




Determination of chemical profiles and antioxidant activity of the essential oil of *Thymus cherlerioides* Vis.

Züleyha Özer, Sema Çarıkçı, Turgut Kılıç, Selami Selvi & Ahmet C. Gören

To cite this article: Züleyha Özer, Sema Çarıkçı, Turgut Kılıç, Selami Selvi & Ahmet C. Gören (2025) Determination of chemical profiles and antioxidant activity of the essential oil of *Thymus cherlerioides* Vis., Journal of Essential Oil Bearing Plants, 28:6, 1391-1398, DOI: [10.1080/0972060X.2025.2598366](https://doi.org/10.1080/0972060X.2025.2598366)

To link to this article: <https://doi.org/10.1080/0972060X.2025.2598366>

 View supplementary material 

 Published online: 10 Dec 2025.

 Submit your article to this journal 

 Article views: 56

 View related articles 

 View Crossmark data 

Determination of chemical profiles and antioxidant activity of the essential oil of *Thymus cherlerioides* Vis.

Züleyha Özer^{1*}, Sema Çarıkçı^{2,3}, Turgut Kılıç^{4,5}, Selami Selvi⁶ and Ahmet C. Gören^{7,8*}

¹ Department of Chemistry and Chemical Processing Technologies, Altınoluk Vocational School, Balıkesir University, 10870-Edremit, Balıkesir, Türkiye

² Vocational School, İzmir Demokrasi University, 35330-Izmir, Türkiye

³ The Sustainable Environmental Studies Application and Research Centre, İzmir Demokrasi University, İzmir, Türkiye

⁴ Necatibey Education Faculty, Department of Science Educations, Balıkesir University, 10100-Balıkesir, Türkiye

⁵ Çevrimiçi A.Ş. Kozmetik Teknolojileri, Küçükbostancı Mah. Küçükbostancı İç Sokak No: 9 Altıeylül/Balıkesir, Türkiye

⁶ Department of Plant and Animal Production, Altınoluk Vocational School, Balıkesir University, 10870-Edremit, Balıkesir, Türkiye

⁷ Department Chemistry, Faculty of Basic Sciences, Gebze Technical University, 41400-Gebze Kocaeli, Türkiye

⁸ Troyasil HPLC Column Technologies, Doruk Analitik, Mehmet Akif Mah. Yumurcak Sok. No:43 Ümraniye İstanbul, Türkiye

*Corresponding Authors

Züleyha Özer
zuleyhaozer@balikesir.edu.tr
Ahmet C. Gören
acgoren@gtu.edu.tr

Received 22 August 2025

Revised 18 November 2025

Accepted 27 November 2025

Supplementary data

Figure S1 (GC-MS chromatogram of the essential oil isolated from *T. cherlerioides*), Figure S2 (*T. cherlerioides*) and Table S1 (DPPH free radical scavenging activity of the *T. cherlerioides* essential oil, BHA, BHT and curcumin) are available as part of the online supplementary file.

Abstract

Thymus species is one of the most important medicinal plants due to its ethnobotanical uses and bioactive constituents in the Mediterranean area. Although *Thymus* species are used for diverse medicinal purposes, there is limited information on *T. cherlerioides* in the literature. After hydrodistillation, the chemical composition of the essential oil extracted from the aerial parts of *T. cherlerioides*, which were collected during the flowering season in Mount Kazdağı, Balıkesir Province, was analyzed using gas chromatography/mass spectrometry (GC-MS). A total of thirty compounds were identified, accounting for 99.9% of the total oil. Oxygenated monoterpenes (97.1%) were the primary chemical class for the volatile organic compounds in the essential oil and α -terpinyl acetate (96.1%) was found to be the major compound. The antioxidant capacity of the essential oil was evaluated for the first time using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and CUPRAC assays. The essential oil of *T. cherlerioides* exhibited significant DPPH free radical scavenging activity compared to the standard compounds. This study presents the first comprehensive report on the constituents and biological activities of the essential oil of *T. cherlerioides*. This work supports the use of *T. cherlerioides* essential oil in antioxidant studies based on natural products offering new insights into its potential for use in food, medicine, and cosmetics.

