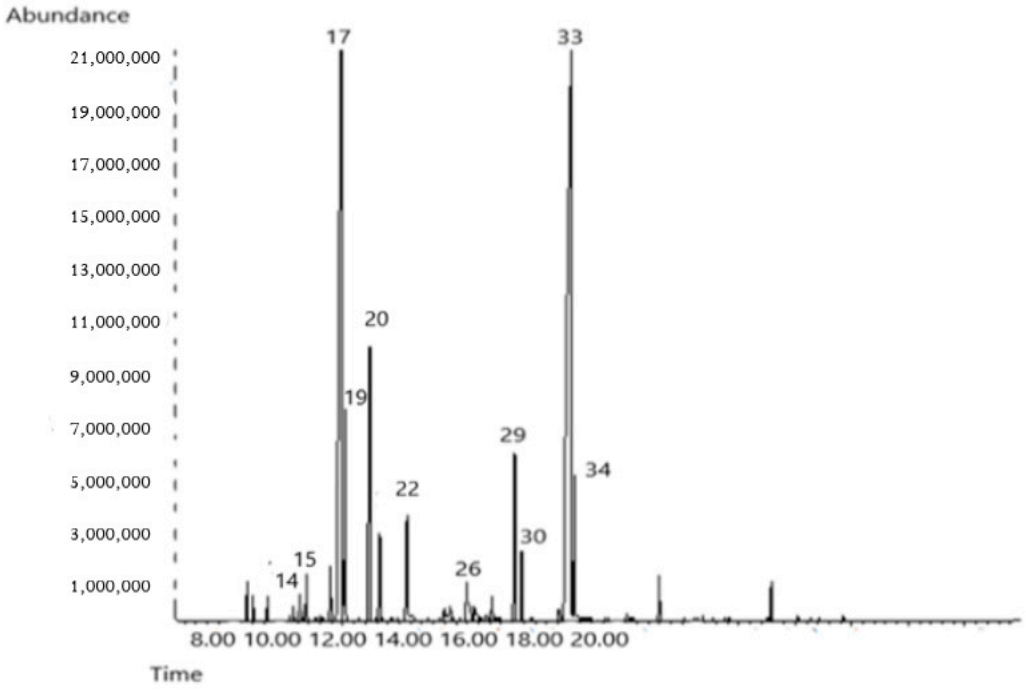


Figure 2. Chromatogram of garden thyme essential oil, sample 2 (peak numbers correspond to those shown in Table 1).

