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Chemical Composition of the Essential Oil of *Thymus longicaulis* C. Presl. subsp. *longicaulis*

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Abstract: In this study, the chemical composition of the essential oils obtained from the aerial parts of *Thymus longicaulis* subsp. *longicaulis* naturally grown in Turkey were analyzed by GC and GC-MS and chemical differences in terms of chemotaxonomy were discussed. The main compounds in the essential oils of *T. longicaulis* subsp. *longicaulis* essential oils were 1,8-cineole (30.1%), linalool (18.0%), *β*-pinene (17.3%) and *(E)*-*β*-ocimene (%12.6%) Hierarchical cluster analysis was performed by examining essential oil studies of 34 samples belonging to the genus *Thymus*, including the Sinop sample. The results of the study were discussed with other taxa belonging to the genus.

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1. INTRODUCTION

The *Thymus* genus is a member of the Lamiaceae family, which attracts attention with its many wild plant species and has aromatic properties. Most of the taxa of this genus are native to the Mediterranean region (Azaz *et al.,* 2004). The Lamiaceae family consists of 240 genera and approximately 7200 species (Harley, 2012; Maciel *et al.,* 2022). There are more than 250 taxa belonging to the genus *Thymus* and they are divided into 8 sections. It is characteristic that the breed has a high degree of hybridization (Baser *et al.,* 1992a). This genus, which has a high polymorphic feature, is included in the Flora of Turkey with 39 species and 64 taxa, the endemism rate is 47% compared to the flora of Turkey (Elkiran & Avsar, 2020).

Some of the thyme species show remarkable health benefits that can be endorsed due to their nutritional value. The main nutrients in this species are namely vitamins, minerals, volatile oils, and antioxidants. Most of them have strong disease-preventing activities as well as health-promoting properties (Badi *et al*., 2004; Özgüven & Tansi, 1998; Penalver *et al*., 2005; Hossain *et al*., 2022).

Treatment effects such as antiseptic, expectorant, and spasmolytic are the general characteristics of essential oils and flavonoids. The studies have shown that thyme essential oils also have therapeutic properties such as antibacterial, antimycotic, antioxidant, and food

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preservatives (Gortzi *et al.,* 2006). Taxa belonging to the genus *Thymus*, which are generally called "kekik" in Turkish, have widespread use among the people for spice, herbal tea, and healing purposes (Baytop, 1999).

In the literature review, essential oil compositions of *T. longicaulis* subsp. *longicaulis* previously reported (Tümen *et al.,*1997; Azaz *et al.,* 2004; Chorianopoulos *et al.,* 2004; Sarikurkcu *et al.,* 2010). In this study, chemical similarities and differences between some taxa of the genus *Thymus* and groups of taxa were revealed by subjecting the results of essential oil analysis to clustering. In this study, in addition to the results of the current study, cluster analysis was carried out by considering the results of essential oil studies on taxa belonging to the genus *Thymus* in Turkey and different regions of the world.

This study aims to reveal the taxonomic similarities and differences by determining the chemical components of the essential oils of *T. longicaulis* subsp. *longicaulis*. At the same time, it is to reveal new results chemotaxonomical within the genus.

2. MATERIAL and METHODS

2.1. Plant materials

T. longicaulis subsp. *longicaulis* plant (Elkiran 1210) was collected during the flowering period from plants grown at Bürnük village (altitude of 1250-1300 m), Sinop-Boyabat / Turkey, in June 2016 identified by Dr. Elkiran with Flora of Turkey and East Aegean Islands (Davis, 1982). An average of 15 samples were collected for essential oil studies from aerial parts (stem, leaf, and flowers). The plant was studied at Sinop University, Turkey.

2.2. Isolation of the essential oils

The collected plant samples were dried at room temperature in a cool environment. Clevenger apparatus was used by hydrodistillation method to obtain essential oil from aerial parts (100g) of plant samples. This process to 3 hours. Essential oil yield was 0.5 (v/w). The essential oil samples were kept at 4°C until chemical composition analysis.

2.3. GC analysis

Chemical analyzes of essential oils were performed using HP 6890 GC and FID detector. HP-5 MS column was used in the study. GC-FID was used when calculating the percentages of chemical components.

