Article

Morphological and Chemical Traits as Quality Determinants of Common Thyme (*Thymus vulgaris* L.), on the Example of ‘Standard Winter’ Cultivar

Olga Kosakowska , Katarzyna Bączek , Jarosław L. Przybył , Anna Pawełczak, Katarzyna Rolewska and Zenon Węglarz

Department of Vegetable and Medicinal Plants, Institute of Horticultural Sciences, Warsaw University of Life Sciences–SGGW, 02-787 Warsaw, Poland; katarzyna_baczek@sggw.edu.pl (K.B.); jaroslaw_przybyl@sggw.edu.pl (J.L.P.); anna_pawelczak@sggw.edu.pl (A.P.); katrol@onet.pl (K.R.); zenon_weglarz@sggw.edu.pl (Z.W.)

* Correspondence: olga_kosakowska@sggw.edu.pl; Tel.: +48-225-932-247

Received: 8 June 2020; Accepted: 23 June 2020; Published: 25 June 2020

Abstract: Common thyme is regarded as one of the most important culinary plants. The purpose of the work was to determine the intraspecific variability of common thyme with respect to morphological and chemical characters including the content and composition of essential oil and phenolic compounds in the herb. The objects of the study were 12 clones, vegetatively multiplied from randomly selected individual plants of cultivar ‘Standard Winter’. The morphological observations and harvest of raw materials were carried out in the first year plants’ vegetation. The highest differences between clones were on fresh and dry weight of herb (CV = 0.38 and 0.36, respectively), width of leaves (CV = 0.21), and density of glandular trichomes on the abaxial surface of leaves (CV = 0.29). Examined clones were also differentiated as to the chemical features. Essential oil content (performed by hydrodistillation) and composition (by GC-MS and GC-FID) were determined and they ranged from 2.10 to 4.38 g × 100 g^{−1} DW. Here, thymol, γ-terpinen, and *p*-cymen were the dominant compounds. Clone no. 4 was distinctive as to the highest content of essential oil followed by the highest share of thymol (54.59%). The total content of phenolic acids and flavonoids (determined according to PPh 6th) also differed among clones (CV = 0.38 and 0.36, respectively). Using a validated HPLC-DAD method, the following compounds were identified: caffeic, rosmarinic, *p*-coumaric acids, luteolin 7-*O*-glucoside, naryngenin, and (−)-epicatechin. Here, rosmarinic acid followed by luteolin 7-*O*-glucoside were present in the highest amounts (611.47–2675.59 and 46.77–325.11 mg × 100 g^{−1} DW, respectively). The highest differences between clones were the contents of *p*-coumaric acid (CV = 0.59), luteolin 7-*O*-glucoside (CV = 0.50) and rosmarinic acid (CV = 0.40). Such a high range of variability can provide problems with raw material standardization. Nevertheless, it opens possibilities for breeders, whereas individual plants/clones may become valuable components for breeding.

Keywords: intraspecific variability; morphological traits; glandular trichomes; essential oil composition; phenolic compounds

1. Introduction

The genus *Thymus*, one of the most important taxon of the *Lamiaceae* family, is widely distributed in the Old World, with the center of origin located in the Mediterranean region. The genus is represented by 214 species and 36 subspecies, divided into eight sections: *Micantes*, *Mastichina*, *Piperella*, *Teucrioides*, *Pseudothymbra*, *Thymus*, *Hyphodromi* and *Serpyllum* [1]. *Thymus* species have been known for ages

as medicinal plants, and currently some like *Thymus serpyllum* L. (belonging to section *Serpyllum*), *Thymus vulgaris* L., and *Thymus zygis* L. (section *Thymus*) are listed in the European Pharmacopeia (EPH 7th) [2]. Here, *T. vulgaris* (common thyme) is regarded to be the most valuable species of the genus. This aromatic, perennial subshrub is cultivated all over the world, and used both as a spice and a medicine. Common thyme herb (*Thymi herba*) is rich in essential oil and according to EPH 7th requirements it is not lower than 1.2% [2,3]. The species creates different chemotypes distinguished on the bases of the dominant compound in the essential oil. Six main chemotypes were described here: phenolic (thymol, carvacrol) and non-phenolic (linalool, geraniol, α -terpineol, trans-thujan-4-ol/terpinen-4-ol) [4–10]. The domination of 1.8 cineol in the essential oil was reported in previous findings [11]. Common thyme is regarded as one of the most polymorphic plants given its terpenic composition. Listed chemotypes are created on the bases of one biosynthetic pathway, where dominant monoterpenes are the final products of its various branches. Such a terpenic polymorphism is under strong genetic control, where an epistatic series of five loci are involved [12,13]. The ‘thymol’ chemotype occurs in the case of plants homozygous for recessive alleles at all five loci. With regards to wild growing populations of common thyme, the relation between chemotype and the type of habitat has been noticed. Phenolic chemotypes are common close to the Mediterranean Sea, whereas non-phenolic ones predominate in inland sites, particularly 400 m above sea level. Moreover, non-phenolic chemotypes showed a significantly better regrowth after winter survival than phenolic chemotypes [5,14,15]. Interestingly, chemotypes’ occurrence is also related to the sexual polymorphism of common thyme. The species, like other *Lamiaceae*, is characterized by gynodioecy, where populations contain two types of plants: females (male sterile) and hermaphrodites. Here, populations with a high female frequency tend to be dominated by non-phenolic chemotypes, while hermaphrodites by phenolic ones [5].

Common thyme herb also contains non-volatile phenolic compounds, mainly phenolic acids and flavonoids. Rosmarinic and caffeic acids are dominants within the phenolic acids fraction, while flavones (e.g., luteolin and apigenin derivatives), and methylated flavones are the main compounds in the flavonoids group [16–19]. Given such a wide spectrum of biologically active compounds, *Thymi herba* has various pharmacological activities, such as: spasmolytic, antioxidant, antitussive, and antimicrobial [20–22]. Therefore, the raw material is commonly used for the treatment of respiratory and digestive system diseases [20–23]. The non-medicinal usage of the species includes its application as a food preservative, cosmetic component, and, most importantly—as a culinary herb. Its pleasant aroma as well as high dietetic and sensory value make common thyme one of the most traded spices, consumed both as a dry and fresh herb [24].

In the past, common thyme was collected from natural sites. However, due to limited occurrence in nature currently, it has been introduced into cultivation. Nowadays many ecotypes, varieties, landraces, and cultivars are available for stakeholders. Nevertheless, when given the high economic importance of the species, there is a need to improve its quality by the creation of new forms [10,25–28]. Compared to traditional food crops, breeding of medicinal and aromatic plants (MAPs), including common thyme, is at its infancy. Selection is traditionally the most common and efficient method of MAPs genetic improvement [29–31]. In the case of common thyme, hybrid breeding has also been introduced, which resulted in the creation of hybrid cultivar ‘Varico 3’ [26,27]. According to Shmeit et al. [32] in vitro-induced polyploidization may also be applied in order to generate new variability within common thyme. Nevertheless, selection is still the background for breeders, since it is a low-cost method providing a high effect in relatively short time. Common thyme is extremely variable in terms of morphological traits, chemical composition, and presents high sexual polymorphism (gynodioecy), which together gives almost unlimited possibilities in breeding programmes. Thus, wild growing populations, germplasm collections, as well as contemporary used cultivars are a source of huge natural variability within common thyme species [6,26–28,33,34]. However, when taking into consideration recent requirements concerning standardization of herbal products, such a high intraspecific variability seems to be a factor that decreases its homogeneity, and potentially, the quality of raw material [35].