Keywords

CUPRAC, DPPH, Essential oil, GC-MS, α -Terpinyl acetate, *Thymus cherlerioides*

INTRODUCTION

Essential oils have garnered scientific and popular attention due to their antioxidant, antimutagenic, antibacterial, antidiabetic, non-toxic, and antimycotic properties, which make them promising for use as bioactive compounds in health, nutrition, and food preservation. Members of the *Lamiaceae* (Labiatae) family are among the most popular medicinal plants due to their diverse secondary metabolites, especially essential oils. This family includes about 221 taxa and 192 species in Türkiye. Historically, they have been used in traditional medicine and as culinary herbs¹.

One of the most economically important members of the *Lamiaceae* family is the genus *Thymus* L.¹. Members of this genus are predominantly found in the Mediterranean region, North Africa and the Canary Islands to the Northwest of Greenland, the Ethiopian Mountains and Southern Europe. The Latin name *Thymus* originates from the Greek word “thyo,” meaning “perfume,” and is related to the Greek word “thymos” (meaning “courage” or “strength”). These plants grow in hot, sunny, and dry regions, often thriving in rocky and stony areas^{2,3}. In Türkiye, the genus *Thymus* consists of 42

species (44 taxa), and 17 of these taxa are endemic³. Members of this genus are called “kekik” in Turkish and are widely used in traditional medicine to treat digestive and respiratory diseases such as bronchitis, indigestion, expectorant, antibacterial, antifungal, asthma, nausea, chronic obstructive pulmonary disease, dysentery, and gastrointestinal disorders. Additionally, fresh or dried aerial parts of *Thymus* species are traditionally used in daily life in the form of herbal tea and spices¹. Essential oils of *Thymus* species have areas of usage in the pharmaceutical and food industries due to numerous biological activities such as antioxidant, antimicrobial, insecticidal, antiprotective, antibacterial properties and they also exhibit cytotoxic effects on some cancer cells⁴.

To determine the antioxidant capacity of plant materials, it is important to characterize their chemical components. *Thymus* species are rich sources of various natural antioxidant compounds, including phenolics and terpenoids⁴. Several studies have demonstrated the antioxidant properties of *Thymus* essential oils and aqueous extracts through various *in vitro* tests⁵. In particular, the antioxidant properties of *Thymus* essential oils make them valuable raw materials for improving natural antibiotic formulations, as well as for potential applications in the cosmetic industry⁴.

Numerous studies on species of the genus *Thymus* have been conducted because of the intensive use of these species as folk medicine. The studies mainly focus on volatile components and their biological activities. Terpenes, terpene alcohols, esters, and phenolic derivatives were identified as the main compounds in the *Thymus* essential oils⁵. *Thymus* essential oils are characterized by high concentrations of thymol and/or carvacrol, which are isomeric phenolic monoterpenes. A total of 34 *Thymus* species grown in Türkiye have been reported to exhibit a range of chemotypes, including thymol, carvacrol, linalool, geraniol, α -terpinyl acetate, β -caryophyllene, borneol, linalyl acetate, and *p*-cymene⁶. The chemical composition of the essential oil of *Thymus* species may be influenced by factors such as developmental stage, geographic area, harvest season, and habitat. It was previously reported that the oil of *T. migricus* contained α -terpineol, thymol, and α -terpinyl acetate; *T. fallax* is rich in *cis*-carveol (29.6%) and α -terpineol (10.8%); *T. pubescens* var. *pubescens* contains carvacrol, *p*-cymene, and (*E*)- β -ocimene⁷. Also, as in a previous review, the chemical composition of the essential oil of various *Thymus* species from Iran

and their biological activities, with a focus on the microbial and antioxidant properties, were reported⁴. Moreover, there are many studies in the literature about the phenolic compounds and bioactivities of the extracts of *Thymus* species⁴.

