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Essential Oil Composition of Ten Species from Sect. *Serpyllum* of Genus *Thymus* Growing in Bulgaria

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Abstract: GC-MS/FID analysis of the essential oils of 10 *Thymus* species, belonging to Sect. *Serpyllum*, led to identification of 118 compounds accounting for 97.79–99.69% of the total oil. *Thymus moesiacus*, *T. jankae*, *T. vandasii*, *T. longicaulis* and *T. sibthorpii* were characterized by the presence of linalool (19.37–35.21%) as the major or dominant component, but differed significantly in the content of the other prominent components: linalyl acetate, geraniol, geranyl acetate, α-terpinyl acetate, myrcen-8-yl acetate, myrcen-8-ol, etc. α-Terpinyl acetate (66.79%), thymol (63.96%), carvacrol (42.65%) and germacrene D (42.15%) were the principal components of *T. pulegioides*, *T. glabrescens*, *T. callieri* and *T. pannonicus*, respectively. β-Myrcene (16.53%), *cis*-sabinene hydrate (13.58%), τ-cadinol (13.24%) and elemol (11.29%) determined the oil from *T. thracicus* as a mixed mono-/sesquiterpene chemotype. The obtained results revealed the existence of new chemotypes of *T. moesiacus*, *T. thracicus*, *T. sibthorpii* and *T. longicaulis*. The essential oil content of *T. callieri* and endemic *T. vandasii* is reported for the first time. The variations in the essential oils of different *Thymus* species from Sect. *Serpyllum* were examined by principal component analysis (PCA) and cluster analysis (CA).

Keywords: *Thymus* species; section *Serpyllum*; essential oil composition; GC-FID/MSD; chemotypes; PCA and CA

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1. Introduction

Essential oils are aromatic oily liquids derived from different plant parts (flowers, buds, seeds, leaves, twigs, bark) and represent complex mixtures of volatile constituents, including monoterpenes, sesquiterpenes and their oxygenated derivatives. They play a significant role in plant resistance against pests, herbivores, fungi and bacteria [1]. Essential oils have various applications in health, agriculture, cosmetic and food industries. The use of essential oils in traditional medicine has been practiced since ancient times in human history due to their wide range of biological properties such as antimicrobial, antiviral, antimutagenic, anticancer, antioxidant, anti-inflammatory, immunomodulatory and antiprotozoal activities [2]. Plants producing essential oils belong to various genera distributed to around 60 families, among which are the species of the genus *Thymus* (Lamiaceae).

Genus *Thymus* includes about 220 species distributed almost everywhere in Eurasia, as well as southern Greenland and Africa. The taxonomy of this genus is still challenging due to high population variability and chemical polymorphism [3]. Bulgarian flora is represented by 21 species belonging to two sections—*Hyphodromi* and *Serpyllum* [4]. Of them, six species are endemic to the Balkan Peninsula.

Recently, we have started a detailed study of the volatile compounds of *Thymus* species growing in Bulgaria. The investigation of five species belonging to sect. *Hyphodromi* has shown a considerable variation in the chemical composition of the species and the existence of new chemotypes [5]. Continuing our study, we focused on 10 species belonging to sect. *Serpyllum*. *Thymus jankae* Čelak., *T. sibthorpii* Benth., *T. thracicus* Velen. and *T. vandasii* Velen. are characterized by relatively limited distribution on the Balkan Peninsula and the Asian part of Turkey; *T. callieri* Borbás ex Velen. is spread out from the Balkans to Ukraine and Transcaucasia (Azerbaijan, Armenia and Georgia); while *T. pannonicus* All., *T. longicaulis* C. Presl, *T. moesiacus* Velen., *T. pulegioides* L. and *T. glabrescens* Willd. (or Benth., nom. Illeg) are widely distributed in Europe [6]. The literature survey on the volatile components of these species, summarized in Table S1 (Supplementary Material) [7–73], revealed the existence of chemotypes of *T. moesiacus*, *T. sibthorpii*, *T. jankae* and *T. thracicus* and a high level of polymorphism with more than 10 chemotypes of *T. pannonicus*, *T. glabrescens*, *T. longicaulis* and *T. pulegioides*. It has been reported that different genetic and environmental factors may also influence the chemical composition of *Thymus* essential oils [3,74]. To our knowledge, the volatile constituents of *T. vandasii* and *T. callieri* have not been investigated yet. There are no data on the essential oil composition of the Bulgarian populations of these species except for two reports for *T. moesiacus* [39] and *T. jankae* [11]. Thus, the aim of this study was to characterize their volatile components, to get additional insights about their relationship in Sect. *Serpyllum* and to compare the results with the essential oil composition of these species from other origins reported so far in the literature.

2. Materials and Methods

2.1. Plant Material

Plant material was collected in July 2019 in the full flowering stage from the native populations in Bulgaria and air dried in the shade at room temperature. Voucher specimens have been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences. The collection site and voucher specimens are given in Table 1.

Table 1. Collection site, voucher specimen and essential oil yield of *Thymus* species.

Species	Code	Collection Site	GPS Coordinates	Voucher Specimen	Yield [%, *w*/*w*]
T. vandasii	**TV**	Seven Rila lakes, Rila Mts.	42°13′11.32″/ 23°19′11.52″	SOM 1433	0.98
T. pannonicus	**TPa**	Eastern Rhodopes Mts., road between Asenovgrad and Kardzhali	41°39′36.08″/ 25°20′30.32″	SOM 1429	0.39
T. longicaulis	**TL**	Western Rhodopes Mts.	42° 3′39.50″/ 23°49′51.89″	SOM 1427	2.23
T. moesiacus	**TM**	Znepol region	42°27′29.68″/ 22°38′16.66″	SOM 1428	1.81
T. callieri	**TC**	Struma River Valley	42° 0′11.58″/ 23° 7′56.80″	SOM 1424	2.11
T. pulegioides	**TPu**	Vlahina Mts.	41°59′18.88″/ 22°55′39.02″	SOM 1430	1.18
T. glabrescens	**TG**	North-Eastern Bulgaria, near Dobrich	43°31′10.37″/ 27°45′16.11″	SOM 1425	1.69
T. jankae	**TJ**	Seven Rila lakes, Rila Mts.	42°13′11.32″/ 23°19′11.52″	SOM 1426	0.97
T. sibthorpii	**TS**	Danube plain	43°22′17.64″/ 24°39′41.95″	SOM 1431	0.83
T. thracicus	**TT**	Pirin Mts., Orelek peak	41°32′51.80″/ 23°37′19.28″	SOM 1432	0.09

2.2. Isolation of the Essential Oils

An amount of 5 grams of each sample were subjected to a micro hydrodistillation extraction in a Likens–Nickerson apparatus for 2.5 h using diethyl ether as a solvent [75]. The essential oil dissolved in diethyl ether was dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under N_2 flow, and the essential oil was stored at 4 °C before analysis. The yield of essential oil from the aerial parts of *Thymus* species is given in Table 1.

2.3. Gas Chromatography–Flame Ionization Detection/Mass Spectrometry (GC-FID/MSD)

Analyses were carried out with an Agilent 7890B gas chromatograph equipped with flame ionization detector and mass selective detector Agilent 5977A. HP-5MS capillary column (5%-phenyl)-methylpolysiloxane, 30 m × 0.25 mm; 0.25 µm film thickness) was used with a helium carrier gas at 1.2 mL/min. GC's oven temperature was kept at 60 °C for 10 min and programmed to 200 °C at a rate of 3 °C/min, and then held at 200 °C for 10 min. The oils were analyzed with a split ratio of 10:1. The injector temperature was 270 °C. Temperatures of the MSD and the source were 230 and 150 °C, respectively. Mass spectra were taken at 70 eV, and the mass range was from *m/z* 35 to 450. Relative percentage amounts of the compounds were calculated from FID chromatograms.

2.4. Identification of Compounds

Relative retention indices (RRI) of the oil components were calculated using retention times of C_8-C_{30} n-alkanes under the same chromatographic conditions. The individual components were identified by their MS, and RRI referring to known compounds from the literature [76,77], and also, by comparison with those of NIST 14 Library and home-made MS databases.

2.5. Statistical Analysis

Principal component analysis (PCA) and cluster analysis (CA) were performed using the PAST 4.0 software to determine the chemical variation and relationship between the species.