2.4. GC-MS analysis

Essential oil samples taken into vials were analyzed using GC-MS with the help of the HP system. In our study, 6890 GC and HP-Agilent 5973N GC-MS systems were used. HP-5 MS column (30mx0.25mmi.d., film thickness 0.25 um) was used as the column in the system, and helium was used as the carrier gas. Injector temperature was 250°C, split flow is 1ml/min. GC oven temperature was 2 min. It was kept at 70°C, increased to 150°C in 10°C increments per minute. After 15 minutes at 150°C, it was increased to 240°C at 50°C/min. Alkanes were used as reference points in the calculation of the RRI. In MS, the Electron energy is 70 eV and the mass range is 35-425 m/z. Chemical components were identified using electronic libraries of spectrometry (WILEY, NIST). The resulting components are given in Table 1 and the chromatogram is given in Figure 1.

2.5. Hierarchical Cluster Analysis

Hierarchical clustering analysis was performed using multivariate statistical package software (MVSP) considering essential oil yields of thirty-four taxa belonging to the genus *Thymus*. Euclidean distance was selected as a measure of similarity, and the nearest neighborhood method was used for cluster definition. The thirteen major compound characters were examined to obtain cluster definitions of oils (Figure 2) (Cheng *et al*., 2006).

3. RESULTS

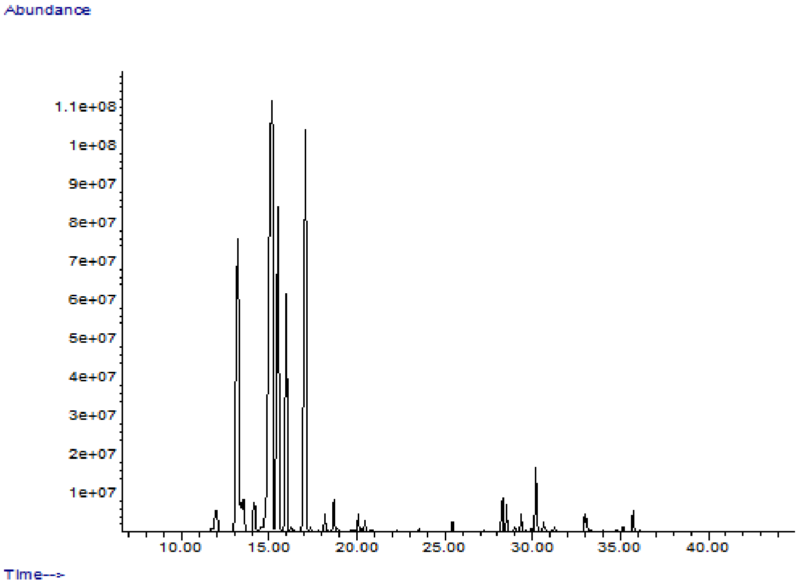
In the present work, the chemical composition of the essential oils of *T. longicaulis* subsp. *longicaulis* growing naturally in Sinop (Turkey) was determined. The chemical contents of essential oils obtained from aerial parts of plants were determined by analysis with GC and GC-MS. The amount of essential oil obtained from *T. longicaulis* subsp. *longicaulis* was 1 ml., 30 chemical components were identified, representing 98.4% of the total oil. Among the chemical components, 1,8-cineole (30.1%) has the highest concentration, while the other high components are linalool (18.0%), *β*-pinene (17.3%), and *(E)*-*β*-ocimene (12.6%) (Table 1, Figure 1).

Table 1. Constituents of the essential oils from *T. longicaulis* subsp. *longicaulis*.

Peak No	RI	Compounds	Percentage (%)
1	1107	*β*-thujene	0.1
2	1112	*α*-pinene	1.2
3	1139	*β*-pinene	17.3
4	1146	Myrcene	1.4
5	1160	*α*-phellandrene	1.3
6	1169	*α*-terpinene	0.2
7	1182	1,8-cineole	30.1
8	1190	*(E)*-*β*-ocimene	12.6
9	1200	Terpinolene	7.4
10	1206	Sabinene hydrate	0.1
11	1224	Linalool	18.0
12	1230	3-carene	0.1
13	1248	allo-ocimene	0.5
14	1260	trans-pinocarveol	0.8
15	1290	4-terpineol	0.5
16	1298	Cryptone	0.2
17	1366	Lavandulyl acetate	0.1
18	1408	Elixene	0.2
19	1470	*(E)*-caryophyllene	1.1
20	1476	*γ*-elemene	0.7
21	1485	*β*-farnesene	0.1
22	1494	Humulene	0.5
23	1507	*β*-curcumene	0.1
24	1512	*β*-cubebene	1.9
25	1522	*γ*-gurjunene	0.4
26	1536	*δ*-cadinene	0.2
27	1573	4-epi-cubebol	0.4
28	1576	Spathulenol	0.3
29	1621	tau-muurolol	0.1
30	1634	Apiol	0.5
Total			98.4

RI: Retention Indices

Figure 1. GC chromatogram of essential oil of *T. longicaulis* subsp. *longicaulis*.