Given the use of common thyme variability in breeding programmes, many traits are taken into consideration, e.g., content and composition of essential oil, developmental features, yield of herb, resistance to biotic and abiotic stresses (especially frost), as well as the ability for regrowth. In Europe, a few ecotypes of common thyme that vary as to their morphological, physiological, and chemical traits, have been distinguished and cultivated. Here, ‘German type’ appeared as a result of selection managed by generations of farmers [26]. This type is frost resistance, cultivated as a perennial, characterized by erect type of growth and greyish-green color of leaves. ‘French type’, the one with narrow leaves, is considered as frost sensitive and cultivated as an annual plant. ‘Mediterranean type’, so called ‘Valdonian type’, was introduced into cultivation from a population growing wild in Italy. This type is distinguished by grayish-blue leaves, low frost resistance, lignified stems, and superior essential oil content and composition [26,36,37]. Another intraspecific division was mentioned by Melchior and Kastner [38] where two forms of common thyme were selected on the bases on the leaf blade shape and size. Here, *T.vulgaris* f. *capitatus* Willk. et. Lange is characterized by oblong-lanceolate leaves, 4–5 mm long and 1–2 mm wide, while *T.vulgaris* f. *verticillatus* Willk. et. Lange by elliptic and/or oblong-obovate leaves, 5–8 mm long and 3–4 mm wide. Both forms can cross with each other and give fertile progeny, being intermediate forms. Rumińska [37] claims that f. *capitatus* covers the ‘French type’ of common thyme, while f. *verticillatus* corresponds to ‘German type’.

Up to now, a few cultivars of common thyme have been evaluated, e.g., ‘English Winter’, ‘French Summer’, ‘Deutscher Winter’, ‘Summer Thyme the Provence’, ‘Krajovy’ [10,28,33]. However, ‘Standard Winter’ cultivar, commonly cultivated in Central Europe, has not yet been studied.

The aim of the study was to determine the intraspecific variability of common thyme ‘Standard Winter’ cultivar, with respect to selected morphological traits and the chemical composition of the herb, including the content and composition of essential oil, flavonoids and phenolic acids.

2. Materials and Methods

2.1. Plant Material

The experiment was performed at the experimental field of the Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences (WULS-SGGW) (52°10'18" N; 21°05'23" E), on alluvial soil. Common thyme seeds (cultivar ‘Standard Winter’, commonly cultivated in Central Europe) were purchased from the Jelitto company (Schwarmstedt, Germany). Seeds were sown in the second week of February into multi-pots filled with a peat substrate, in a greenhouse. In the first week of May, 100 seedlings were randomly selected and planted. In August, 12 individual (maternal) plants were chosen and marked for vegetative multiplication. In December, the plants were transferred into the greenhouse. In February of the second year, green, soft cuttings were collected from each plant and rooted. Well rooted cuttings were planted out into the field, in a randomized block design (20 cuttings per plot; in 3 replications), with a spacing of 20 × 40 cm. Thus, the object of the study were 12 clones of common thyme, vegetatively multiplied from individual plants selected within the cultivar. The observations of morphological traits (Section 2.2) followed by harvest of herb was carried out on 1-year old plants (clones), at the beginning of plant blooming. The herb (upper, not woody parts of shoots) was cut from each plot, at a height of about 10 cm above ground. The fresh and dry weight of the herb was estimated (g per plant). After drying at 35 °C, leaves were separated from stems, then weighed. Leaves (so-called rubbed herb) were ground for chemical analysis (Section 2.3).

2.2. Morphological Observations

Morphological traits of the clones were estimated according to the List of Descriptors for *Thymus vulgaris* L. elaborated by the Medicinal and Aromatic Plants Working Group of European Cooperative Programme for Plant Genetic Resources (MAPs WG ECP/GR) [39]. Observations were performed directly before harvest of raw material, on 10 plants per one clone. Plant growth habit, plant height (cm), plant diameter (mm), foliage density, leaf shape and color, leaf length (mm), and width

(mm), were determined. Moreover, observations concerning density of glandular trichomes on abaxial and adaxial surface of leaves were estimated. Samples of leaves (5 per plant) were collected, and directly subjected to microscopic observations. Stereoscope microscope Nikon SMZ 745 T (Tokyo, Japan) equipped with Camera Invenio 3SPixel CMOS and Coolview v.1.6 Programme (Precoptic Co, Warsaw, Poland) was used. The number of glandular trichomes was counted on both sides of the leaf, then expressed as number per 1 mm². Photographic documentation was performed (Figures 1 and 2).

Figure 1. (a) Plant of clone no. 2; (b) Plant of clone no 5.

Figure 2. (a) Shape of leaves: 1–oblong-ovate; 2–lanceolate; (b) Glandular trichomes on the adaxial leaf surface.

2.3. Chemical Analysis

2.3.1. Content of Essential Oil

30 g of air-dried raw material was used for hydrodistillation for 3 h using a Clevenger-type apparatus. The content of essential oil was expressed as g $\times$ 100 g^{−1} of dry weight (DW). Obtained essential oils were stored in amber vials, at 4 °C.

2.3.2. Analysis of Essential Oils by GC-MS and GC-FID

The qualitative and quantitative analysis was performed by usage an Agilent Technologies 7890A gas chromatograph equipped with a flame ionization detector (FID) and MS Agilent Technologies 5975C Inert XL_MSD with Triple Axis Detector (Agilent Technologies, Wilmington, DE, USA). Capillary,

polar column HP 20M (25 m × 0.32 mm × 0.3 µm film thickness) (Agilent Technologies, Wilmington, DE, USA) was applied. Operation conditions were described previously by Bączek et al. [40].

2.3.3. Total Content of Phenolic Acids and Flavonoids

Both groups of compounds were determined according to the Polish Pharmacopoeia 6th edition [41]. Phenolic acids content was determined by Arnov's method, and expressed as caffeic acid equivalent ($\text{g} \times 100 \text{ g}^{-1} \text{ DW}$), while flavonoids were determined by the aluminum chloride colorimetric method and expressed as quercetin equivalent ($\text{g} \times 100 \text{ g}^{-1} \text{ DW}$). A detailed description of these methods has already been given by Kosakowska et al. [42].

2.3.4. Analysis of Phenolic Acids and Flavonoids by HPLC-DAD

Validation

The standards were purchased from Merck (Darmstadt, Germany) and ChromaDex® (Irvine, CA, USA) and separately dissolved with MeOH in a 25 mL volumetric flask according to the ChromaDex's Tech Tip 0003: Reference Standard Recovery and Dilution and used as standard stock solutions [43]. Working solutions were prepared by diluting 10 µL and 100 µL of standard stock solutions with methanol in 10 mL volumetric flasks, 500 µL and 1000 µL in 5 mL volumetric flasks as well as 1000 µL in 2 mL volumetric flasks. The working solutions and undiluted stock solutions were injected (1 µL) on a column in six replicates ($n = 6$) using SIL-20AC HT. Six-point calibration curves were plotted according to the external standard method by correlating concentration with peak area. Curves parameters were calculated with Microsoft Excel 14. Signal-to-noise ratio approach were used to determine LOD (S/N of 3:1) and LOQ (S/N of 10:1) (Table 1). The peak table and UV-spectra library (190–450 nm) of individual compounds were also created.

Table 1. Validation parameters of the HPLC-DAD analysis ($n = 6$).

Compound	Precision Intraday (CV, %)	Precision Interday (CV, %)	Calibration Equation	R^2 ($n = 6$)	Linear Range ($\text{mg} \times \text{mL}^{-1}$)	LOD ($\mu\text{g} \times \text{L}^{-1}$)	LOQ ($\mu\text{g} \times \text{L}^{-1}$)	Recovery (%)
Caffeic acid	1.00	1.72	$y = 2592.9x + 379.6$	0.9996	1.00–998.40	0.03	0.08	95.7
(–)-Epicatechin	0.68	1.51	$y = 7345.1x - 5643.8$	0.9995	0.47–23.40	0.10	0.34	95.6
<i>p</i> -Coumaric acid	0.28	0.65	$y = 6196.40x - 537.45$	0.9999	1.01–504.70	0.07	0.23	101.9
Luteolin 7-O-glucoside	0.36	2.67	$y = 2022.2x - 1149.4$	0.9997	0.19–19.08	0.05	0.18	101.4
Rosmarinic acid	1.24	2.12	$y = 2017.9x + 1100.4$	0.9999	0.43–434.02	0.03	0.09	102.6
Naringenin	1.21	1.89	$y = 1304.8x + 1983.8$	0.9998	1.98–396.8	0.03	0.09	102.9

Sample Preparation

Raw material (1.000 g) was homogenized and extracted with 100 mL of methanol in Extraction System B-811 (Büchi Labortechnik AG, Flawil, Switzerland). Soxhlet hot extraction with twenty-five extraction cycles, flushing and drying was used. After evaporation of solvent, the residue was dissolved in 5 mL of methanol. The obtained extracts were filtered with ISO-Disc™ Filters PTFE-25-2, diameter 25 mm, pore size 0.20 µm (Supelco Analytical™, Bellefonte, PA, USA) and subjected to HPLC-DAD.