3. Results and Discussion

GC-MS/FID analysis of *T. vandasii* (**TV**), *T. pannonicus* (**TPa**), *T. longicaulis* (**TL**), *T. moesiacus* (**TM**), *T. callieri* (**TC**), *T. pulegioides* (**TPu**), *T. glabrescens* (**TG**), *T. jankae* (**TJ**), *T. sibthorpii* (**TS**) and *T. thracicus* (**TT**) essential oils led to the identification of 118 compounds accounting for 97.79–99.69% of the total oil. Of them, 115 compounds were unambiguously identified, and 3 were determined as a sesquiterpene hydrocarbon and 2 sesquiterpene alcohols based on their mass-spectral fragmentation (Table 2). The volatile compounds identified in the oils belong to six classes, namely monoterpene hydrocarbons (MH), oxygenated monoterpenes (MO), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (SO), aromatic compounds (AR) and others. It has been found that studied samples afforded specific chemical profiles with high variation in the type of compounds: MH (0.28–22.70%), MO (3.66–86.78%), SH (5.06–74.20%), SO (0.37–30.64%), AR (0.11–78.17%) and others (0.26–5.07%) (Figure 1). The essential oils differed significantly in the content of the individual components, too.

In the essential oil from *T. moesiacus*, 36 individual components were detected (Table 2). This oil was characterized by the highest amounts of O-containing monoterpenes (MO) −86.78%. The main MO in the oil were linalool, geraniol and geranyl acetate (35.21, 32.89 and 15.47%, respectively). Sesquiterpene hydrocarbons (SH) accounted 10.90% of the total oil, while all other types of compounds did not exceed 1%. Geraniol and geranyl acetate were found as the main components in two *T. moesiacus* essential oils from N. Macedonia [40], and linalool was the major component in another N. Macedonian sample [40] (Table S1). However, these samples were also rich in carvacrol (12.3–13.3%), while this component was detected in our sample only in traces. The study of essential oils of the species from Kosovo also showed the presence of linalool and geraniol in amounts between 6.9 and 13.8% along with carvacrol and thymol [8]. Linalool and thymol were the major components in *T. moesiacus* from Bosnia [39], while linalyl acetate (52.4%) was the most abundant component of *T. moesiacus* from another Bulgarian population [39]. Therefore, this essential oil could be characterized as a new linalool/geraniol/geranyl acetate chemotype.

Table 2. Essential oil composition (%) from the aerial parts of *Thymus* species.

RRI *	RRI **	Compound	TV #	TJ #	TL #	TM #	TT #	TC #	TG #	TS #	TPu #	TPa #*
923	925	α-Thujene	-	-	0.06	0.01	0.24	0.25	0.34	0.12	0.04	0.11
929	929	α-Pinene	0.04	0.47	0.06	0.08	2.63	0.24	0.23	0.25	0.07	0.67
944	946	Camphene	0.05	0.05	0.09	0.19	0.15	0.37	0.09	0.27	0.10	1.14
972	970	Sabinene	0.01	-	0.09	-	0.38	0.01	-	0.06	0.37	0.09
973	973	β-Pinene	0.01	-	-	-	1.36	0.11	0.11	0.23	0.20	0.18
978	980	1-Octen-3-ol	0.23	0.45	0.15	0.15	1.04	0.51	0.90	0.86	0.11	1.63
985	986	3-Octanone	-	-	0.04	-	-	0.13	0.16	0.18	0.32	0.16
991	991	β-Myrcene	1.09	1.50	0.38	-	**16.53**	0.65	0.84	1.99	0.12	0.27
995	994	3-Octanol	-	-	0.09	0.12	0.20	0.21	0.09	0.22	-	3.07
1015	1017	α-Terpinene	-	-	0.11	-	0.23	1.35	0.99	0.21	0.17	0.15
1023	1022	p-Cymene	0.02	-	0.76	-	0.15	7.17	7.16	3.40	1.52	0.36
1028	1030	Limonene	0.39	1.42	0.48	-	0.36	0.31	0.30	0.64	1.39	3.09
1030	1032	Eucalyptol	-	0.31	-	0.16	-	0.77	0.91	0.73	-	0.79
1037	1037	*cis*-β-Ocimene	0.26	0.22	0.15	-	-	-	-	-	-	-
1042	1045	Benzene acetaldehyde	0.05	-	-	-	0.14	-	-	-	-	0.30
1049	1049	*trans*-β-Ocimene	0.85	0.51	0.18	-	-	-	-	-	-	-
1056	1054	γ-Terpinene	0.05	0.07	0.69	-	0.82	12.04	4.48	0.65	1.86	0.91
1065	1065	*cis*-Sabinene hydrate	0.06	0.11	0.08	-	**13.58**	1.14	1.04	0.45	0.06	0.19
1071	1070	*cis*-Linalool oxide furanoid	0.10	0.26	-	0.20	-	-	-	0.29	-	-
1081	1080	1-Nonen-3-ol	-	-	-	-	-	0.14	0.04	0.14	-	0.22
1085	1080	3-Nonanone	-	-	-	-	0.17	-	-	-	-	-
1087	1086	*trans*-Linalool oxide furanoid	-	-	-	-	-	-	-	0.33	-	-
1087	1088	Terpinolene	0.15	0.31	0.16	-	-	-	0.11	-	0.24	0.07
1096	1098	*trans*-Sabinene hydrate	-	-	-	-	1.01	0.30	0.22	-	0.09	0.12
1099	1099	Linalool	**19.37**	**29.29**	**13.37**	**35.21**	-	0.12	0.36	**19.72**	0.12	0.09
1100	1100	Undecane	-	-	-	-	0.34	-	-	-	-	-
1102	1102	Nonanal	-	-	-	-	0.29	-	-	-	-	-
1116	1111	*cis*-2-*p*-Menthen-1-ol	-	-	-	-	0.18	-	-	-	-	-
1125	1123	3-Octanol acetate	0.02	-	0.04	-	-	-	0.08	-	0.24	-
1138	1140	*trans*-2-*p*-Menthen-1-ol	-	-	-	-	0.11	-	-	-	-	-
1141	1145	Camphor	0.04	0.27	0.04	-	0.30	-	-	-	-	3.16
1143	1142	*trans*-Verbenol	-	0.10	-	-	0.12	-	-	-	-	-
1148	1145	Myrcenone	-	0.18	-	-	-	-	-	-	-	-
1163	1167	Borneol	0.10	0.18	0.40	1.29	-	4.59	0.68	1.01	0.47	1.37
1165	1167	*p*-Mentha-1,5-dien-8-ol	0.03	-	-	-	-	-	-	-	-	-
1175	1177	Terpinen-4-ol	0.11	0.13	0.12	0.06	2.30	0.45	0.37	0.26	0.13	0.30
1183	1183	*p*-Cymen-8-ol	-	-	-	-	-	-	-	0.14	-	-
1188	1189	α-Terpineol	2.76	3.27	1.47	0.06	0.44	0.14	0.07	0.13	2.30	0.18
1217	1219	Coumaran	-	-	-	-	0.26	-	-	-	-	-
1220	1221	*cis*-Sabinene hydrate acetate	-	-	-	-	-	-	-	0.43	-	-
1226	1228	Citronellol	-	-	-	-	0.49	-	-	-	-	-
1230	1226	Myrcen-8-ol	-	-	-	-	-	-	-	**14.71**	-	-
1230	1230	Nerol	0.55	1.16	-	0.90	-	-	-	-	0.25	-
1232	1235	Thymol methyl ether	-	-	0.89	0.23	-	0.18	1.12	0.97	0.33	0.13
1242	1240	Neral	0.07	1.04	-	0.42	1.14	-	-	-	-	-
1243	1244	Carvacrol methyl ether	-	-	0.67	0.04	0.34	3.93	4.34	0.79	0.15	0.01
1252	1250	Thymoquinone	-	-	-	-	-	0.16	0.67	0.25	-	-
1254	1255	Geraniol	0.05	-	**25.28**	**32.89**	0.20	-	-	2.75	7.27	-
1256	1257	Linalyl acetate	**45.84**	**20.58**	**12.72**	-	-	-	-	-	-	-
1273	1270	Geranial	0.22	1.75	-	-	1.63	-	-	-	-	-
1284	1285	Bornyl acetate	0.05	-	0.27	0.06	-	-	-	-	0.43	0.09
1292	1291	Thymol	0.03	1.22	8.27	0.18	-	**13.38**	**63.96**	10.67	6.70	0.26
1300	1299	Carvacrol	0.01	0.31	0.04	0.07	-	**42.65**	0.41	0.19	0.22	0.08
1303	1305	6-Ethyl-3,4-dimethylphenol	-	-	-	-	-	-	-	0.31	-	-
1309	1309	*p*-Vinylguaiacol	-	-	-	-	0.31	-	-	-	-	-
1335	1338	δ-Elemene	0.23	0.23	0.08	0.08	-	-	0.09	-	-	0.13
1349	1351	α-Cubebene	-	-	-	-	-	0.01	-	-	-	0.30
1349	1350	α-Terpinyl acetate	0.05	4.22	**17.46**	0.06	-	-	-	-	**66.79**	-
1351	1348	Myrcen-8-yl acetate-	-	-	-	-	-	-	-	**22.03**	-	-
1355	1357	Eugenol	-	-	-	-	-	-	0.05	-	-	0.09
1365	1364	Neryl acetate	0.85	1.41	0.31	-	-	-	-	-	-	-
1371	1372	Ylangene	-	-	-	-	-	0.01	0.06	-	-	0.13
1373	1376	α-Copaene	-	-	-	-	-	0.01	0.11	0.07	-	0.49
1382	1384	β-Bourbonene	-	-	-	0.50	0.08	0.16	0.08	0.24	-	0.82
1385	1382	Geranyl acetate	1.58	1.70	6.64	**15.47**	0.10	-	-	0.96	0.48	-
1389	1389	β-Cubebene	-	-	-	-	-	-	-	-	-	0.67
1390	1391	β-Elemene	0.10	0.08	-	-	0.38	-	-	-	-	1.82
1407	1409	α-Gurjunene	0.06	0.23	-	-	-	-	-	-	-	-
1419	1419	β-Caryophyllene	4.51	3.47	2.77	4.44	6.49	3.97	1.74	3.25	2.48	**12.28**
1426	1432	β-Copaene	-	-	0.03	0.12	-	-	0.12	0.13	-	2.25
1436	1440	Aromandendrene	-	-	-	-	-	-	0.26	-	-	-
1442	1442	2-Hydroxy-5-methoxyacetophenone	-	-	-	-	-	0.17	0.16	-	-	-
1441	1440	Isogermacrene D	-	-	0.05	0.08	-	-	-	-	-	-
1444	1440	Cadina-3,5-diene	-	0.22	-	-	0.90	-	-	-	-	-
1451	1454	Humulene	0.27	0.27	0.21	0.28	3.80	0.28	-	0.53	0.13	-
1454	1454	Sesquisabinene	-	-	-	-	-	-	-	-	-	2.52
1457	1457	*trans*-β-Famesene	-	0.11	0.06	-	-	-	-	-	-	0.92
1458	1461	Alloaromadendrene	-	0.52	-	-	-	-	0.15	-	-	-
1461	1463	*cis*-Muurola-4(15),5-diene	-	0.40	-	-	1.30	-	-	-	-	-
1460	1464	9-*epi*-β-Caryophyllene	0.48	-	-	-	-	-	-	-	-	-