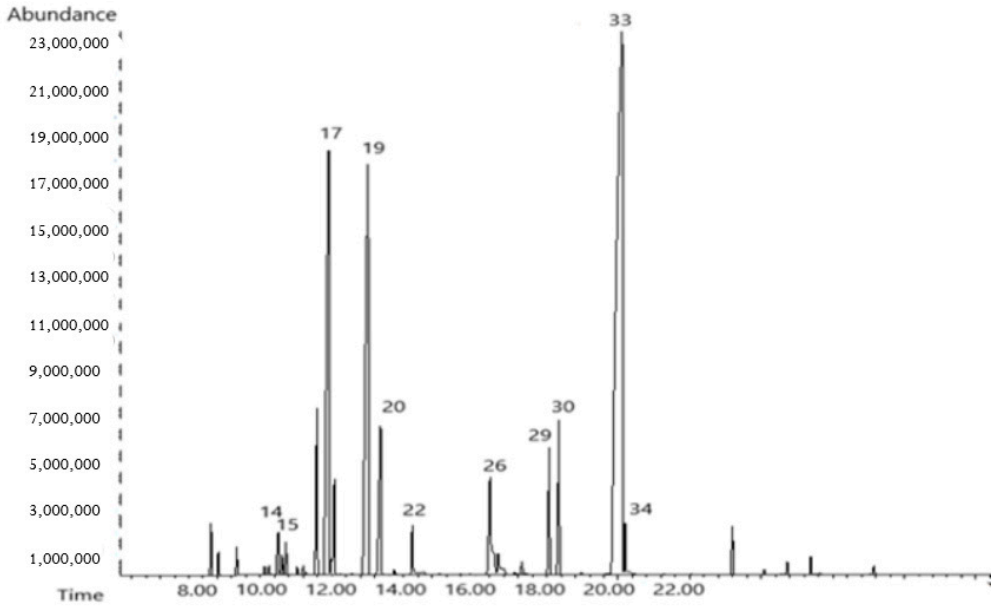
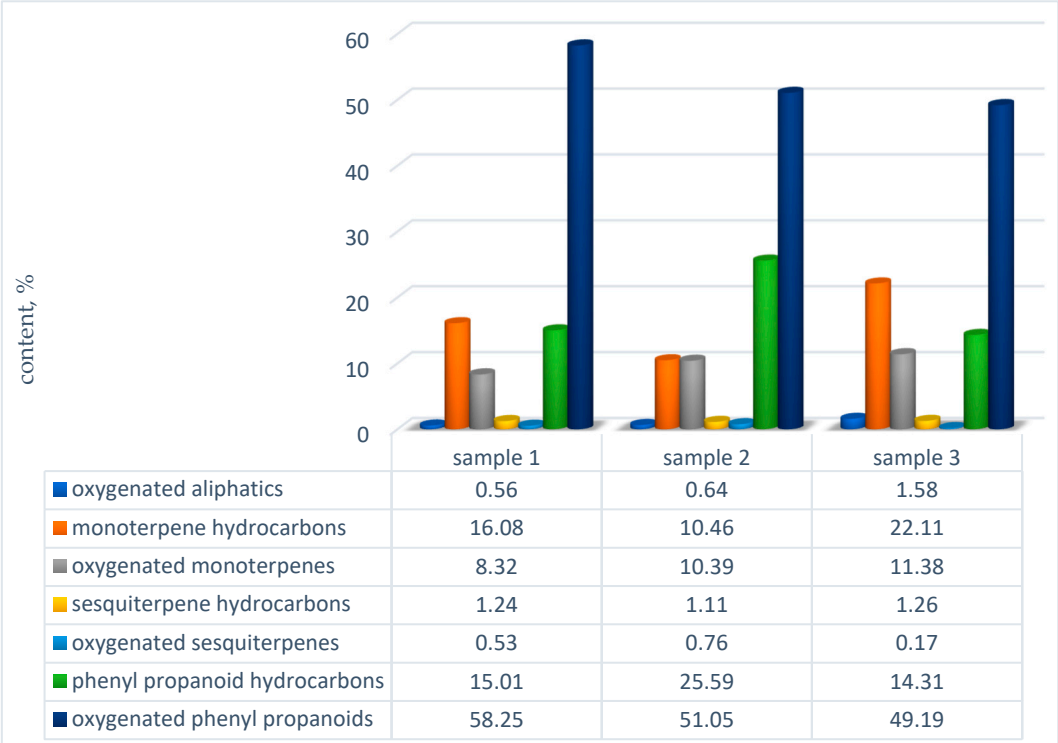


Figure 3. Chromatogram of garden thyme essential oil, sample 3 (peak numbers correspond to those shown in Table 1).



	sample 1	sample 2	sample 3
oxygenated aliphatics	0.56	0.64	1.58
monoterpene hydrocarbons	16.08	10.46	22.11
oxygenated monoterpenes	8.32	10.39	11.38
sesquiterpene hydrocarbons	1.24	1.11	1.26
oxygenated sesquiterpenes	0.53	0.76	0.17
phenyl propanoid hydrocarbons	15.01	25.59	14.31
oxygenated phenyl propanoids	58.25	51.05	49.19

Figure 4. Distribution of the components by groups in the garden thyme essential oils, %.

The distribution of the identified components by groups of compounds is shown in Figure 4.

3.2. Antioxidant Activity

The antioxidant activity of the three garden thyme essential oils is presented in Table 2. The data show that the studied essential oils are active in the DPPH and ABTS assays. The comparative analysis shows that sample 1 has a higher value of the DPPH radical compared to the other two samples, i.e., it has a lower antioxidant activity. The measured

values from the three samples of garden thyme essential oils using the ABTS assay are comparable.

Table 2. Antioxidant activity of three samples of garden thyme essential oils.