Parameters of Separation

The work were performed using a Shimadzu Prominence chromatograph equipped with auto sampler SIL-20AC HT, photodiode array detector SPD-M20A and LCsolution 1.21 SP1 chromatography software (Shimadzu, Kyoto, Japan). Separations were achieved using a 100 mm × 4.60 mm, C18 reversed-phase column, 2.6 µm particles with solid core and porous outer layer (Kinetex™, Phenomenex,

Torrance, CA, USA). Binary gradient of mobile phase A (deionised water adjusted to pH 2 with phosphoric acid) and B (ACN) was used as follows: 0 min—12.5% B; 4.0 min—23% B; 6.0 min—50% B; 6.01 min—12.5% B; 8 min—stop. The HPLC conditions were as follows: flow rate $1.5 \text{ mL} \times \text{min}^{-1}$, oven temperature 40°C , injection volume $1 \mu\text{L}$.

Parameters of Integration

Peak identification was carried out by comparison of retention time as well UV-spectra with standards. The content of the determined compounds was calculated in $\text{mg} \times 100 \text{ g}^{-1} \text{ DW}$.

2.4. Statistical Analysis

Data were subjected to statistical analysis using Statistica software. The mean values were compared by using the one way analysis of variance (ANOVA) followed by Tukey's multiple range test. The differences between individual means were deemed to be significant at $p < 0.05$. Standard deviation ($\pm\text{SD}$) and coefficient of variation (CV) was estimated.

3. Results and Discussion

Investigated clones differed in respect of morphological traits. They were characterized by the sub-erect (7 clones) or erect (5 clones) type of growth habit (Table 2). The plants' heights ranged from 19.75 (clone no. 4) to 28.00 cm (clone no. 6), with the CV at the level of 0.08, while plants' diameters ranged from 306.7 (clone no. 12) to 456.7 cm (clone no. 10) (Table 3). Dalir and Safarnejad [34] claim that the minimum diameter of the common thyme plant is 19.42 cm, while the maximum is 29.26 cm. Listed features, especially type of growth habit and plant's height, are important when suitability for mechanical harvesting is taken into consideration. However, a high and stable yield seems to be the most desirable trait from the practice point of view. In the present study, fresh weight of herb ranged from 48.33 to 129.33 g per plant, while dry weight ranged from 9.27 to 31.23 g per plant. Clones no. 5, 7 and 10 gave the highest fresh and dry weight of harvested herb. These features differentiated the clones at a relatively high degree (CV = 0.38 for fresh weight; CV = 0.36 for dry weight) (Table 3). This corresponds to the results obtained by Mewes et al. [33], where dry weight of common thyme herb varied from 20 to 47 g per plant, and a high diversity (CV = 0.44) among investigated accessions was observed. When given common thyme, the mass of leaves is usually estimated, since dry leaves themselves (so-called rubbed herb) are a commercial and traded product. In our work, the weight of leaves ranged from 5.80 (clone no. 12) to 19.83 g per plant (clone no. 10), CV = 0.33. This trait, related to foliar density, is one of the most important descriptors recommended by ECP/GR for common thyme evaluation [39]. Here, clones no. 7, 8, 9 were characterized by sparse, clones no. 1, 4, 5, 10, 12 by dense, while clones no. 2, 3, 6 and 11—medium foliar density (Table 2).

Table 2. Morphological traits of investigated clones.

Clone	Plant Habit	Foliage Density	Leaf Shape	Leaf Colour
1	sub-erect	dense	oblong-obovate	dark green
2	sub-erect	medium	lanceolate	pale green
3	sub-erect	medium	oblong-obovate	dark green
4	sub-erect	dense	oblong-lanceolate	greyish-green
5	sub-erect	dense	obovate	greyish-blue
6	sub-erect	medium	elliptic	green
7	erect	sparse	oblong-lanceolate	pale green
8	sub-erect	sparse	obovate	green
9	erect	sparse	lanceolate	green
10	erect	dense	oblong-lanceolate	green
11	erect	medium	obovate	green
12	erect	dense	elliptic	green

Table 3. Morphological traits of investigated clones cd.

Clone	Plant Height (cm)	Plant Diameter (mm)	Fresh Weight of Herb (g × plant ⁻¹)	Dry Weight of Herb (g × plant ⁻¹)	Dry Weight of Leaves (g × plant ⁻¹)
1	25.75 ± 2.75 c	320.0 ± 20.0 d	70.99 ± 7.63 b	19.50 ± 2.29 b	12.33 ± 2.08 c
2	26.00 ± 1.83 b	323.3 ± 25.2 d	59.33 ± 8.50 bc	14.87 ± 1.86 c	9.07 ± 1.10 e
3	27.75 ± 1.71 a	363.3 ± 25.2 c	68.67 ± 6.03 b	19.17 ± 1.26 b	10.20 ± 0.72 d
4	19.75 ± 1.71 e	360.0 ± 30.0 c	50.67 ± 4.04 c	13.33 ± 1.04 d	8.37 ± 0.64 e
5	23.75 ± 1.00 d	443.3 ± 40.4 a	119.33 ± 14.01 ab	29.10 ± 1.91 a	16.17 ± 0.76 b
6	28.00 ± 1.63 a	402.3 ± 16.6 b	73.00 ± 7.21 b	16.23 ± 1.75 c	11.13 ± 0.81 d
7	25.75 ± 0.96 c	343.3 ± 40.4 cd	127.00 ± 14.73 a	29.73 ± 2.53 a	12.13 ± 1.78 c
8	25.75 ± 2.75 c	403.3 ± 30.6 b	70.17 ± 7.18 b	19.83 ± 1.26 b	10.43 ± 0.60 d
9	26.75 ± 0.50 ab	353.3 ± 15.3 c	50.27 ± 4.24 c	13.77 ± 1.78 d	8.27 ± 1.08 e
10	25.25 ± 1.71 c	456.7 ± 4.04 a	129.33 ± 9.07 a	31.23 ± 3.22 a	19.83 ± 1.89 a
11	24.75 ± 2.50 cd	315.0 ± 15.0 d	69.33 ± 9.29 b	18.83 ± 1.76 b	10.50 ± 0.50 d
12	26.50 ± 1.29 b	306.7 ± 30.6 d	48.33 ± 4.73 c	9.27 ± 0.68 e	5.80 ± 0.85 f
Mean	25.48	365.9	78.04	19.57	11.19
CV	0.08	0.14	0.38	0.36	0.33

Means marked with different letters differ at $p < 0.05$

In present work, leaves of investigated clones were subjected to observations concerning colour and shape of the leaf blade, as well as its length and width. Most were characterized by green leaves (6 clones), however there also were dark green (2 clones), pale green (2 clones), greyish-green (1 clone) and grayish-blue (1 clone) (Table 2, Figure 1a,b). The shape was described as oblong-obovate (clones no. 1 and 3), obovate (clones no. 5, 8, 11), oblong-lanceolate (clones no. 4, 7, 10), lanceolate (clones no. 2 and 9) and elliptic (clones 6 and 12) (Table 2). The length of the leaf blade varied from 5.29 (clone no. 12) to 7.91 mm (clone no. 1) (CV = 0.14), while its width from 2.38 (clone no. 7) to 4.79 (clone no. 1) (CV = 0.21). On the bases of these results, clones no. 1, 3, 5, 8, 11 can be classified as ‘broad-leaved’, clones no. 2, 4, 7, 9, 12 as ‘narrow-leaved’, while clones no. 6 and 10 can be considered as intermediate forms (Table 4, Figure 2a). These results correspond to divisions mentioned by Melchior and Kastner [38].

Table 4. Leaves parameters (mm) and density of glandular trichomes on leaves (number per 1 mm²).