Table 2. *Cont.*

RRI *	RRI **	Compound	TV #	TJ #	TL #	TM #	TT #	TC #	TG #	TS #	TPu #	TPa #*
1475	1477	γ-Muurolene	-	-	-	-	-	0.52	0.47	0.19	0.39	-
1481	1481	Germacrene D	0.22	0.24	0.51	2.18	0.89	0.26	-	1.07	-	**42.15**
1494	1493	Epicubebol	-	-	-	-	-	0.28	-	-	-	0.91
1493	1492	Valencene	-	-	-	-	-	-	0.49	-	-	-
1494	1495	Bicyclogermacrene	0.58	0.62	0.17	0.24	0.16	-	0.13	0.28	0.10	-
1498	1500	α-Muurolene	0.24	0.19	-	-	-	0.14	0.06	0.08	-	1.01
1507	1509	β-Bisabolene	-	0.38	2.80	2.81	-	0.78	2.25	3.42	1.88	6.15
1517	1517	6-*epi*-Shyobunol	2.66	1.91	-	-	-	-	-	-	-	-
1513	1513	γ-Cadinene	-	2.26	-	-	5.52	0.42	0.45	-	-	0.80
1518	1520	*trans*-Calamenene	-	-	-	-	0.38	-	-	-	-	-
1521	1525	Dihydroactinidiolide	-	-	-	-	0.14	-	-	-	-	-
1522	1522	β-Cadinene	-	2.03	0.12	0.12	0.20	0.42	0.75	0.44	0.08	1.65
1526		$C_{15}H_{24}$ (MW 204)	2.19	-	-	-	-	-	-	-	-	-
1540	1538	*trans*-α-Bisabolene	-	-	0.35	0.06	-	-	0.29	-	-	0.10
1550	1549	Elemol	4.92	2.01	-	-	**11.29**	-	-	0.07	-	0.20
1554	1561	Thymohydroquinone	-	-	-	-	-	0.49	0.30	-	-	-
1561	1562	Geranyl butyrate	-	-	0.15	-	-	-	-	-	0.11	-
1578	1576	Spathulenol	0.20	-	0.07	0.09	-	-	0.28	0.23	-	-
1574	1574	Germacrene D-4-ol	0.62	-	-	-	0.37	-	-	-	-	0.21
1582	1581	Caryophyllene oxide	0.39	-	0.19	0.61	0.41	0.47	0.30	0.61	0.29	0.21
1608	1604	Geranyl isovalerate	0.12	-	0.12	-	-	-	-	-	0.08	-
1592	1595	Salvial-4(14)-en-1-one	-	-	-	-	-	-	-	-	-	0.11
1607		$C_{15}H_{24}O$ (MW 220)	-	-	-	-	-	-	-	0.20	0.11	0.09
1616	1617	Junenol	-	-	-	-	-	-	-	-	-	0.09
1612	1614	Di-*epi*-1,10-cubenol	-	0.72	-	-	1.78	-	-	-	-	0.11
1630	1627	*epi*-Cubenol	-	-	-	-	-	-	-	-	-	0.11
1630	1631	γ-Eudesmol	0.17	-	-	-	0.44	-	-	-	-	-
1634	1637	Caryophylladienol-II	-	-	0.03	0.06	-	-	-	0.09	0.09	0.07
1640	1640	τ-Cadinol	-	6.07	-	-	**13.24**	-	-	0.28	-	0.55
1643	1642	τ-Muurolol	0.22	-	-	-	-	-	-	0.10	-	-
1645	1645	δ-Cadinol	0.04	-	-	-	-	-	-	-	-	0.20
1649	1649	β-Eudesmol	0.17	0.07	-	-	0.50	-	-	-	-	0.08
1652	1653	α-Eudesmol	0.20	-	-	-	-	-	-	-	-	0.98
1653	1653	α-Cadinol	0.34	0.84	-	-	1.01	-	0.30	-	-	-
1663		$C_{15}H_{26}O$ (MW 222)	-	-	-	-	1.25	-	-	-	-	-
1684	1680	*ent*-Germacra-4(15),5,10(14)-trien-1β-ol	-	-	0.09	0.01	-	-	0.10	0.30	-	1.03
1686	1684	α-Bisabolol	-	0.19	-	-	0.36	-	-	-	-	-
1689	1689	Shyobunol	3.78	2.76	-	-	-	-	-	0.10	-	0.05
		Total	**97.79**	**98.32**	**99.36**	**99.53**	**98.41**	**99.69**	**99.07**	**98.02**	**98.31**	**98.48**
		Number of identified compounds	**56**	**51**	**49**	**36**	**54**	**42**	**50**	**54**	**40**	**63**

* Relative retention index (RRI) determined relative to a homologous series of *n*-alkanes (C_8-C_{25}) on HP-5MS column; ** Relative retention index from the literature; # For abbreviation see Table 1.

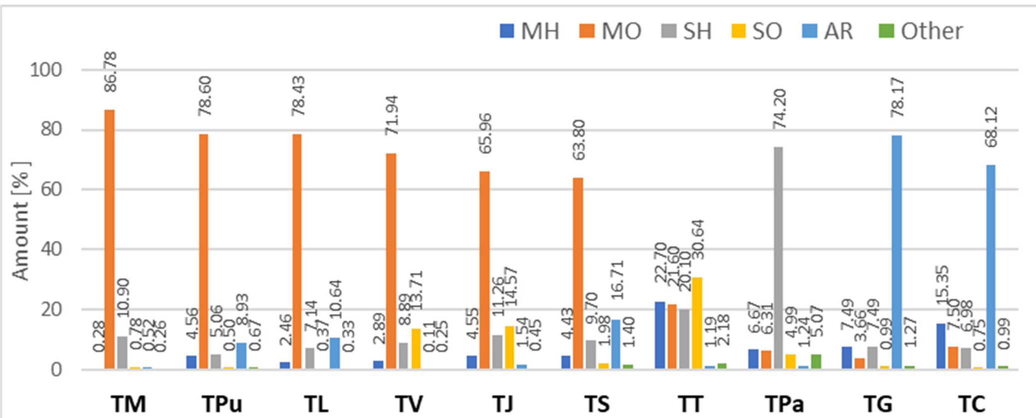

Figure 1. Main classes of compounds detected in *Thymus* essential oils (for abbreviation see Table 1).