Values	Sample 1	Sample 2	Sample 3
DPPH, mM TE/g	165.91 ± 1.50 [a]	144.93 ± 1.33 [b]	143.20 ± 1.32 [b]
ABTS, mM TE/g	127.65 ± 1.20 [a]	126.99 ± 1.22 [a]	121.68 ± 1.20 [a]

Values with different letters in the same row indicate significant differences ($p < 0.05$).

4. Discussion

The yields of essential oils are 1.0% for sample 1, 0.7% for sample 2, and 0.6% for sample 3, which corresponds to the data in the literature [1,2,4,7,11,31,49]. However, they are lower than Zhekova's data [48], which is explained by the fact that samples 2 and 3 are wild. The places where the plants are harvested have different soils and altitudes, which also affect the yield of the essential oils. The difference in the amount of essential oil between sample 1 and the data from the literature for the same Bulgarian variety of garden thyme [48] can also be explained by the long period of time (14 years) during which the study was carried out and the varietal changes that probably occurred. Similar differences in the amounts of essential oil obtained from the same variety, but in different years, were also established by other authors for different essential oil plants [1,49]. The lower amount of essential oil obtained from the wild garden thymes (samples 2 and 3) compared to the cultivated garden thyme (sample 1) can be explained by the agrotechnical care that was applied in the experimental field of the Institute of Roses, Essential and Medical Cultures, Kazanlak.

The obtained essential oils are easily mobile light yellow to yellow-orange liquids with a characteristic warm, intense, and spicy herbal smell, which coincides with the data from the literature [1,49].

A comparative analysis of the data shows that the same components were identified in all the essential oils in different quantities (there was a statistically significant difference). The difference in the amount of the identified components between the individual samples examined can be explained by the fact that sample 1 is cultivated and samples 2 and 3 are wild, as well as by the type of soil [1,49,56]. The varying amounts of some of the components in the three researched samples of essential oils can also be explained by the different altitudes of the places where the plants were harvested [1,49].

The main component in all the samples is the phenol thymol (the data are statistically distinguishable), which assigns them to the thymol chemotype.

The obtained data on the composition of the essential oils of the species *T. vulgaris*, originating in Bulgaria, confirm the data in the literature, described in the text. The established differences in chemical composition (data from Table 1 and Figures 1–3) can be explained by the peculiarities of the soil and climatic conditions in which the plants thrive—the cultivation and fertilization of the soil of cultivated plants, altitude, air temperature, precipitation, chemotype and time of harvesting from the field, type of processed plant part—whole plant or only flowers and with the method of processing—water or steam distillation, etc. [1,2,49].

It has been found that changes in temperature and climatic conditions—humidity, wind, and sunlight—affect cell metabolism and hence, the synthesis of essential oil. The higher temperature during the growing season provokes the synthesis of more monoterpenes and less phenolic compounds. A weaker synthesis of monoterpene compounds is reported with frequent changes in air temperature during the active period of plant development [1,57].

Essential oils contain various aromatic substances, most of which can cause allergic reactions. Very often, they are expressed in swelling, redness, itching, and dermatitis [58–61]. Allergy-inducing aromatic substances included in various perfumery or cosmetic products are described in the Regulation 2023/1545 [62].

Of the allergens described in the Regulation 2023/1545, the following were found in the tested garden thyme essential oils: γ-terpinene (6.58–15.51%), α-terpinene (1.01–3.24%), ß-linalool (0.98–2.31%), ß-caryophyllene (0.88–0.98%), α-pinene (0.37–0.46%), α-limonene (0.23–0.29%), ß-pinene (0.16–0.20%), terpinen-4-ol (0.35–0.60%), α-terpineol (0.16–0.47%), terpinolene (0.06–0.14%), geranial (0.06%), and camphor (0.03–0.08%).

Their distribution by structure and functional groups is as follows: monoterpene hydrocarbons (α- and γ-terpinene, terpinolene, α- and ß-pinene, and limonene); monoterpene oxygen derivatives—alcohols (linalool, α-terpineol, and terpinen-4-ol), aldehydes (geranial), and ketones (camphor); and sesquiterpene hydrocarbons (ß-caryophyllene).