Clone	Length of Leaf Blade (mm)	Width of Leaf Blade (mm)	Density of Glandular Trichomes on Adaxial Surface of Leaf (Number per 1 mm ²)	Density of Glandular Trichomes on Abaxial Surface of Leaf (Number per 1 mm ²)
1	7.91 ± 0.54 a	4.79 ± 0.40 a	14.32 ± 0.95 cd	5.16 ± 0.50 c
2	7.40 ± 0.48 a	2.45 ± 0.16 d	13.08 ± 0.97 d	5.88 ± 0.64 bc
3	5.54 ± 0.41 b	3.59 ± 0.43 b	13.30 ± 1.16 d	4.55 ± 0.87 d
4	7.25 ± 0.25 a	3.06 ± 0.12 c	16.72 ± 0.95 b	9.36 ± 1.39 a
5	5.99 ± 0.37 b	3.51 ± 0.31 b	15.36 ± 0.65 c	4.16 ± 0.36 d
6	5.49 ± 0.49 b	2.86 ± 0.20 cd	13.20 ± 0.73 d	6.65 ± 0.62 b
7	6.91 ± 0.49 ab	2.38 ± 0.12 d	16.52 ± 1.15 b	6.20 ± 0.89 b
8	5.82 ± 0.75 b	3.23 ± 0.25 b	13.16 ± 1.58 d	5.36 ± 0.65 c
9	5.87 ± 0.35 b	2.54 ± 0.26 d	15.85 ± 1.48 c	4.90 ± 1.48 c
10	6.98 ± 0.29 ab	3.04 ± 0.31 c	13.32 ± 0.88 d	3.96 ± 0.26 d
11	5.34 ± 0.25 bc	3.37 ± 0.29 b	18.36 ± 1.61 a	8.80 ± 1.61 a
12	5.29 ± 0.33 bc	2.61 ± 0.38 d	16.45 ± 1.42 b	6.40 ± 0.69 b
Mean	6.32	3.12	14.97	5.95
CV	0.14	0.21	0.12	0.29

Means marked with different letters differ at $p < 0.05$.

As it was mentioned before, the examined clones originate from individual plants selected from the cultivar ‘Standard Winter’. Thus, differences between clones may represent a range of variability within this cultivar. It seems that morphological traits, typical European ecotypes and forms of common

thyme [37,38], appear within the investigated cultivar as a result of phenotypical plasticity of the species, caused probably by its allogamous way of reproduction.

With regard to aromatic plants, including common thyme, the issue concerning the content and composition of essential oil is one of the most important, since this substance determines both the sensory value and pharmacological activity of raw material. In common thyme (as well as in other *Lamiaceae*), essential oil is stored in multicellular epidermal glands, the glandular trichomes, situated on the surface on both sides of the leaves. The glandular trichomes are composed of the epidermal and stalk cells. The head of the gland is formed by 10–14 secreted cells, where essential oil is produced before being transferred and stored in sub-cuticular space. Glandular trichomes are subdivided into two groups: peltate and capitate [44,45]. There is a direct correlation between glands number and essential oil production. Moreover, the distribution of glandular trichomes have valuable taxonomical significance at the species level [46]. Our results show that the examined common thyme clones differed with respect to number of glandular trichomes per 1 mm² of a leaf. The density of these glands on the adaxial surface of the leaf varied from 13.08 to 18.36 (CV = 0.12), while on the abaxial surface from 3.96 to 9.36 (CV = 0.29) per 1 mm² (Table 4, Figure 2b). Trichome density was related to essential oil content and it was observed that clones no. 4 and 11, characterized by the highest number of glandular trichomes on leaves, were also distinguished by the highest essential oil content (4.38 and 4.15 g × 100 g⁻¹ DW, respectively) (Tables 4 and 5). In general, the content of essential oil ranged from 2.10 to 4.38 g × 100 g⁻¹ DW, and corresponds with results obtained by others [10,27,28,33]. The content of essential oils in aromatic plants, including common thyme, depends on various factors, both internal (genetic, ontogenetic, morphogenetic) and external (environmental such as temperature, day length, solar radiation, and postharvest treatment) [30,47–49].

Table 5. The total content (g × 100 g⁻¹) and gas chromatographic composition (% peak area) of essential oil samples.

No.	Compound	RI	1	2	3	4	5	6	7	8	9	10	11	12
1	α-thujene	1022	0.34	0.00	0.00	0.31	0.29	0.95	0.00	0.54	0.00	0.25	0.95	0.47
2	α-pinene	1028	0.19	0.26	0.00	0.00	0.24	0.00	0.24	0.00	0.37	0.24	0.32	0.00
3	camphene	1074	2.38	2.64	2.28	2.03	2.55	2.48	1.93	2.67	2.64	2.84	2.21	2.34
4	β-pinene	1115	0.56	0.95	0.89	0.52	0.92	0.46	0.35	0.85	0.94	1.76	0.43	0.29
5	sabinene	1124	0.23	0.32	0.23	0.27	0.25	0.27	0.21	0.00	0.37	0.27	0.24	0.25
6	3-carene	1150	2.25	2.17	1.90	1.79	1.87	1.90	1.82	1.85	1.91	2.00	1.91	2.20
7	β-myrcene	1165	2.28	2.68	1.87	1.77	2.10	2.25	1.75	2.15	1.94	2.01	2.27	0.44
8	α-terpinene	1181	0.43	0.49	0.44	0.38	0.40	0.38	0.39	0.41	0.39	0.46	0.41	0.42
9	limonene	1204	0.00	1.02	0.00	0.84	0.29	0.63	0.35	0.81	1.32	0.00	0.54	0.55
10	γ-terpinene	1247	22.31	27.17	16.17	14.63	19.65	21.67	14.03	21.17	18.27	19.12	19.35	20.88
11	p-cymene	1271	15.85	15.38	14.22	12.49	14.94	14.51	14.06	17.84	14.51	10.69	11.63	12.14
12	1-octen-3-ol	1446	0.30	0.00	1.51	1.60	1.00	0.81	2.01	0.72	1.45	0.02	1.22	1.36
13	β-cubebene	1539	0.37	0.41	2.21	0.54	0.69	1.25	0.61	2.13	2.06	0.57	0.59	0.62
14	linalool	1542	1.90	1.99	2.63	2.29	2.55	1.53	2.05	1.54	1.81	2.71	2.09	2.40
15	β-copaene	1581	0.98	1.07	1.16	1.07	1.00	0.97	1.17	0.90	0.97	1.00	0.94	0.95
16	terpinen-4-ol	1597	0.29	0.51	0.00	0.00	0.66	0.00	0.00	0.50	1.11	0.74	0.00	0.00
17	bornyl acetate	1575	0.00	0.33	0.00	0.00	0.28	0.00	0.00	0.00	0.00	0.38	0.00	0.00
18	β-caryophyllene	1592	0.98	1.03	5.17	1.35	2.33	4.12	0.49	5.72	5.51	1.12	1.07	0.82
19	γ-elemene	1641	0.00	0.00	0.00	0.43	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00
20	borneol	1687	0.00	0.00	0.00	0.42	0.00	0.36	0.54	0.00	0.35	0.00	0.00	0.00
21	geranial	1724	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00
22	α-cadinene	1770	0.72	1.15	1.72	0.61	1.10	0.58	0.55	1.04	0.86	2.83	0.84	0.45
23	caryophyllene oxide	1976	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.47	0.00	0.00	0.00	0.00
24	germacrene-D-4-ol	2027	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.51	0.54	0.52
25	thymol	2165	44.28	37.03	46.01	54.59	44.07	42.71	54.02	36.74	41.22	46.70	49.34	48.18
26	carvacrol	2216	2.68	2.29	1.52	2.02	2.30	1.55	2.96	1.27	1.34	2.56	2.62	2.56
Monoterpene hydrocarbons			46.82	53.08	38.00	35.03	43.50	45.50	35.13	48.29	42.66	39.64	40.26	39.98
Oxygenated monoterpenes			2.19	2.83	2.63	2.71	3.49	1.89	2.59	2.04	3.49	3.83	2.09	2.40
Phenolic monoterpenes			46.96	39.32	47.53	56.61	46.37	44.26	56.98	38.01	42.56	49.26	51.96	50.74
Sesquiterpene hydrocarbons			3.05	3.66	10.26	4.00	5.12	6.92	3.15	9.79	9.40	5.52	3.44	2.84
Oxygenated sesquiterpenes			0.00	0.05	0.00	0.00	0.00	0.34	0.00	0.47	0.29	0.51	0.54	0.52
Others			0.30	0.00	1.51	1.60	1.00	0.81	2.01	0.72	1.45	0.02	1.22	1.36
Total identified			99.32	98.94	99.93	99.95	99.48	99.72	99.86	99.32	99.85	98.78	99.51	97.84
Essential oil content			3.11	2.82	2.93	4.38	2.53	3.20	3.20	3.00	2.10	2.43	4.15	4.10