The essential oil from *T. vandasii* contained 56 components (Table 2), and MO were the major class, followed by SO and SH. Linalool and linalyl acetate were the principal compounds (Figure 1). The content of linalyl acetate was twice higher than that of linalool (45.84% vs. 19.37%). β-Caryophyllene (4.51%) and elemol (4.92%) were the main sesquiterpenoids. The essential oil from *T. vandasii* was poor in MH (2.89%) and AR (0.11%). Therefore, *T. vandasii* belongs to linalyl acetate/linalool chemotype. This is the first report on the essential oil composition of *T. vandasii.* The results correlated with those found for the alpine populations of *Thymus praecox* Opiz ssp. *polytrichus* (Kern. ex Borb.) Ronn., emend. Jalas, a synonym of *T. vandasii* [6]. The study of the essential oils from 141 individual plants of *Thymus praecox* Opiz ssp. *polytrichus* revealed a high polymorphism with 12 different oil types, among which the most abundant chemotype was linalool/linalyl acetate type [78].

Oxygenated monoterpenes (MO) were also dominant in *T. jankae* essential oil, followed by SO and SH. The essential oil was poor in MH (4.55%) and AR (1.54%) (Figure 1). Among detected 51 components (Table 2), linalool (29.29%) and linalyl acetate (20.58%) were the major compounds, and τ-cadinol (6.07%) was the main sesquiterpenoid. The studied essential oil could be determined as linalool/linalyl acetate chemotype. Comparison of the chemical composition with the literature data for the Balkan populations of *T. jankae* (Table S1) showed similarities and differences. Thus, linalool seems to be a characteristic component of *T. jankae* as it was found in the essential oils from Bulgaria [11], Serbia [11], N. Macedonia [9], Kosovo [8] and Bosnia [10]. Linalyl acetate was reported in the essential oils from Bulgaria [11], Kosovo [8] and Bosnia [10] in amounts between 7.6 and 28.7%. The studied sample differed in the content of other components such as α-terpinyl acetate, geranial, (*E*)-caryophyllene, carvacrol and thymol. Thus, α-terpinyl acetate was the major component (20.1%) in another sample from Bulgaria [11], while its content in the studied sample was only 4.22%. *T. jankae* subsp. *jankae* from N. Macedonia [9] also contained a significant amount of α-terpinyl acetate (11.3%). Geranial was the second most abundant component (15.3–24.9%) in three varieties of *T. jankae* from N. Macedonia [9], while its content in our sample was only 1.75%. (*E*)-Caryophyllene (14.6%) and thymol (10.7%) were dominant compounds in the essential oil from Serbia [11], and carvacrol (13.8%) in the sample from Kosovo [8]. As can be seen from Table 2, the content of (E)-caryophyllene, thymol and carvacrol was 3.47, 1.22 and 0.31%, respectively. Finally, the studied population of *T. jankae* was very similar to that from Bosna with linalool and linalyl acetate as the main constituents [10].

The essential oil of *T. longicaulis* was also characterized by a high percentage of MO (Figure 1). Geraniol (25.28%), α-terpinyl acetate (17.46%), linalool (13.37%) and linalyl acetate (12.72%) were the main components (Table 2). This essential oil differed from the oils described above by the relatively high percentage of thymol (8.27%). Geraniol was found to be the main component in samples from Turkey [36], Greece [24,25] and Italy [26], α-terpinyl acetate-in samples from Serbia [28] and Turkey [29,36], linalool and linalyl acetate-in *T. longicaulis* ssp. *longicaulis* var. *subisophyllus* [38] and linalool and geraniol-in the sample from Kosovo [8]. Most of the studied *T. longicaulis* essential oils from Italian, Greek, Turkish and Croatian populations belonged to the thymol/*p*-cymene and carvacrol/*p*-cymene chemotypes (Table S1). Therefore, the studied essential oil could be described as a new geraniol/α-terpinyl acetate/linalool/linalyl acetate chemotype.

In the essential oil of *T. pulegioides*, 40 components were detected (Table 2). This oil was characterized by an extremely high content of α-terpinyl acetate (66.79%). In addition, geraniol (7.27%), thymol (6.70%), β-caryophyllene (2.48%) and α-terpineol (2.30%) were the compounds registered in amounts greater than 2%. This essential oil could be described as α-terpinyl acetate chemotype. It is worth mentioning that the European *T. pulegioides* individuals are predominantly phenolic (thymol/carvacrol) chemotypes, and individuals of α-terpinyl acetate chemotype are rare (Table S1). So far, α-terpinyl acetate was found to be the dominant component in essential oils of *T. pulegioides* growing wild in France at 1700 m a.s.l. (64.8–88.0%) [69] and in Lithuania (50.0–70.0%) in slopes with southern exposure [60,79]. Data in the literature showed only several species of genus *Thymus* containing α-terpinyl acetate in amounts over 45%, namely *T. willkommii* Ronniger (36–69%) [80], *T. zygis* (65–73%) [81], *T. glabrescens* Willd. (47.6%) [19], *T. munbyanus* subsp. *ciliatus* (43–82%) [82], *T. longicaulis* subsp. *longicaulis* (82.1%) [36] and *T. pannonicus* (35.7–48.8%) [46]. Recently, it has been found that α-terpinyl acetate essential oil from *T. pulegioides* possessed a high antimicrobial effect against fungi and dermatophytes and could be used as a potential source for developing preventive measures or/and drugs against mycosis [79].

In the oil of *T. sibthorpii*, 54 compounds were identified (Table 2). MO (63.80%) were the most abundant class of compounds followed by AR (16.71%), SH (9.70%) and MH (4.43%) (Figure 1). Among individual compounds, myrcen-8-yl acetate (22.03%), linalool (19.72%), myrcen-8-ol (14.71%) and thymol (10.67%) were detected in significant concentrations. While linalool and thymol are common components for *Thymus* species, the presence of myrcen-8-yl acetate and myrcen-8-ol is very rare in essential oils (Table S1). To our knowledge, myrcen-8-yl acetate was found in significant amounts in *T. praecox* Opiz ssp. *arcticus* (E. Durand) Jalas [83] and *T. vulgaris* L. [84], while myrcen-8-ol-in *T. willkommii* Ronn [80] and *T. vulgaris* L. [84]. The obtained data differed significantly from those for Greek and Turkish populations of the species for which geraniol/linalool/citronellyl acetate, geraniol/thymol/p-cymene, thymol/p-cymene and carvacrol/p-cymene chemotypes were described [38,70,71]. Therefore, the studied **TS** oil is a new myrcen-8-yl acetate/linalool/myrcen-8-ol chemotype.

The essential oil of *T. pannonicus* was the richest in components (63 compounds, Table 2). This sample was characterized by the highest content of SH (74.20%) and almost equal amounts of MH, MO and SO (5.07–6.76%). The main components, germacrene D (42.15%) and β-caryophyllene (12.28%), determined the oil as a germacrene D/β-caryophyllene chemotype. Similar amounts of germacrene D and β-caryophyllene (43.4 and 15.0%) were found in the essential oil of the plant growing on the Bakony Hills, Hungary [43]. Germacrene D was detected as a major component also in the essential oils of *T. pannonicus* from Serbia (36.9%) [50], Hungary (29.7%) [43] and Kosovo (18%) [14], but differed in the second most dominant compound. Although the sesquiterpenoids are not characteristic for species of genus *Thymus*, that type of compounds were detected in many *T. pannonicus* populations in significant amounts (Table S1) [8,14,43,48,50].

In the essential oil of *T. thracicus*, 54 compounds were identified (Table 2). This oil contained similar amounts of mono- and sesquiterpenoids (44.30 and 50.74%) (Figure 1). The main components were β-myrcene (16.53%), *cis*-sabinene hydrate (13.58%), τ-cadinol (13.24%) and elemol (11.29%), which determined the oil as β-myrcene/*cis*-sabinene hydrate/τ-cadinol/elemol chemotype. This oil differed significantly from those originating from Turkey [29,72] and from *Thymus alsarensis* Ronn. (syn. *T. thracicus* Velen.) from N. Macedonia [73], in which thymol/carvacrol and geraniol were the most abundant components. As can be seen from Table 2, these compounds were absent or were found in a negligible amount of *T. thracicus* essential oil. Therefore, this oil was a new chemotype.