Their quantities should be taken into account when using the oil in perfumery or cosmetic products. In case of the improper storage of the oil (presence of water, light, heat, and other factors), the contained ester, geranyl propionate, can hydrolyze, and the released acid can increase the values of the acid number, and the alcohol (geraniol) is an allergen.

Essential oils that contain thymol are known to exhibit antioxidant activity [1,24,39,63], which the authors explain by its presence.

According to some authors [21,37,38,64], the antioxidant activity of garden thyme essential oils is due to the synergistic action of the two phenols thymol and carvacrol, and in the oils we studied, the amount of thymol is high (42.88% for sample 3, 44.09% for sample 2, and 53.55% for sample 1) and that for carvacrol is lower (0.73% for sample 3, 2.33% for sample 2, and 2.00% for sample 1).

Other authors [27,38] consider that the antioxidant activity of garden thyme essential oil is due to non-polar compounds (-CH-), for example, the hydrocarbons γ-terpinene, p-cymene, and α-terpinene, and in the essential oil we studied, the amount of the first two components is high (γ-terpinene: 6.58% for sample 2, 11.13% for sample 1, and 15.51% for sample 3; p-cymene: 14.25% for sample 3, 14.96% for sample 1, and 25.51% for sample 2).

The differences in the established values of antioxidant activity can be explained by the specifics of the two methods used: for the DPPH assay, the reducing ability of the antioxidant in relation to this radical is taken into account, and for the ABTS assay, the radical scavenging ability of the antioxidant in relation to the stable cation radical is measured.

The essential oil sample 3 has a weaker antioxidant activity compared to the other samples despite the high thymol content. This may be due to the presence of other aromatic substances in the essential oil with different structures and functional groups affecting its activity [41].

The comparative analysis with the data from the literature shows differences in the measured values, for example, the % inhibition of DPPH assay 30.50 by 0.2 g/L garden thyme essential oil and 82.00 by 2 g/L garden thyme essential oil [27]; of DPPH assay (149.8 μmol of TE g^{-1}) and of ABTS assay (192.4 μmol of TE g^{-1}) [43]; of DPPH assay (IC_{50}, 159.59 μg/mL) [14]; of DPPH assay (IC_{50} 0.08 g/L) [65]; and of DPPH assay (EC50 0.14 mg/mL) [66], which is explained by the origin of the plants (soil and climatic conditions), as well as by the way they are processed.

The obtained values are higher than those of pure thymol published in the literature, determined by DPPH assay: 0.538 μg/mL [39] and 28.82 μg/mL [24], indicating that it is a stronger antioxidant.

The comparative analysis of the three thyme samples studied shows that the wild-growing plants contain less essential oil, in which the presence of phenol thymol is lower, compared to the cultivated variety "German Winter". The different content of thymol and other oxygen components explains the difference in the determined antioxidant activity.

5. Conclusions

The wild and cultivated garden thyme (*Thymus vulgaris* L.) originating in Bulgaria, collected from different regions, has a high thymol content, which determines the essential oils obtained from them by hydrodistillation as thymol chemotype. Oxygenated phenyl propanoids predominate in the composition of all the essential oils. The other groups of

aromatic compounds are represented with different participation in the composition of oils, depending on the different regions of plant habitat.

The assessment of the antioxidant activity of the thyme essential oils based on the results of two different methods (DPPH and ABTS) shows that these oils have antioxidant activity, being slightly more pronounced in the cultivated plant (sample 1) than in the wild plants (samples 2 and 3). Sample 1 also has the highest content of thymol and oxygenated phenyl propanoids. The essential oils exhibit antioxidant activity, which is a prerequisite for their use in various food products, and the subject of our subsequent studies.

Author Contributions: Conceptualization, K.D. and M.D.; methodology, K.D. and M.D.; software, M.D.; validation, K.D.; formal analysis, T.V.; investigation, K.D., M.D. and A.S.; resources, T.V. and I.I.; data curation, K.D., S.D. and A.S.; writing—original draft preparation, K.D. and A.S.; writing—review and editing, K.D., A.S. and I.I.; visualization, K.D. and A.S.; supervision, O.B.O.; project administration, O.B.O. All authors have read and agreed to the published version of the manuscript.

Funding: This study is financed by the European Union—NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0006.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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