In the present work, 26 compounds were detected in essential oils, comprising 97.84–99.95% of the total identified fraction. Monoterpenes were present in the highest amounts. This class of compounds was subdivided into three groups: phenolic monoterpenes with the domination of thymol (from 36.74 to 54.59%), monoterpene hydrocarbons (up to 53.08%) where γ -terpinen and *p*-cymen were present in the highest amounts, and oxygenated monoterpenes (up to 3.83%) (Table 5, Figure 3). The fraction of sesquiterpenes was represented by sesquiterpene hydrocarbons (e.g., β -caryophyllene, α -cadinene, β -copaene), and trace amounts of oxygenated sesquiterpenes. Aliphatic alcohol 1-octen-3-ol was classified as the ‘other’ compound (Table 5). The predominance of thymol led to qualifying the examined essential oils as ‘thymol’ chemotype. Here, clones no. 4 and 7 were characterized by the highest amount of this compound (54.59, 54.02%, respectively). Due to its high pharmacological activity, mainly antimicrobial and antioxidant, thymol is considered as the most important constituent of thyme herb [21,22]. It is also responsible for the sensory profile of thyme herb, conditioning its pleasant, herbal and spicy aroma [50]. The fluctuation of thymol content in common thyme essential oil was found to depend on various factors, and the chemotype seems to be one of the most important [4,6–9,51–55].

Figure 3. Chemical structures of compounds dominating in essential oil (1–3) and phenolic fraction (4,5): 1: thymol, 2: γ -terpinen, 3: *p*-cymen, 4: rosmarinic acid, 5: luteolin 7-*O*-glucoside.

In general, the creation of chemotypes is not limited only to terpenes. Such phenomenon covers non-volatile phenolic compounds, as well. Although these two groups of compounds are derived from distinct metabolic pathways, they serve complementary roles in mediating plant interactions with the environment. Here, both terpenes and phenolics play a crucial role in plant physiology, since they are considered to take part in mechanisms of protection, adaptation, competition, signaling, and defense [30,47,48,56]. On the other hand, terpenes and phenolics reveal a wide range of pharmacological activities, which make them one of the most important secondary metabolites. In the present work, investigated common thyme clones were compared as to the total content and composition of non-volatile phenolic compounds, namely phenolic acids and flavonoids. The total content of phenolic acids ranged from 0.59 to 1.65 g $\times$ 100 g^{−1} DW (CV = 0.38) (Table 6). Among this group, three phenolic acids were identified: caffeic, rosmarinic and *p*-coumaric acid. Here, rosmarinic acid appeared to be a clear dominant, and its content ranged from 611.47 to 2675.59 mg $\times$ 100 g^{−1} DW (CV = 0.40). It is worth noting that clones no. 3 and 12 were distinguished by the highest content of this compound. Caffeic and *p*-coumaric acids were present in smaller quantities, ranging from 9.71–37.53 and 0.16–4.13 mg $\times$ 100 g^{−1} DW, respectively (Table 6, Figure 3). It was observed that *p*-coumaric acid differentiated investigated clones at the highest degree (CV = 0.59). The listed phenolic acids represent cinnamic acids derivatives, where caffeic and *p*-coumaric acids are free compounds, while rosmarinic acid (ester of caffeic and 3, 4-dihydroxyphenyl lactic acids) belongs to depsides. The presence of these compounds in common thyme herb were reported earlier by other authors [18,57–59]. Common thyme

herb contains other caffeic and rosmarinic acids derivatives, i.e.: salvianolic acid (trimeric form of caffeic acid), rosmarinic acid glucoside, methyl rosmarinate. Rosmarinic acid is common in plants and is a dominant compound in the *Lamiaceae* species, including the *Thymus* genus, where it is reported to account for about 70% of total polyphenols [18]. However, its content is quite variable: in common thyme it oscillated between 3.4 and 22 mg $\times$ 1 g⁻¹ [18,60]. According to Lukas et al. [61], the content of rosmarinic acid in wild growing populations of common oregano (*Origanum vulgare* L. ssp. *vulgare*) varied from 0.6 to 37.2 mg $\times$ 1 g⁻¹. Such a wide range of content suggests that this compound may be used as a marker of intraspecific variability. Moreover, when taking into account its extremely high antioxidant activity, a considerable amount of rosmarinic acid in plant material appears to be a desirable trait from a pharmacological point of view [62].

Table 6. The total content (g $\times$ 100 g⁻¹ DW) and chemical composition of phenolic acids (mg $\times$ 100 g⁻¹ DW).

Clone	Total Content	Caffeic Acid	Rosmarinic Acid	<i>p</i> -Coumaric Acid
1	1.65 $\pm$ 0.09 a	14.56 $\pm$ 1.27 d	1163.30 $\pm$ 88.33 c	0.50 $\pm$ 0.13 e
2	0.64 $\pm$ 0.03 d	26.82 $\pm$ 1.06 b	611.47 $\pm$ 67.36 e	2.40 $\pm$ 0.25 c
3	1.40 $\pm$ 0.19 ab	9.71 $\pm$ 0.61 e	2110.45 $\pm$ 100.31 ab	4.13 $\pm$ 0.79 a
4	1.13 $\pm$ 0.09 b	25.99 $\pm$ 1.67 b	1809.21 $\pm$ 155.62 b	3.89 $\pm$ 0.33 a
5	0.65 $\pm$ 0.19 d	21.01 $\pm$ 1.17 c	1058.66 $\pm$ 87.49 c	0.59 $\pm$ 0.06 e
6	1.22 $\pm$ 0.24 b	21.58 $\pm$ 0.90 c	1647.90 $\pm$ 138.23 b	3.11 $\pm$ 0.23 b
7	0.75 $\pm$ 0.11 c	36.21 $\pm$ 0.94 a	919.80 $\pm$ 80.99 d	2.37 $\pm$ 0.32 c
8	0.59 $\pm$ 0.10 d	29.61 $\pm$ 1.49 ab	944.28 $\pm$ 50.25 d	2.44 $\pm$ 0.37 c
9	0.66 $\pm$ 0.02 d	12.97 $\pm$ 1.00 d	1568.56 $\pm$ 126.17 bc	0.16 $\pm$ 0.01 f
10	0.71 $\pm$ 0.13 cd	37.53 $\pm$ 1.63 a	1176.99 $\pm$ 79.86 c	1.47 $\pm$ 0.02 d
11	0.86 $\pm$ 0.27 c	22.71 $\pm$ 1.76 c	1565.11 $\pm$ 80.53 bc	2.23 $\pm$ 0.32 c
12	0.79 $\pm$ 0.07 c	15.64 $\pm$ 1.17 d	2675.59 $\pm$ 169.78 a	2.42 $\pm$ 0.15 c
Mean	0.92	22.86	1437.61	2.14
CV	0.38	0.39	0.40	0.59

Means marked with different letters differ at $p < 0.05$.