The species *T. cherlerioides* Vis., a perennial aromatic herb endemic to specific high-altitude regions such as Anatolia and parts of the eastern Mediterranean, is characterized by its compact morphology, small glandular leaves, and high essential oil content. *T. cherlerioides*, which is a rare species, named “Kazkekiği” in the vernacular, grows especially in Mount Kazdağı (Ida)². In previous studies, the essential oil of *T. cherlerioides* var. *isauricus* Jalas was reported to be rich in α -linalool, borneol, γ -terpinene and *p*-cymene rich⁸. Özek *et al.*⁹ reported that (-) and (+)-linalool were isolated from the essential oil of *T. cherlerioides*. Moreover, micromorphological and anatomical studies of *T. cherlerioides*² and genetic and taxonomic studies of *T. cherlerioides* var. *isauricus*¹⁰ were reported in the literature. Our preliminary phytochemical investigations indicate that this species produces a unique profile of monoterpenes and phenolic compounds, which may differ from those of the more extensively reported data of the species and the genus⁸. Such chemical variability makes *T. cherlerioides* a promising candidate for further exploration in food preservation, natural product chemistry, and medicinal plant research.

Due to the limited number of comprehensive studies on the phytochemical and biological properties of *T. cherlerioides*, this study aimed to determine the essential oil composition and antioxidant capacity of this species from the Ida Mountain (Kaz Dağı). Thus, we aimed to create a dataset for the sustainable use of *T. cherlerioides* in pharmaceutical and industrial applications.

MATERIALS AND METHODS

Plant material

The aerial parts of *T. cherlerioides* were collected during the flowering stage in June 2023 from B1 Balıkesir: Sarıkız peak, bare areas, 1674 m, 39°41.484'N, 26°52.546'E, Türkiye. Identification of the species was carried out by Dr. Selami Selvi. The voucher sample is stored at the Altınoluk Vocational School, Balıkesir University (AMYO 212). The plant samples were dried in the shade at room temperature for 15 days.

Isolation of the essential oil

The dried aerial parts of *T. cherlerioides* (120 g) were chopped and subjected to hydrodistillation for 4 hours using a modified Clevenger-type apparatus and the extract was collected in a 5 mL vial. The Clevenger apparatus walls were washed with diethyl ether (2 x 1 mL) via a glass Pasteur pipette and collected in the same vial. The essential oil was dried over anhydrous CaCl₂, freed of solvent and, approximately 0.58 mL of essential oil was stored at +4°C until experimental procedures were performed.

Determination of the chemical composition

Fifty µL of the essential oil was dissolved in 1400 µL dichloromethane, and the prepared solution was analyzed using Thermo Scientific Trace GC 1300 connected to Thermo TSQ 9610 MS-MS system on a DB-5 (Restek Corp., Phase: RTX-5 SUMS) capillary column (30 m x 0.25 mm, 0.25 µm film thickness) and helium as carrier gas (1.0 mL/min). GC oven temperature was maintained at 40°C for 1 min, increased to 250°C at a rate of 3°C/min, and then held constant at 250°C for 10 min. The total analysis time was 81 min. The split ratio was set at 30:1. One µL of the solution was injected twice. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 20 to 650¹¹.

GC-FID analysis was also performed using the same Thermo Scientific Trace GC 1310 system, with the FID detector set at 280°C by splitting the injection on the instrument. To ensure consistency with GC-MS data, simultaneous duplicate auto-injections were conducted under identical conditions using the same column. Compound percentages were calculated from FID data. Alkanes (C₆-C₂₄) were used as reference points in the calculation of relative retention indices of the compounds (RI). Compound identification was based on co-injections with the terpene mixture Ultra Kit WRK 105, as well as comparison of retention times and mass spectra with those obtained from standard samples and/or with NIST and Wiley spectra and literature data. Relative percentages of separated compounds were calculated from total ion chromatography and GC-FID chromatogram with a computerized integrator^{12,13}.