Aromatic compounds (68.12%) were the prevailing class of compounds in the essential oil of *T. callieri*. The major components carvacrol (42.65%), thymol (13.38%) and γ-terpinene (12.04%) determined the oil is a carvacrol/thymol/γ-terpinene chemotype. Another aromatic compound p-cymene is also in significant concentration 7.17%. This is the first report on the essential oil composition of *T. callieri* from Bulgaria. Aromatic compounds were also the predominant class in the essential oil of *T. roegneri* (syn. of *T. callieri*) from Turkey with thymol (56.23%) and p-cymene (12.94%) as the main components [85]. Carvacrol was 8.59% and γ-terpinene was not detected in the Turkish sample.

The essential oil of *T. glabrescens* was the richest in aromatic compounds (78.17%). The oil contained equal amounts of MH and SH (7.49%), while MO and SO were 3.66 and 0.99%, respectively. Thymol (63.96%) was the main component, followed by p-cymene (7.17%) and γ-terpinene (4.48%), similar to some Hungarian [15,44], Serbian [11,20,21] and Romanian [18] oils. Generally, the presence of thymol and its precursors (p-cymene and γ-terpinene) was characteristic of *T. glaberescens* populations, although some variations in the main components from different locations (Table S1).

To demonstrate the relationship between the studied *Thymus* species from Section *Serpyllum,* the composition data were analyzed by principal component analysis (PCA) and cluster analysis (CA). The PCA conducted on the content of all constituents of each sample showed that the first 3 principal axes accounted for 65.77% of the total variations (Figure 2A,B). Considering the contributions to the loadings, PC 1 (25.91% of the total variations) accounted for positive contributions of thymol, α-terpinyl acetate, carvacrol, p-cymene, γ-terpinene, carvacrol methyl ether, borneol, α-terpinene, *cis*-sabinene hydrate, thymol methyl ether and eucalyptol and negative contributions of α-terpineol, 6-*epi*-shyobunol, germacrene D, τ-cadinol, β-caryophyllene, shyobunol, elemol, geranyl acetate, geraniol, linalyl acetate and linalool. PC 2 (24.73% of the total variations) was positively related to thymol, carvacrol, linalool, germacrene D, linalyl acetate, myrcen-8-yl acetate, p-cymene, γ-terpinene, β-myrcene, carvacrol methyl ether, myrcen-8-ol, β-caryophyllene, *cis*-sabinene hydrate, elemol and τ-cadinol and negatively related to 3-octanol acetate, sabinene, bornyl acetate, limonene, geranyl acetate, α-terpineol, geraniol and α-terpinyl acetate. As can be seen from Figure 2A, *T. pulegioides* was placed in the most isolated position (group I) due to the extremely high content of α-terpinyl acetate (66.79%). In contrast, the relatively high concentration of linalool, geraniol and their acetates grouped *T. vandasii, T. jankae, T. moesiacus* and *T. longicaulis* in group II. *T. glabrescens* and *T. callieri* formed group III and were discriminated from other species by the content of the aromatic compounds (thymol and carvacrol). The results do not agree with some recent taxonomic classifications [6], considering *T. pannonicus* as a subspecies of *T. pulegioides*. In the present study, the two taxa were clearly distinct (Figure 2), and in other studies they were even classified in different subsections [3].

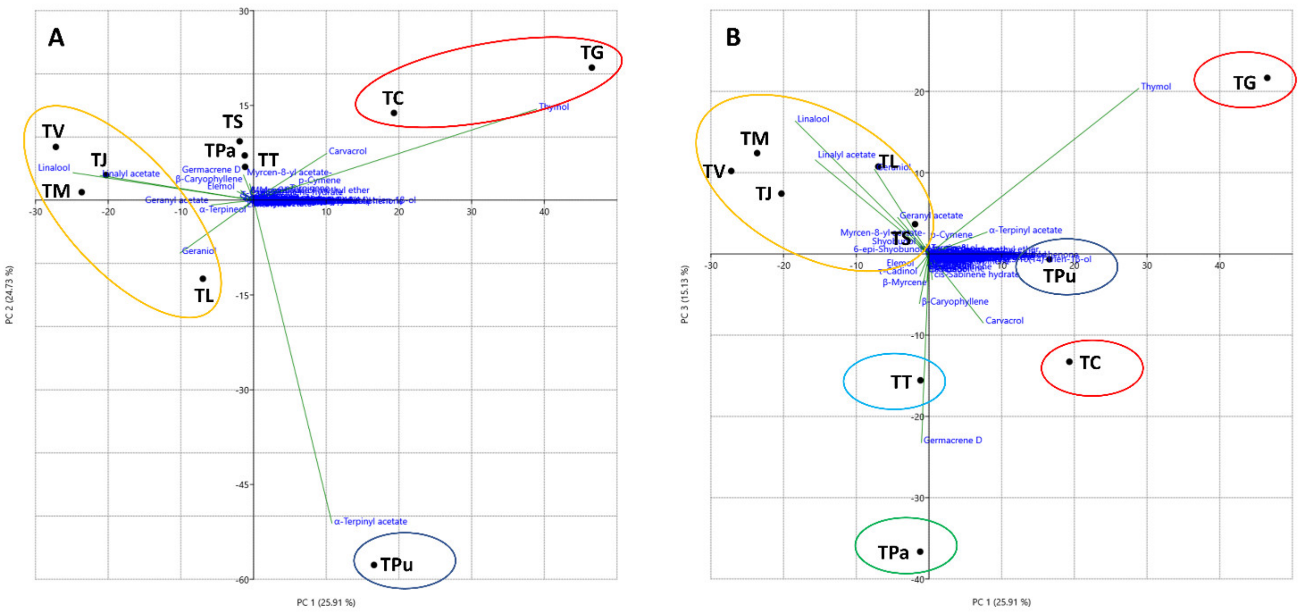

Figure 2. (**A**): PCA Biplot (PC1/PC2) and (**B**) PCA Biplot (PC1/PC3) for the whole set of data of the ten *Thymus* species from Sect. *Serpyllum* (for abbreviation see Table 1).

Further, PC 3 (15.13% of the total variations) was positively related to linalool, geraniol, linalyl acetate, thymol, α-terpinyl acetate, myrcen-8-yl acetate and geranyl acetate and negatively related to β-myrcene, *cis*-sabinene hydrate, carvacrol, elemol, τ-cadinol, β-caryophyllene and germacrene D. The influence of these additional components allowed the re-grouping of *T. sibthorpii*, *T. pannonicus* and *T. thracicus* as follows: **TS** in group II due to the high percentage of linalool, myrcen-8-ol and myrcen-8-yl acetate, **TPa** in group IV with the highest content of sesquiterpenoids [germacrene D (42.15%) and β-caryophyllene (12.28%)] and **TT** in group V with almost equal amounts of β-myrcene (16.53%), *cis*-sabinene hydrate (13.58%), τ-cadinol (13.24%) and elemol (11.29%). Finally, group III was divided into 2 subgroups IIIa (**TG**) and IIIb (**TC**), depending on the dominating component–thymol and carvacrol. The cluster analysis (UPMGA, Euclidean distance) performed on the same data matrix (Figure 3) supported the differentiation of the samples obtained by the PCA analysis.

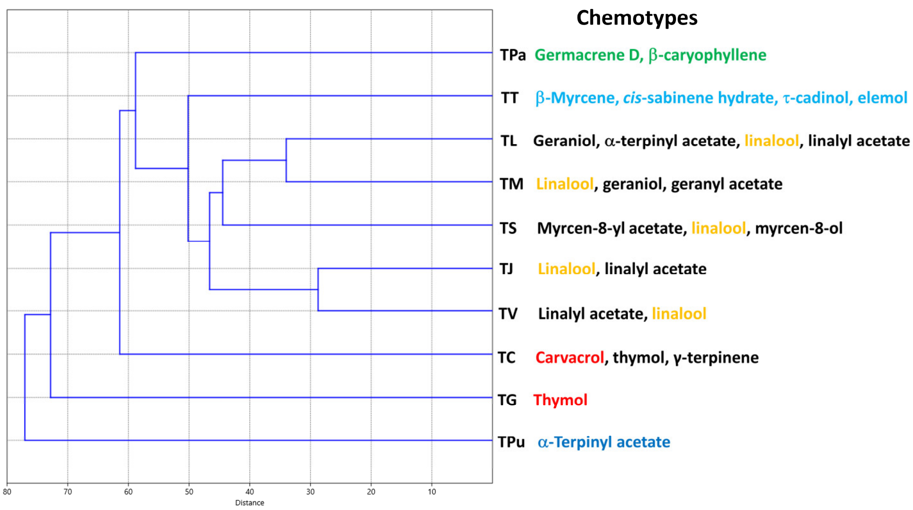

Figure 3. Dendrogram obtained by cluster analysis (UPGMA, Euclidean distance) for the whole set of data of the 10 *Thymus* species from Sect. *Serpyllum* (for abbreviation see Table 1).