The investigated common thyme clones differed as to the total content and composition of flavonoids. Total content of these substances ranged from 0.19 to 0.47 g $\times$ 100 g⁻¹ DW (CV = 36). Three compounds were identified within the group, namely luteolin 7-*O*-glucoside, naryngenin, and (–)-epicatechin. Luteolin 7-*O*-glucoside was present in the highest amount (from 46.77 to 325.11 mg $\times$ 100 g⁻¹ DW (CV = 0.50)), followed by naryngenin (from 59.22 to 185.12 mg $\times$ 100 g⁻¹ DW (CV = 0.38)). In turn, content of (–)-epicatechin was lower, and ranged from 32.70 to 78.32 mg $\times$ 100 g⁻¹ DW (CV = 0.26). Among examined clones, clone no. 3 was characterized by the highest content of luteolin 7-*O*-glucoside and (–)-epicatechin (Table 7, Figure 3). The presence of listed flavonoids in common thyme herb were demonstrated earlier [19,59,63,64]. According to literature data, other flavones and methylated flavones have also been noticed in this raw material, e.g., luteolin and its derivatives (luteolin *O*-diglucoside, luteolin acetyl-*O*-glycoside), apigenin, apigenin 7-glucuronide, apigenin 7-*O*-rutinoside, cirsilinol, eriodictyol, thymonin, etc. [16]. In general, flavones are regarded as a health promoting agents. Here, luteolin and its derivatives play a remarkable role in preventing a wide range of chronic diseases. Due to its antioxidant, anti-inflammatory, antitumor and antimicrobial activities, it prevents and reduces incidences of common diseases like: cancer, heart failure, and arteriosclerosis [65–67]. Thus, flavones determine the pharmacological activity of common thyme herb and, due to its physiological and chemical stability, it may be a good chemotaxonomic marker within the species [16].

Table 7. The total content ($\text{g} \times 100 \text{ g}^{-1} \text{ DW}$) and chemical composition of flavonoids ($\text{mg} \times 100 \text{ g}^{-1} \text{ DW}$).

Clone	Total Content	Luteolin 7-O-Glucoside	Naryngenin	(-)-Epicatechin
1	$0.21 \pm 0.05 \text{ c}$	$200.51 \pm 20.59 \text{ b}$	$77.12 \pm 4.32 \text{ c}$	$71.33 \pm 3.17 \text{ ab}$
2	$0.44 \pm 0.01 \text{ a}$	$117.32 \pm 1.66 \text{ d}$	$127.48 \pm 4.53 \text{ ab}$	$49.07 \pm 5.07 \text{ b}$
3	$0.19 \pm 0.00 \text{ c}$	$325.11 \pm 13.30 \text{ a}$	$96.96 \pm 5.63 \text{ bc}$	$78.32 \pm 8.55 \text{ a}$
4	$0.25 \pm 0.02 \text{ bc}$	$157.43 \pm 13.04 \text{ c}$	$175.22 \pm 8.82 \text{ a}$	$46.24 \pm 2.21 \text{ bc}$
5	$0.33 \pm 0.00 \text{ b}$	$155.81 \pm 7.71 \text{ c}$	$167.19 \pm 19.34 \text{ a}$	$42.49 \pm 2.49 \text{ bc}$
6	$0.19 \pm 0.00 \text{ c}$	$46.77 \pm 2.40 \text{ e}$	$59.22 \pm 3.12 \text{ c}$	$37.61 \pm 1.81 \text{ c}$
7	$0.28 \pm 0.02 \text{ bc}$	$156.26 \pm 12.38 \text{ c}$	$68.37 \pm 2.73 \text{ c}$	$54.76 \pm 6.09 \text{ b}$
8	$0.43 \pm 0.01 \text{ a}$	$186.96 \pm 11.19 \text{ b}$	$153.60 \pm 11.49 \text{ ab}$	$32.70 \pm 1.68 \text{ c}$
9	$0.47 \pm 0.00 \text{ a}$	$219.46 \pm 31.39 \text{ b}$	$185.12 \pm 9.53 \text{ a}$	$65.65 \pm 2.68 \text{ ab}$
10	$0.19 \pm 0.01 \text{ c}$	$91.40 \pm 5.41 \text{ de}$	$116.43 \pm 7.92 \text{ b}$	$52.30 \pm 2.38 \text{ b}$
11	$0.34 \pm 0.02 \text{ b}$	$110.45 \pm 9.52 \text{ d}$	$67.76 \pm 1.89 \text{ c}$	$49.92 \pm 6.12 \text{ b}$
12	$0.47 \pm 0.03 \text{ a}$	$60.76 \pm 1.85 \text{ e}$	$118.64 \pm 16.71 \text{ b}$	$47.71 \pm 6.65 \text{ b}$
Mean	0.32	152.35	117.76	52.34
CV	0.36	0.50	0.38	0.26

Means marked with different letters differ at $p < 0.05$.

4. Conclusions

The results presented in this study indicate high level variability in common thyme. The examined clones, being representative of single plants within the cultivar ‘Standard Winter’ appeared to be strongly diverse, both in morphological and chemical traits. Here, even though a quite significant homogeneity was noticed with regard to type of growth habit and height of plants, there was a strong differentiation in the frame of weight of the herb, shape, colour, length and width of leaf blade, as well as density of glandular trichomes, which influenced variability in the essential oil content. The analyzed clones represent ‘thymol’ chemotype, however a relatively high diversity within content of this constituent in the essential oil was noticed. Among phenolic acids and flavonoids, rosmarinic acid, followed by luteolin 7-O-glucoside, were the dominant compounds. However, the highest differences between investigated clones concerned *p*-coumaric acid ($\text{CV} = 0.59$) content. The obtained results indicate that except from association between number of glandular trichomes and essential oil content, there was no clear relationship between other investigated characters, not morphological nor chemical.

Results of our work suggest that ‘Standard Winter’ cultivar is not homogenous in respect of traits important from the industrial point of view. It is worth noting that in practice, besides cultivars, many varieties, landraces, and even populations of common thyme are cultivated by farmers. It can be suspected that in these cases, the level of heterogeneity may be much higher. Such a high range of variability may create problems with raw material standardization. From another side, it opens a huge possibilities for breeders, where single plants/clones may become valuable components for breeding. Moreover, clones distinguished by a desirable traits (e.g., a high content of thymol and/or rosmarinic acid), can be vegetatively multiplied on a smaller scale and directly consumed as a fresh culinary herb.

Author Contributions: Conceptualization, O.K. and Z.W.; methodology of chemical analysis, O.K., J.L.P.; validation, J.L.P.; investigation, O.K., K.B., A.P., K.R., Z.W.; writing—original draft preparation, O.K.; references, K.R.; writing—review and editing, Z.W. and K.B.; supervision, Z.W.; project administration, K.B.; funding acquisition, K.B. All authors have read and agreed to the published version of the manuscript.

Funding: Part of the studies were supported by the Polish Ministry of Agriculture and Rural Development, within the Multiannual Programme “Creating the Scientific Basis of the Biological Progress and Conservation of Plant Genetic Resources as a Source of Innovation to Support Sustainable Agriculture and Food Security of the Country”—task 1.2. Project title: “Managing the collection of genetic resources of medicinal and aromatic plants (including spice crops) with the exception of species protected in Poland”.

Acknowledgments: The technical help of Analytical Centre (WULS-SGGW) is gratefully acknowledged.