A hierarchical cluster analysis was performed using the results of 34 taxa belonging to the genus *Thymus*, which essential oils were studied before, by scanning the literature (Figure 2). The chemotaxonomy and essential oil diversity of the genus were revealed according to the results of other studies compared with the results of the current study (Kilic & Bagci, 2016). Based on their common constituents, cluster analysis of the identified compounds classified the aerial part oils into five main groups, including the first group (29 to 34), the second group (18), the third group (19 and 22), the fourth group (17 and 26), and the fifth group (24) samples.

According to the results of chemotaxonomic classification made for the genus *Thymus*, it was found that thymol was the most common chemotype, and *o*-cymene and linalool chemotypes were less common. For this reason, it is possible to argue that the general chemotype of the genus *Thymus* is thymol. These results also show that the chemotype of taxa of the same genus or the same taxa can change in different environmental conditions (Table 2).

Figure 2. The dendrogram was obtained by cluster analysis of the percentage composition of essential oils of thirty-four samples of the *Thymus*.

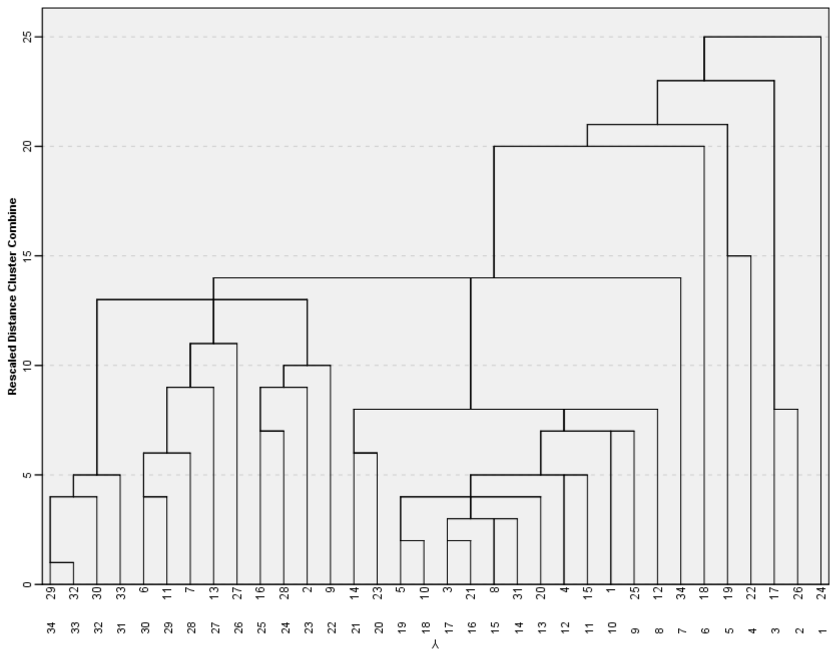


Table 2. Common constituents of *Thymus* taxa from literature and studied sample (%).