The cluster analysis (UPMGA, Euclidean distance) performed on the same data matrix (Figure 3) supported the differentiation of the samples obtained by the PCA analysis. As can be seen, six main chemotypes: α-terpinyl acetate, thymol, carvacrol, linalool, germacrene D/β-caryophyllene and β-myrcene/*cis*-sabinene hydrate/τ-cadinol/elemol were clearly distinguished. Inside the linalool chemotype, the contribution of the other main components resulted in the formation of additional chemotypes.

4. Conclusions

The obtained results showed considerable variation in the chemical composition of the species in this study: *T. moesiacus*, *T. pulegioides*, *T. longicaulis*, *T. jankae*, *T. vandasii* and *T. sibthorpii* were rich in monoterpenoids; *T. callieri* and *T. glabrescens* in aromatic compounds; *T. pannonicus* in sesquiterpenoids; while *T. thracicus* contained almost equal amounts of mono- and sesquiterpenoids. The chemical profile of *T. moesiacus*, *T. thracicus*, *T. sibthorpii* and *T. longicaulis* differed from that reported previously, and therefore, they formed new chemotypes. The essential oil content of endemic *T. vandasii* and *T. callieri* is reported for the first time. The described essential oil composition contributed to the phytochemistry of these species and confirmed the chemical polymorphism, a widespread phenomenon within the genus *Thymus*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15060759/s1, Table S1: Literature survey of the main components in the essential oils of different *Thymus* species from sect. *Serpyllum.*

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References

1. Harkat-Madouri, L.; Asma, B.; Madani, K.; Bey-Ould Si Said, Z.; Rigou, P.; Grenier, D.; Allalou, H.; Remini, H.; Adjaoud, A.; Boulekbache-Makhlouf, L. Chemical composition, antibacterial and antioxidant activities of essential oil of *Eucalyptus globulus* from Algeria. *Ind. Crops Prod.* **2015**, *78*, 148–153. [CrossRef]
2. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef]
3. Stahl-Biskup, E.; Sáez, F. *Thyme: The genus Thymus*; Taylor & Francis Group: Abingdon, UK, 2002; ISBN 9780203216859.
4. Aneva, I.; Zhelev, P.; Stoyanov, S.; Marinov, Y.; Georgieva, K. Survey on the distribution, diversity and phyochemistry of genus *Thymus* in Bulgaria. *Ecol. Balk.* **2018**, *10*, 101–110.
5. Trendafilova, A.; Todorova, M.; Ivanova, V.; Zhelev, P.; Aneva, I. Essential Oil Composition of Five *Thymus* Species from Bulgaria. *Chem. Biodivers.* **2021**, *18*, e2100498. [CrossRef]
6. Thymus | Euro+Med-Plantbase. Available online: https://europlusmed.org/cdm_dataportal/taxon/86dabab4-03af-416d-8d9f-1a0110d69207 (accessed on 16 February 2023).
7. Hatipi Ibrahimi, M.; Papajani, V.; Zeljkovic, S.C.; Matevski, V. Essential oil analysis of two *Thymus* spp. growing wild in Kosovo. *J. Essent. Oil-Bear. Plants* **2014**, *17*, 832–837. [CrossRef]
8. Nedanova, V.; Flurim, N.; Karaandzova, M.; Cvetkovikj, I.; Stefkov, G.; Kulevanova, S. Chemical composition of the essential oils of some *Thymus* spp. (Lamiaceae) from Kosovo. *Maced. Pharm. Bull.* **2016**, *62*, 513–514.
9. Kulevanova, S.; Ristic, M.; Stafilov, T.; Matevski, V. Composition of the essential oils of *Thymus jankae* Chel. var. *jankae*, *T. jankae* var. *pantotrichus* Ronn. and *T. jankae* var. *patentipilus* Lyka from Macedonia. *J. Essent. Oil Res.* **1998**, *10*, 191–194. [CrossRef]
10. Ćavar, S.; Vidic, D.; Maksimović, M. Essential oil profile of *Thymus jankae* Celak. from Bosnia. *Planta Med.* **2009**, *75*, PB10. [CrossRef]
11. Stojanović, G.; Jovanović, O.; Petrović, G.; Mitić, V.; Jovanović, V.S.; Jovanović, S. Composition of headspace volatiles and essential oils of three *Thymus* species. *Nat. Prod. Commun.* **2014**, *9*, 1609–1612. [CrossRef]
12. Kuštrak, D.; Martinis, Z.; Kuftinec, J.; Blažević, N. Composition of the essential oils of some *Thymus* and *Thymbra* species. *Flavour Fragr. J.* **1990**, *5*, 227–231. [CrossRef]
13. Pluhár, Z.; Sárosi, S.; Novák, I.; Kutta, G. Essential Oil Polymorphism of Hungarian Common Thyme (*Thymus glabrescens* Willd.) Populations. *Nat. Prod. Commun.* **2008**, *3*, 1934578X0800300. [CrossRef]
14. Simkó, H.; Sarosi, S.; Ladanyi, M.; Marton, B.; Radacsi, P.; Csontos, P.; Gosztola, B.; Kun, R.; Pluhár, Z. Studies on occurence, essential oil data and habitat conditions of Hungarian *Thymus pannonicus* and *Thymus glabrescens* populations. *Med. Aromat. Plants* **2013**, *2*, 1000119. [CrossRef]
15. Pluhár, Z.; Simkó, H.; Sárosi, S.; Boros, B.; Dörnyei, Á.; Felinger, A.; Horváth, G. Determination of essential oil and polyphenolic compounds in *Thymus* species. *Acta Hortic.* **2015**, *1099*, 661–670. [CrossRef]
16. Rădulescu, V.; Pavel, M.; Teodor, A.; Tănase, A.; Ilieş, D.C. Analysis of volatile compounds from infusion and hydrodistillate obtained from the species *Thymus pulegioides* L. (Lamiaceae). *Farmacia* **2009**, *57*, 282–289.
17. Pavel, M.; Ristić, M.; Stević, T. Essential oils of *Thymus pulegioides* and *Thymus glabrescens* from Romania: Chemical composition and antimicrobial activity. *J. Serb. Chem. Soc.* **2010**, *75*, 27–34. [CrossRef]
18. Imbrea, I.; Prodan, M.; Nicolin, A.; Butnariu, M.; Imbrea, F. Valorising *Thymus glabrescens* Willd. from the Aninei mountains. *Res. J. Agric. Sci.* **2010**, *42*, 260–263.
19. Varga, E.; Bardocz, A.; Belak, A.; Maraz, A.; Boros, B.; Felinger, A.; Böszörményi, A.; Horváth, G. Antimicrobial activity and chemical composition of thyme essential oils and the polyphenolic content of different *Thymus* extracts. *Farmacia* **2015**, *63*, 357–361.
20. Maksimović, Z.; Stojanović, D.; Šoštarić, I.; Dajić, Z.; Ristić, M. Composition and radical-scavenging activity of *Thymus glabrescens* Willd. (Lamiaceae) essential oil. *J. Sci. Food Agric.* **2008**, *88*, 2036–2041. [CrossRef]
21. Dajic-Stevanovic, Z.; Sostaric, I.; Marin, P.D.; Stojanovic, D.; Ristic, M. Population variability in *Thymus glabrescens* Willd. from Serbia: Morphology, anatomy and essential oil composition. *Arch. Biol. Sci.* **2008**, *60*, 475–483. [CrossRef]
22. Ilić, B.S.; Kocić, B.D.; Ćirić, V.M.; Cvetković, O.G.; Miladinović, D.L. An in vitro synergistic interaction of combinations of *Thymus glabrescens* essential oil and its main constituents with chloramphenicol. *Sci. World J.* **2014**, *2014*, 826219. [CrossRef] [PubMed]
23. Vladimir-Knežević, S.; Kosalec, I.; Babac, M.; Petrović, M.; Ralić, J.; Matica, B.; Blažeković, B. Antimicrobial activity of *Thymus longicaulis* C. Presl essential oil against respiratory pathogens. *Cent. Eur. J. Biol.* **2012**, *7*, 1109–1115. [CrossRef]
24. Chorianopoulos, N.G.; Evergetis, E.T.; Aligiannis, N.; Mitakou, S.; Nychas, G.J.E.; Haroutounian, S.A. Correlation between chemical composition of Greek essential oils and their antibacterial activity against food-borne pathogens. *Nat. Prod. Commun.* **2007**, *2*, 419–426. [CrossRef]
25. Chorianopoulos, N.; Kalpoutzakis, E.; Aligiannis, N.; Mitaku, S.; Nychas, G.J.; Haroutounian, S.A. Essential oils of *Satureja*, *Origanum*, and *Thymus* species: Chemical composition and antibacterial activities against foodborne pathogens. *J. Agric. Food Chem.* **2004**, *52*, 8261–8267. [CrossRef] [PubMed]