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

References

- Jalas, J. Notes on *Thymus* L. (Labiatae) in Europe. Supraspecific classification and nomenclature. *Bot. J. Linn. Soc.* **1971**, *64*, 199–215.
- European Pharmacopoeia*, 7th ed.; European Directorate for the Quality of Medicines and Health Care (EDQM), Council of Europe: Strasbourg, France, 2010.
- Stahl Biskup, E.; Sáez, F. *Thyme. The Genus Thymus*; Medicinal and Aromatic Plants—Industrial Profiles Series; Taylor and Francis: London, UK; New York, NY, USA, 2002.
- Morgan, R.K. Chemotypic characteristics of *Thymus vulgaris* L. in Central Otago, New Zealand. *J. Biogeogr.* **1989**, *16*, 483–491. [[CrossRef](#)]
- Thompson, J.D. Population structure and the spatial dynamic of genetic polymorphism in thyme. In *Thyme. The Genus Thymus*; Medicinal and Aromatic Plants—Industrial Profiles Series; Stahl-Biskup, E., Sáez, F., Eds.; Taylor and Francis: London, UK; New York, NY, USA, 2002; pp. 44–74.
- Thompson, J.D.; Chalchat, J.C.; Michet, A.; Linhart, Y.B.; Ehlers, B. Qualitative and quantitative variation on monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *J. Chem. Ecol.* **2003**, *29*, 859–880. [[CrossRef](#)] [[PubMed](#)]
- Torras, J.; Grau, M.D.; Lopez, J.F.; de las Heras, F.X. Analysis of essential oils from chemotypes of *Thymus vulgaris* in Catalonia. *J. Sci. Food Agric.* **2007**, *87*, 2327–2333. [[CrossRef](#)]
- Chizzola, R.; Michitsch, H.; Franz, C. Antioxidant properties of *Thymus vulgaris* leaves: Comparison of different extracts and essential oil chemotypes. *J. Agric. Food Chem.* **2008**, *56*, 6897–6904. [[CrossRef](#)]
- Saty, P.; Murray, B.L.; Mcfeeters, R.L.; Setzer, W.N. Essential oil characterization of *Thymus vulgaris* from various geographical locations. *Foods* **2016**, *5*, 70. [[CrossRef](#)] [[PubMed](#)]
- Wesołowska, A.; Jadczyk, D. Comparison of the chemical composition of essential oils isolated from two thyme (*Thymus vulgaris* L.) cultivars. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2019**, *47*, 829–835. [[CrossRef](#)]
- Guillen, M.D.; Manzanos, M.J. Study of the composition of different parts of a Spanish *Thymus vulgaris* L. plant. *Food Chem.* **1998**, *63*, 373–383. [[CrossRef](#)]
- Passet, J. *Thymus vulgaris* L. Chémotaxonomie et Biogénèse Monoterpénique. Ph.D. Thesis, Faculté de Pharmacie, Université de Montpellier, Montpellier, France, 1971; p. 153.
- Vernet, P.; Gouyon, P.H.; Valdeyron, G. Genetic control of the oil content in *Thymus vulgaris* L.: A case of polymorphism in a biosynthetic chain. *Genetica* **1986**, *69*, 227–231. [[CrossRef](#)]
- Gouyon, P.H.; Vernet, P.; Guillermin, J.L.; Valdeyron, G. Polymorphisms and environment: The adaptive value of the oil polymorphisms in *Thymus vulgaris* L. *Heredity* **1986**, *57*, 59–66. [[CrossRef](#)]
- Amiot, J.; Salmon, Y.; Collin, C.; Thompson, J.D. Differential resistance to freezing and spatial distribution in a chemically polymorphic plant *Thymus vulgaris* L. *Ecol. Lett.* **2005**, *8*, 370–377. [[CrossRef](#)]
- Vila, R. Flavonoids and further polyphenols in the genus *Thymus*. In *Thyme. The Genus Thymus*; Medicinal and Aromatic Plants—Industrial Profiles Series; Stahl-Biskup, E., Sáez, F., Eds.; Taylor and Francis: London, UK; New York, NY, USA, 2002; pp. 144–177.
- Bazylo, A.; Strzelecka, H. A HPTLC densitometric determination of luteolin in *Thymus vulgaris* and its extracts. *Fitoterapia* **2007**, *78*, 391–395. [[CrossRef](#)] [[PubMed](#)]
- Pereira, O.R.; Cardoso, S.M. Overview on *Mentha* and *Thymus* Polyphenols. *Curr. Anal. Chem.* **2013**, *9*, 382–396. [[CrossRef](#)]
- Alu'datt, M.H.; Rababah, T.; Johargy, A.; Gammoh, S.; Ereifej, K.; Alhamad, M.N.; Brewer, M.S.; Saati, A.A.; Kubow, S.; Rawshdeh, M. Extraction, optimisation and characterisation of phenolics from *Thymus vulgaris* L.: Phenolic content and profiles in relation to antioxidant, antidiabetic and antihypertensive properties. *Int. J. Food Sci. Technol.* **2015**, 1–11. [[CrossRef](#)]
- EMA (European Medicines Agency). *Assessment Report on Thymus vulgaris L., vulgaris zygis L., herba*; EMA/HMPC/342334; EMA: Amsterdam, The Netherlands, 2013.
- Reddy, P.; Kandisa, R.V.; Varsha, P.V.; Satyam, S. Review on *Thymus vulgaris* traditional uses and pharmacological properties. *Med. Aromat. Plants* **2014**, *3*, 2167–0412. [[CrossRef](#)]
- Dauqan, E.M.A.; Abdullah, A. Medicinal and functional values of thyme (*Thymus vulgaris* L.) herb. *JABB* **2017**, *5*, 17–22. [[CrossRef](#)]
- WHO. *Herba Thymi*. In *WHO Monographs of Selected Medicinal Plants*; WHO: Geneva, Switzerland, 1999; pp. 259–266.

24. Lawrence, B.M.; Tucker, A.O. The genus *Thymus* as a source of commercial products. In *Thyme. The Genus Thymus; Medicinal and Aromatic Plants—Industrial Profiles Series*; Stahl-Biskup, E., Sáez, F., Eds.; Taylor and Francis: London, UK; New York, NY, USA, 2002; pp. 252–263.
25. Jackson, S.A.L.; Hay, R.K.M. Characteristics of varieties of thyme (*Thymus vulgaris* L.) for use in the UK: Oil content, composition and related characters. *J. Hortic. Sci.* **1994**, *69*, 275–281. [\[CrossRef\]](#)
26. Rey, C.; Sáez, F. Field culture, in vitro culture and selection of *Thymus*. In *Thyme. The Genus Thymus; Medicinal and Aromatic Plants—Industrial Profiles Series*; Stahl-Biskup, E., Sáez, F., Eds.; Taylor and Francis: London, UK; New York, NY, USA, 2002; pp. 117–197.
27. Carlen, C.; Schaller, M.; Carron, C.A.; Vouillamoz, J.F.; Baroffio, C.A. The new *Thymus vulgaris* L. hybrid cultivar ‘Varico 3’ compared to five established cultivars from Germany, France and Switzerland. *Acta Hortic.* **2010**, *860*, 161–166. [\[CrossRef\]](#)
28. Dušková, E.; Dušek, K.; Smékalová, K.; Nosálková, M.E.S.L. Genetic resources of *Thymus vulgaris* L. and *T. vulgaris* x *T. Marschallianus* Willd. in the Czech Republic. In Proceedings of the 6th International Symposium Breeding Research on Medicinal and Aromatic Plants, BREEDMAP 6, Quedlinburg, Germany, 19–23 June 2016; pp. 56–58. [\[CrossRef\]](#)
29. Carlen, C. Breeding and cultivation of medicinal plants. In *Herbal Medicines for Human Health*; Bagetta, G., Cosentino, M., Corasaniti, M.T., Sakurada, S., Eds.; Taylor and Francis: London, UK, 2011; pp. 79–91.
30. Başer, K.H.C.; Bouchbauer, G. *Handbook of Essential Oils: Science, Technology and Applications*; Chemical Rubber Company Press: London, UK, 2009.
31. De Chandra, L. Breeding of medicinal and aromatic plants—An overview. *IJBR* **2017**, *7*, 25–34.
32. Shmeit, H.; Fernández, Y.; Novy, E.; Kloucek, P.; Orosz, P.; Kokoska, L. Autopolyploidy effect on morphological variation and essential oil in *Thymus vulgaris* L. *Sci. Hortic.* **2019**. [\[CrossRef\]](#)
33. Mewes, S.; Krüger, H.; Pank, F. Physiological, morphological, chemical and genomic diversities of different origins of thyme (*Thymus vulgaris* L.). *Genet. Resour. Crop Evol.* **2008**, *55*, 1303–1311. [\[CrossRef\]](#)
34. Dalir, M.; Safarnejad, A. Morphological, molecular and phytochemical variation in some thyme genotypes. *JMBP* **2017**, *1*, 41–52.
35. Marzec, M.; Polakowska, C.; Chilczuk, R.; Kołodziej, B. Evaluation of essential oil content, its chemical composition and price of thyme (*Thymus vulgaris* L.) raw material available in Poland. *Herba Pol.* **2010**, *56*, 37–52.
36. Flannery, H. A study of the taxa of *Thymus* cultivated in the United States. Ph.D. Thesis, Cornell University, Ithaca, NY, USA, 1982.
37. Rumińska, A. *Rośliny Lecznicze. Podstawy Biologii i Agrotechniki*; Państwowe Wydawnictwo Naukowe: Warsaw, Poland, 1983.
38. Melchior, H.; Kastner, H. *Przyprawy. Badania Botaniczne i Chemiczne*; Wydawnictwa Naukowo-Techniczne: Warsaw, Poland, 1978.
39. ECP/GR. Draft Descriptor List *Thymus vulgaris* L. 2011. Available online: http://www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/NW_and_WG_UPLOADS/MAP_Descriptors/Thymus_vulgaris_DRAFT_DESCRIPTOR_LIST_FINAL.pdf (accessed on November 2011).
40. Bączek, K.; Kosakowska, O.; Przybył, J.L.; Kuźma, P.; Ejdyś, M.; Obiedziński, M.; Węglarz, Z. Intraspecific variability of yarrow (*Achillea millefolium* L. s.l.) in respect of developmental and chemical traits. *Herba Pol.* **2015**, *61*, 37–52. [\[CrossRef\]](#)
41. *Polish Pharmacopoeia*, 6th ed.; Office of Registration of Medicinal Products, Medical Devices and Biocidal Products. Polish Pharmaceutical Society: Warsaw, Poland, 2002.
42. Kosakowska, O.; Bączek, K.; Przybył, J.L.; Ejdyś, M.; Kuźma, P.; Obiedziński, M.; Węglarz, Z. Intraspecific variability in the content of phenolic compounds, essential oil and mucilage of small-leaved lime (*Tilia cordata* Mill.) from Poland. *Ind. Crops Prod.* **2015**, *78*, 58–65. [\[CrossRef\]](#)
43. ChromaDex. Tech Tip 0003—Recovery & Dilution Procedures. Available online: https://www.chromadex.com/media/2126/techtip0003-recoverydilutionprocedures_nl_pw.pdf (accessed on 26 June 2018).
44. Stahl Biskup, E. Essential oil chemistry of the genus *Thymus*—A global view. In *Thyme. The Genus Thymus; Medicinal and Aromatic Plants—Industrial Profiles Series*; Stahl-Biskup, E., Sáez, F., Eds.; Taylor and Francis: London, UK; New York, NY, USA, 2002; pp. 75–125.
45. Hazzoumi, Z.; Moustakime, Y.; Joutei, K.A. Essential oil and glandular hairs: Diversity and roles. In *Essential Oils—Oils of Nature*; InTech Open: London, UK, 2019. [\[CrossRef\]](#)