Constituents/ Plants	*p*-cymene	1,8-cineole	*γ*-terpinene	*α*-terpineol	Linalool	Geraniol	Thymol	Carvacrol	*o*-cymene	Limonene	Isothymol methyl ether	*β*-pinene	*(E)*-*β*-ocimene
1	2.26	*	2.97	*	*	*	79.74	5.49	*	*	*	*	*
2	3.11	1.20	1.96	9.60	4.02	21.85	19.88	4.37	*	0.44	*	0.16	0.71
3	6.67	2.69	0.89	2.43	0.90	0.65	52.45	6.81	*	0.33	*	0.13	*
4	1.69	2.54	0.54	7.02	0.94	*	46.01	10.20	*	0.24	*	*	*
5	2.16	1.68	0.37	1.11	0.99	0.87	63.33	12.30	*	*	*	*	*
6	1.70	2.06	0.71	5.50	2.56	8.60	19.75	37.10	*	*	*	*	*
7	1.15	1.39	0.38	7.56	8.32	2.22	16.07	42.10	*	*	*	*	*
8	6.63	1.58	2.71	0.54	0.92	3.31	58.96	8.55	*	0.41	*	0.19	*
9	2.69	0.80	1.28	0.57	16.20	22.40	25.80	11.80	*	*	*	*	*
10	0.80	1.27	0.24	1.25	1.56	1.15	64.30	17.20	*	*	*	*	*
11	2.22	4.68	1.00	4.87	1.19	5.84	12.40	36.50	*	*	*	*	0.24
12	1.25	3.37	0.44	6.78	2.96	4.75	34.30	14.90	*	*	*	*	*
13	4.52	1.99	3.17	5.18	4.26	8.80	12.60	20.90	*	0.77	*	0.24	0.59
14	12.80	0.86	10.90	1.63	2.67	0.30	39.50	4.20	*	0.87	*	0.98	0.12
15	5.50	*	6.70	*	0.30	*	55.00	19.70	*	*	*	*	*
16	1.10	1.30	*	*	5.20	34.40	*	*	*	*	*	*	*
17	3.90	*	6.70	0.20	1.80	*	0.30	76.10	*	0.30	*	0.30	*
18	*	*	4.80	0.30	*	*	0.60	9.60	30.60	6.80	7.20	1.00	2.50
19	*	30.10	*	*	18.00	*	*	*	*	*	*	17.30	12.60
20	8.50	*	10.00	*	1.30	*	58.00	2.70	*	*	*	*	*
21	5.33	1.20	*	*	3.08	*	55.42	6.84	*	0.18	*	*	*
22	6.00	35.80	1.40	3.70	3.80	3.00	0.10	1.90	*	1.20	*	2.80	0.50
23	18.70	0.30	11.40	0.10	3.70	*	48.20	3.20	*	0.50	*	0.30	0.10
24	2.40	0.20	6.90	1.60	44.40	0.10	0.10	*	*	3.00	*	0.40	0.10
25	5.07	*	8.38	*	1.01	*	69.61	3.57	*	*	*	0.43	0.02

Table 2. *Continues*

26	*	*	2.08	*	*	*	*	89.24	*	*	*	*	*
27	13.73	*	15.25	*	0.60	7.77	3.60	16.83	*	*	*	*	*
28	2.30	0.30	0.90	*	1.20	28.70	10.90	0.60	*	*	0.90	*	*
29	*	*	*	*	0.40	*	*	*	*	*	*	*	*
30	0.30	0.70	1.00	0.50	2.70	3.30	*	*	*	0.40	*	0.30	7.00
31	6.30	0.60	6.70	*	*	*	57.40	9.80	*	*	*	*	*
32	*	1.63	*	0.26	*	*	1.28	*	*	*	*	0.07	0.45
33	1.90	0.90	1.10	0.40	0.40	2.10	9.60	0.70	*	0.50	0.20	0.50	*
34	0.18	*	14.32	3.14	3.14	*	43.87	2.52	20.35	*	0.47	*	*

1: *T. migricus*; 2: *T. fallax*; 3: *T. serpyllum*; 4: *T. pubescence*; 5: *T. trautvetteri*; 6: *T. transcaspicus*; 7: *T. carmanicus*; 8: *T. daenensis*-1; 9: *T. daenensis*-2; 10: *T. daenensis*-3; 11: *T. fedtschenkoi*-1; 12: *T. fedtschenkoi*-2; 13: *T. fedtschenkoi*-3; 14: *T. vulgaris*-1 (Tohidi *et al.,* 2017); 15:*T. transcaucasicus* (Bektaş *et al.,* 2016(1)); 16: *T. dacicus* (Petrović *et al.,* 2017); 17: *T. capitatus* (Jemaa *et al.,* 2017); 18: *T. leucostomus* (Elkiran & Avsar 2020); 19: *T. longicaulis* subsp. *longicaulis*; 20: *T. nummularis* (Bektaş *et al.,* 2016(2)); 21: *T. vulgaris*-2 (Myszka *et al.,* 2016); 22: *T. hyemalis*; 23: *T. zygis*-1; 24: *T. zygis*-2 (Carrasco *et al.,* 2016); 25: *T. lanceolatus* (Khadir *et al.,* 2016); 26- *T. vulgaris*-3 (Benmoussa *et al.,* 2016); 27-*T. pulegioides* (Vaičiulytė *et al.,* 2017); 28: *T. vulgaris*-4; 29: *T. citriodorus* (Checcucci *et al*., 2016); 30: *T. alternans* (Vitali *et al.,* 2016); 31: *T. daenensis*-4 (Jarrahi *et al.,* 2016); 32: *T. bovei* (Jaradat *et al.,* 2016); 33: *T. praecox* ssp. *polytrichus* (Petrovic *et al.,* 2016); 34: *T. vulgaris*-5 (Lemos *et al.,* 2017).