26. De Martino, L.; Bruno, M.; Formisano, C.; De Feo, V.; Napolitano, F.; Rosselli, S.; Senatore, F. Chemical composition and antimicrobial activity of the essential oils from two species of *Thymus* growing wild in Southern Italy. *Molecules* **2009**, *14*, 4614–4624. [CrossRef]
27. Hatipi Ibrahimi, M.; Papajani, V.; Cavar, S.; Matevski, V. GC/MS Analysis of the essential oil of *Thymus longicaulis* Presl. from Kosovo. *Bull. Chem. Technol. Bosnia Herzeg.* **2013**, *41*, 6–10.
28. Grujic Jovanovic, S.; Marin, P.D.; Dzamic, A.; Ristic, M. Essential oil composition of *Thymus longicaulis* from Serbia. *Chem. Nat. Compd.* **2009**, *45*, 265–266. [CrossRef]
29. Akçin, T.A. Numerical taxonomic studies on some species of the genus *Thymus* L. (Labiatae) in Turkey. *Asian J. Plant Sci.* **2006**, *5*, 782–788. [CrossRef]
30. Tzakou, O.; Verykokidou, E.; Roussis, V.; Chinou, I. Chemical composition and antibacterial properties of T*hymus longicaulis* subsp. *chaoubardii* oils: Three chemotypes in the same population. *J. Essent. Oil Res.* **1998**, *10*, 97–99. [CrossRef]
31. Baser, K.H.; Koyuncu, M. Composition of the essential oils of two varieties of *Thymus longicaulis* C. Presl. subsp. *chaubardii* (Boiss. et Heldr. ex Reichb. fil.) Jalas. *J. Essent. Oil Res.* **1994**, *6*, 207–209. [CrossRef]
32. Azaz, A.D.; Irtem, H.A.; Kurkcuoğlu, M.; Baser, K.H.C. Composition and the in vitro antimicrobial activities of the essential oils of some *Thymus* species. *Z Fur Naturforsch. Sect. C J. Biosci.* **2004**, *59*, 75–80. [CrossRef]
33. Pavela, R.; Bartolucci, F.; Desneux, N.; Lavoir, A.V.; Canale, A.; Maggi, F.; Benelli, G. Chemical profiles and insecticidal efficacy of the essential oils from four *Thymus* taxa growing in central-southern Italy. *Ind. Crops Prod.* **2019**, *138*, 111460. [CrossRef]
34. Bellomaria, B.; Hruška, K.; Valentini, G. Composizione degli olii essenziali di *Thymus longicaulis* C. Presi in varie località dell'Italia centrale. *G. Bot. Ital.* **1981**, *115*, 17–27. [CrossRef]
35. Sarikurkcu, C.; Sabih Ozer, M.; Eskici, M.; Tepe, B.; Can, Ş.; Mete, E. Essential oil composition and antioxidant activity of *Thymus longicaulis* C. Presl subsp. *longicaulis* var. *longicaulis*. *Food Chem. Toxicol.* **2010**, *48*, 1801–1805. [CrossRef]
36. Baser, K.H.C.; Özek, T.; Kirimer, N.; Tümen, G. The occurrence of three chemotypes of *Thymus longicaulis* C. Presl. subsp. *longicaulis* in the same population. *J. Essent. Oil Res.* **1993**, *5*, 291–295. [CrossRef]
37. Baser, K.H.C.; Özek, T.; Kürkçüoglu, M.; Tümen, G. Composition of the essential oil of *Thymus longicaulis* C. Presl. var. *subisophyllus* (Borbas) Jalas from Turkey. *J. Essent. Oil Res.* **1992**, *4*, 311–312. [CrossRef]
38. Tümen, G.; Kirimer, N.; Başer, K.H.C. Composition of the essential oils of *Thymus* species growing in Turkey. *Chem. Nat. Compd.* **1995**, *31*, 42–47. [CrossRef]
39. Ćavar, S.; Vidic, D.; Maksimović, M. GC-MS analiza volatilnih konstituenata Thymus moesiacus Velen. *Glas. Hemičara Tehnol. I Ekol. Repub. Srp.* **2009**, *2*, 27–29.
40. Kulevanova, S.; Ristić, M.; Stafilov, T. Composition of the essential oil from *Thymus moesiacus* from Macedonia. *Planta Med.* **1996**, *62*, 78–79. [CrossRef] [PubMed]
41. Kameníková, M.; Fialová, S.; Ťažký, A.; Čičová, I. Polyphenolic compounds and essential oil analysis of selected species of the genus *Thymus*. *Acta Fac. Pharm. Univ. Comen.* **2015**, *62*, 12–17. [CrossRef]
42. Pluhár, Z.; Héthelyi, É.; Kutta, G.; Kamondy, L. Evaluation of environmental factors influencing essential oil quality of *Thymus pannonicus* All. and *Thymus praecox* Opiz. *J. Herbs Spices Med. Plants* **2007**, *13*, 23–43. [CrossRef]
43. Pluhár, Z.; Sárosi, S.; Pintér, A.; Simkó, H. Essential oil polymorphism of wild growing Hungarian thyme (*Thymus pannonicus*) populations in the Carpathian Basin. *Nat. Prod. Commun.* **2010**, *5*, 1681–1686. [CrossRef]
44. Pluhár, Z.; Kocsis, M.; Kuczmog, A.; Csete, S.; Simkó, H.; Sárosi, S.; Molnár, P.; Horváth, G. Essential oil composition and preliminary molecular study of four Hungarian *Thymus* species. *Acta Biol. Hung.* **2012**, *63*, 81–96. [CrossRef] [PubMed]
45. Berciu, I.; Toma, C.; Burzp, I. Aspecte Histo-anatomice şi biochimice referitoare la organele vegetative aeriene de *Thymus Pannonicus* All. subspeciile auctus şi *pannonicus*. *An. Ştiinţifice Ale Univ. Al. I. Cuza Iaşi* **2008**, *LIV*, 100–105.
46. Boz, I.; Burzo, I.; Zamfirache, M.-M.; Toma, C.; Padurariu, C. Glandular trichomes and essential oil composition of *Thymus pannonicus* All. (Lamiaceae). *An. Univ. Din Oradea Fasc. Biol.* **2009**, *1*, 36–39.
47. Jianu, C.; Mihail, R.; Muntean, S.G.; Pop, G.; Daliborca, V.C.; Horhat, F.G.; Nitu, R. Composition and antioxidant capacity of essential oils obtained from *Thymus vulgaris*, *Thymus pannonicus* and *Satureja montana* grown in Western Romania. *Rev. Chim.* **2015**, *66*, 2157–2160.
48. Boz, I.; Burzo, I.; Tanase, C. The effect of harvesting time on essential oils composition of *Thymus pannonicus* L. *An. Ştiinţifice Ale Univ. Al. I. Cuza Iaşi* **2016**, *62*, 59–64.
49. Maksimović, Z.; Milenković, M.; Vučićević, D.; Ristić, M. Chemical composition and antimicrobial activity of *Thymus pannonicus* All. (Lamiaceae) essential oil. *Cent. Eur. J. Biol.* **2008**, *3*, 149–154. [CrossRef]
50. Sostaric, I.; Arsenijevic, J.; Acic, S.; Stevanovic, Z.D. Essential oil polymorphism of *Thymus pannonicus* All. (Lamiaceae) in Serbia. *J. Essent. Oil-Bear. Plants* **2012**, *15*, 237–243. [CrossRef]
51. Arsenijević, J.; Drobac, M.; Šoštarić, I.; Jevđović, R.; Živković, J.; Ražić, S.; Moravčević, Đ.; Maksimović, Z. Comparison of essential oils and hydromethanol extracts of cultivated and wild growing *Thymus pannonicus* All. *Ind. Crops Prod.* **2019**, *130*, 162–169. [CrossRef]
52. Radonić, A.; Mastelić, J. Essential oil and glycosidically bound volatiles of *Thymus pulegioides* L. growing wild in Croatia. *Croat. Chem. Acta* **2008**, *81*, 599–606.
53. Mastelić, K.; Kravar, A.J.G. The chemical composition of terpene alcohols and phenols from the essential oil and terpene glycosides isolated from *Thymus pulegioides* growing wild in Dalmatia. *Planta Med.* **1992**, *58*, 678–680. [CrossRef]