46. Svidenko, L.; Grygorieva, O.; Vergun, O.; Hudz, N.; Horčinová, E.; Sedláčková, V.; Šimková, J.; Brindza, J. Characteristic of leaf peltate glandular trichomes and their variability of some Lamiaceae family species. *Agrobiodivers. Improv. Nutr. Health Life Qual.* **2018**. [\[CrossRef\]](#)
47. Rohloff, J. Essential oil drug—Sterpene composition of aromatic herbs. In *Production Practices and Quality Assessment of Food Crops Quality Handling and Evaluation*; Dris, R., Jain, S.M., Eds.; Kluwer Academic Publishers: Alphen aan den Rijn, Netherlands, 2004; pp. 73–128.
48. Figueiredo, A.C.; Barroso, J.G.; José, G.; Pedro, L.G.; Scheffer, J.J.C. Factors affecting secondary metabolite production in plants: Volatile components and essential oils. *Flavour Fragr. J.* **2008**, *23*, 213–226. [\[CrossRef\]](#)
49. Verma, N.; Shukla, S. Impact of various factors responsible for fluctuation in plant secondary metabolites. *JARMAP* **2015**, *2*, 105–113. [\[CrossRef\]](#)
50. Clark, G.S. An aroma chemical profile. *Thymol. Perfum. Flavorist* **1995**, *20*, 41–44.
51. Raal, A.; Arak, E.; Orav, A. Comparative chemical composition of the essential oil of *Thymus vulgaris* L. from different geographical sources. *Herba Pol.* **2005**, *51*, 10–17.
52. Jordán, M.J.; Martínez, R.M.; Goodner, K.L.; Baldwin, E.A.; Sotomayor, J.A. Seasonal variation of *Thymus hyemalis* Lange and Spanish *Thymus vulgaris* L. essential oils composition. *Ind. Crops Prod.* **2006**, *24*, 253–263. [\[CrossRef\]](#)
53. Hudaib, M.; Aburjai, T. Volatile components of *Thymus vulgaris* L., from wild growing and cultivated plants in Jordan. *Flavour Fragr. J.* **2007**, *22*, 322–327. [\[CrossRef\]](#)
54. Golparvar, A.R.; Hadipanah, A.; Salehi, S. Comparative effect of harvest time on essential oil and thymol content of (*Thymus vulgaris* L.) and (*Thymus daenensis* Celak) in Iran Province. *Electron. J. Biol.* **2014**, *10*, 85–92.
55. Juárez-Rosete, C.R.; Aguilar-Castillo, J.A.; Rodríguez-Medoza, M.N. Fertilizer source in biomass production and quality of essential oils of thyme (*Thymus vulgaris* L.). *EJMP* **2014**, *4*, 865–871. [\[CrossRef\]](#)
56. Andersen, R.M.; Markham, K.R. *Flavonoids: Chemistry, Biochemistry, and Applications*; Taylor and Francis, CRC Press: Boca Raton, FL, USA, 2006; ISBN 0-8493-2021-6.
57. Zgórk, G.; Głowniak, K. Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. *J. Pharm. Biomed. Anal.* **2001**, *26*, 79–87. [\[CrossRef\]](#)
58. Wang, H.; Provan, G.J.; Helliwell, K. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. *Food Chem.* **2004**, *87*, 307–311. [\[CrossRef\]](#)
59. Hossain, M.B.; Rai, D.K.; Brunton, N.P.; Martin-Diana, A.B.; Barry-Ryan, C. Characterization of phenolic composition in Lamiaceae spices by LC-ESI-MS/MS. *J. Agric. Food Chem.* **2010**, *58*, 10576–10581. [\[CrossRef\]](#)
60. Petersen, M.; Simmons, M.J. Rosmarinic acid. *Phytochemistry* **2003**, *62*, 121–125. [\[CrossRef\]](#)
61. Lukas, B.; Schmiderer, C.; Novak, J. Phytochemical diversity of *Origanum vulgare* L. subsp. *vulgare* (Lamiaceae) from Austria. *Biochem. Syst. Ecol.* **2013**, *50*, 106–113. [\[CrossRef\]](#)
62. Adomako-Bonsu, A.G.; Chan, S.L.F.; Pratten, M.; Fry, J.R. Antioxidant activity of rosmarinic acid and its principal metabolites in chemical and cellular systems: Importance of physico-chemical characteristics. *Toxicol. In Vitro* **2017**, *40*, 248–255. [\[CrossRef\]](#)
63. Kulisic, T.; Dragovic-Uzelac, V.; Milos, M. Antioxidant activity of aqueous tea infusions prepared from oregano, thyme and wild thyme. *Food Technol. Biotechnol.* **2006**, *44*, 485–492.
64. Fecka, I.; Turek, S. Determination of polyphenolic compounds in commercial herbal drugs and spices from Lamiaceae: Thyme, wild thyme and sweet marjoram by chromatographic techniques. *Food Chem.* **2008**, *108*, 1039–1053. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Lopez-Lazaro, M. Distribution and biological activities of the flavonoid luteolin. *Mini Rev. Med. Chem.* **2009**, *9*, 31–59. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Luo, Y.; Shang, P.; Li, D. Luteolin: A flavonoid that has multiple cardio-protective effects and its molecular mechanisms. *Front. Pharmacol.* **2017**. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Aziz, N.; Kim, M.J.; Cho, Y.J. Antiinflammatory effect of luteolin: A review of in vitro, in vivo and in silico studies. *J. Ethnopharmacol.* **2018**, *225*, 342–588. [\[CrossRef\]](#)