4. DISCUSSION and CONCLUSION

In the study of essential oils from *T. longicaulis* subsp. *longicaulis* collected from Muğla, the total oil yield was found to be 99.61%, the major components were *γ*-terpinene, thymol, and *p*-cymene (Sarikurkcu *et al.,* 2010). Also, two different chemotypes (carvacrol (60.82%) and geraniol (27.35%)) appeared in the study of *T. longicaulis* taken at different times from Peloponnese and Greece. Linalool is among the major components in both studies (Chorianopoulos *et al.*, 2004). In a different study with *T. longicaulis* subsp. *longicaulis* var. *subisophyllus* major components were carvacrol (60.0%), thymol (7.0%), *β*-bisabolene (4.8%) and borneol (4.7%). It is among the 1,8-cineole major components in the study conducted with *T. longicaulis* subsp. *chaubardii* var. *chaubardii* samples collected from Balıkesir and *T. glabrescens* from Croatia like in our study (Kuštrak *et al.,* 1990; Azaz *et al.,* 2004). Moreover, our results differ from previous data on the essential oil of *T. longicaulis* from Serbia, Italy, and Jasenice which demonstrated *α*-terpinyl acetate (Grujic *et al.,* 2009), *p*-cymene (Napoli *et al.,* 2010), geraniol (De Martino *et al.,* 2009) and thymol (Vladimir-Knežević *et al.,* 2012) as the main components of these oils.

Tümen et al. (1997) reported that carvacrol (21.5%), *p*- cymene (17.80%), thymol (14.1%), borneol (8.32%) and *α*-terpinyl acetate (23.80%), linalool (13.6%), borneol (12.8%), thymol (11.31%) were major components of essential oils of *T. leucostomus* (Tümen *et al.,* 1997). In different studies thymol (27%), carvacrol (22%), was determined as the major constituent in the essential oil of *T. leucostomus* var. *argillaceus* (Baser *et al*., 1992b).

In a study with a different variety of essential oil compositions of *T. Leucostomus* var. *gypsaceus*, the major components were thymol (33.2%) and borneol (22.2%) (Baser *et al*., 1999). As well, *o*-cymene was also dominant for *T. vulgaris* in Brazil (Lemos et al., 2017). Elkiran and Avsar reported, *o*-cymene (30,62%), carvacrol (9.66%), isothymol methyl ether (7.22%), and limonene (6.88%) were the main components of the essential oil of *T. leucostomus* from the Sinop (Elkiran & Avsar, 2020). In our study, these components were absent. In addition, in a different study using cluster analysis, as in our study, the main component was found to be carvacrol (Napoli *et al.,* 2010). The results of the study show that the essential oils of the plant may vary according to the geographical conditions of the region where the plant is located.

According to the literature review and the results of the current study, it has been observed that there is a significant variation in the essential oil components of the genus *Thymus*. According to the results of the study, the chemotype of *T. longicaulis* subsp. *longicaulis* are 1,8-cineole, linalool, *β*-pinene and *(E)*-*β*-ocimene. In general studies, it was stated that the chemotypes and dominant compounds of the *Thymus* samples from different regions were linalool, carvacrol, 1,8-cineole, *o*-cymene, thymol, geraniol, carvacrol / *γ*-terpinene, thymol/carvacrol. These differences can be the result of different ecological properties and might have been derived from geographical factors of the plant localities.

Declaration of Conflicting Interests and Ethics

The author declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

Authorship contribution statement

Omer Elkiran: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing -original draft, Methodology, Supervision, and Validation.

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