54. Groendahl, E.; Ehlers, B.K.; Keefover-Ring, K. A new *cis*-sabinene hydrate chemotype detected in large thyme (*Thymus pulegioides* L.) growing wild in Denmark. *J. Essent. Oil Res.* **2008**, *20*, 40–41. [CrossRef]
55. Senatore, F.; De Fusco, R.; Grassia, A.; Moro, C.O.; Rigano, D.; Napolitano, F. Chemical composition and antibacterial activity of essential oils from five culinary herbs of the Lamiaceae family growing in Campania, southern Italy. *J. Essent. Oil-Bear. Plants* **2003**, *6*, 166–173. [CrossRef]
56. Sárosi, S.; Bernáth, J.; Bertoli, A.; Pistelli, L.; Benvenuti, S. Essential oil polymorphism of *Thymus pulegioides* collected in Monti Pisani, Italy. *Acta Hortic.* **2012**, *955*, 59–64. [CrossRef]
57. Mockute, D.; Bernotiene, G. The α-terpenyl acetate chemotype of essential oil of *Thymus pulegioides* L. *Biochem. Syst. Ecol.* **2001**, *29*, 69–76. [CrossRef]
58. Mockute, D.; Bernotiene, G. Chemical composition of the essential oils and the odor of *Thymus pulegioides* L. growing wild in Vilnius. *J. Essent. Oil Res.* **2005**, *17*, 415–418. [CrossRef]
59. Vaičiulyte, V.; Ložiene, K. Metabolomic analysis and effects of meteorological factors on phenolic and non-phenolic chemotypes of *Thymus pulegioides* L. cultured in the same locality. *Ind. Crops Prod.* **2015**, *77*, 491–498. [CrossRef]
60. Vaičiulytė, V.; Ložienė, K. Impact of chemical polymorphism of *Thymus pulegioides* on some associated plant species under natural and laboratory conditions. *Plant Biosyst.* **2020**, *154*, 663–672. [CrossRef]
61. Svediene, J.; Raudoniene, V.; Loziene, K.; Bridziuviene, D.; Paškevicius, A.; Vaiciulyte, V. The effect of various *Thymus pulegioides* chemotypes essential oils and ph on food spoilage microorganisms. *J. Essent. Oil-Bear. Plants* **2015**, *18*, 276–288. [CrossRef]
62. Ložiene, K.; Venskutonis, P.R. Influence of environmental and genetic factors on the stability of essential oil composition of *Thymus pulegioides*. *Biochem. Syst. Ecol.* **2005**, *33*, 517–525. [CrossRef]
63. Ložiene, K. Selection of fecund and chemically valuable clones of thyme (*Thymus*) species growing wild in Lithuania. *Ind. Crops Prod.* **2009**, *29*, 502–508. [CrossRef]
64. Pinto, E.; Pina-Vaz, C.; Salgueiro, L.; Gonçalves, M.J.; Costa-De-Oliveira, S.; Cavaleiro, C.; Palmeira, A.; Rodrigues, A.; Martinez-De-Oliveira, J. Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida, Aspergillus* and dermatophyte species. *J. Med. Microbiol.* **2006**, *55*, 1367–1373. [CrossRef] [PubMed]
65. Salgueiro, L.R.; da Cunha, A.P.; Paiva, J. Chemotaxonomic characterization of a *Thymus* hybrid from Portugal. *Flavour Fragr. J.* **1993**, *8*, 325–330. [CrossRef]
66. Boz, I.; Gille, E.; Necula, R.; Dunca, S.; Zamfirache, M.M. Chemical composition and antibacterial activity of essential oils from five populations of *Thymus pulegioides* L. *Cellul. Chem. Technol.* **2015**, *49*, 169–174.
67. Miladinović, D.L.; Ilić, B.S.; Miladinović, L.C.; Kocić, B.D.; Ćirić, V.M.; Stankov-Jovanović, V.P.; Cvetković, O.G.; Govil, J.N.; Bhattacharya, S. Antibacterial activity of *Thymus pulegioides* essential oil and its synergistic potential with antibiotics: A chemometric approach. *Recent Prog. Med. Plants* **2013**, *38*, 101–136.
68. Mártonfi, P. Polymorphism of essential oil in *Thymus pulegioides* subsp. *chamaedrys* in Slovakia. *J. Essent. Oil Res.* **1992**, *4*, 173–179. [CrossRef]
69. Michet, A.; Chalchat, J.C.; Figuérédo, G.; Thébaud, G.; Billy, F.; Pétel, G. Chemotypes in the volatiles of wild thyme (*Thymus pulegioides* L.). *J. Essent. Oil Res.* **2008**, *20*, 101–103. [CrossRef]
70. Katsiotis, S.T.; Chatzopoulou, P.; Baerheim Svendsen, A. The essential oil of *Thymus sibthorpii* Benth. growing wild in Greece. *Sci. Pharm.* **1990**, *58*, 303–306.
71. Baser, K.H.C.; Özek, T.; Kürkçüoglu, M.; Tümen, G. Characterization of the essential oil of *Thymus sibthorpii* Bentham. *J. Essent. Oil Res.* **1992**, *4*, 303–304. [CrossRef]
72. Baser, K.H.C.; Özek, T.; Kürkçüoglu, M.; Tümen, G. Essential oil of *Thymus thracicus* Velen var. *longidens* (Velen) Jalas. *J. Essent. Oil Res.* **1995**, *7*, 661–662. [CrossRef]
73. Kulevanova, S.; Ristic, M.; Stafilov, T.; Dorevski, K.; Ristov, T. Essential oil analysis of some taxa of genera *Thymus* L. Environment influences. *Bull. Chem. Technol. Maced.* **1996**, *15*, 33–38.
74. Ćavar Zeljković, S.; Maksimović, M. Chemical composition and bioactivity of essential oil from *Thymus* species in Balkan Peninsula. *Phytochem. Rev.* **2015**, *14*, 335–352. [CrossRef]
75. Sandra, P.; Bicchi, C. *Capillary Gas Chromatography in Essential Oil Analysis*; Dr. Alfred Huethig Verlag: Heidelberg, Germany, 1987.
76. Babushok, V.I.; Linstrom, P.J.; Zenkevich, I.G. Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* **2011**, *40*, 043101. [CrossRef]
77. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 4th ed.; Allured Business Media: Carol Stream, IL, USA, 2007; ISBN 978-1-932633-214.
78. Bischof-Deichnik, C.; Holtuijzen, J.; Stahl-Biskup, E. Multivariate statistical analysis of the essential oil composition of *Thymus praecox* Opiz ssp. *polytrichus* (Kern. ex Borb.) Ronn. collected in the Tyrolean Alps. *Flavour Fragr. J.* **2000**, *15*, 1–6. [CrossRef]
79. Vaičiulytė, V.; Ložienė, K.; Švedienė, J.; Raudonienė, V.; Paškevičius, A. α-Terpinyl acetate: Occurrence in essential oils bearing *Thymus pulegioides*, phytotoxicity, and antimicrobial effects. *Molecules* **2021**, *26*, 1065. [CrossRef] [PubMed]
80. Adzet, T.; Cañigueral, S.; Gabalda, N.; Ibañez, C.; Tomas, X.; Vila, R. Composition and variability of the essential oil of *Thymus Willkomii*. *Phytochem.* **1991**, *30*, 2289–2293. [CrossRef]
81. García Martín, D.; García Vallejo, M.C. Chemotypes of *Thymus zygis* (Löfl.) L. of Guadarramma Sierra and other places in Castile (Spain). In Proceedings of the 9th International Essential Oil Congress, Singapore, 13–17 March 1983; pp. 134–140.

82. Benjiali, B.; Hammoumi, M.; Richard, H. Polymorphisme chimique des huiles essentielles de thym du Maroc. 1—Caractérisation des composants. *Sci. Aliment.* **1987**, *7*, 77–91.
83. Schmidt, A.; Bischof-Deichnik, C.; Stahl-Biskup, E. Essential oil polymorphism of *Thymus praecox* subsp. *arcticus* on the British Isles. *Biochem. Syst. Ecol.* **2004**, *32*, 409–421. [CrossRef]
84. Granger, R.; Passet, J. Thymus vulgaris spontane de France: Races chimiques et chemotaxonomie. *Phytochemistry* **1973**, *12*, 1683–1691. [CrossRef]
85. Tümen, G.; Kirimer, N.; Kürkçüoglu, M.; Baser, K.H.C. Composition of the essential oils of *Thymus atticus* and *Thymus roegneri* from Turkey. *J. Essent. Oil Res.* **1997**, *9*, 473–474. [CrossRef]