Antioxidant activity

DPPH free radical scavenging method

The free radical scavenging activity of *T.*

cherlerioides essential oil was determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay^{14,15}. DPPH absorbs at 517 nm in its radical form, but upon reduction by an antioxidant or a radical species, its absorption decreases. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 160 µL of this solution was added to 40 µL of sample solutions in methanol at 10, 25, 50 and 100 µg/mL concentrations. The mixtures were kept in the dark for 30 min. The absorbance was measured at 517 nm. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as standard compounds.

CUPRAC method

The reducing capacity of *T. cherlerioides* essential oil was evaluated using the CUPRAC method¹⁶. Solutions of 1 mM DMF, 10 mM CuCl₂, 7.5 mM neocuproine, 1 M NH₄-CH₃COO (pH 7.0), and distilled water were mixed in a 1:1:1:0.6 volume ratio. Afterward, 180 µl of the mixture was added into the wells, followed by 25 µL diluted compounds (1:20) in ethanol. The samples were held for 30 min at 25°C. The absorbance was measured at 450 nm against a reagent blank. EtOH was used as a negative control and curcumin as a positive control.

Statistical analyses

The results are presented as means±standard deviation, based on three replicates for each measurement. Statistical analyses were performed to evaluate antioxidant activity results using a one-way ANOVA test (GraphPad, Software 8.3.0). *P* < 0.05 was considered the minimum level of significance. IC₅₀ values were determined by plotting the dose-response data and mathematically finding the concentration that results in 50% inhibition (Microsoft Excel 365). Due to the very good repeatability values obtained in GC-MS and GC-FID analyses (<0.2%), it was not necessary to apply any correction factor when reporting the percentage composition of the compounds.

RESULTS AND DISCUSSION

The essential oil of *T. cherlerioides* was obtained by hydrodistillation using a Clevenger-type apparatus, and yielding 0.59 mL of the essential oil (0.49% w/w on a dry weight basis). The essential oil of *T. cherlerioides* was subjected to GC-MS analysis using a RTX-5 SUMS column. The use of gas chromatography in conjunction with mass spectrometry facilitates the acquisition of both qualitative and quantitative data.

Table 1 summarizes the composition of essential oil from aerial parts of *T. cherlerioides*, including percentage content, retention indices and literature retention indices. Thirty compounds were identified in the essential oil of *T. cherlerioides*, which constituted 99.8% of the total essential oil extracted. Oxygenated

monoterpenes (97.1%) predominated, followed by sesquiterpene hydrocarbons (2.7%). *T. cherlerioides* essential oil was found as α -terpinyl acetate (96.1%) type, which is known for its antioxidant¹⁷, antifungal, antimicrobial¹⁸, insecticidal¹⁹, acetylcholinesterase, butyrylcholinesterase²⁰, and phytotoxicity²¹ activities.

Table 1. The chemical composition of the essential oil of *T. cherlerioides*

No	RI ^a	RI ^b	Compound*	Composition \pm SD (%) ^c	Identification method
1	928	932	α -Pinene	t	RI, MS, ST
2	944	946	Camphene	t	RI, MS, ST
3	1031	1026	Eucalyptol	t	RI, MS, ST
4	1064	1054	γ -Terpinene	t	RI, MS, ST
5	1147	1141	(-)-Camphor	t	RI, MS
6	1170	1165	Borneol	0.451 \pm 0.001	RI, MS
7	1182	1162	4-Terpineol	t	RI, MS
8	1196	1186	α -Terpineol	t	RI, MS
9	1284	1284	Bornyl acetate	0.551 \pm 0.001	RI, MS
10	1300	1289	Thymol	t	RI, MS, ST
11	1308	1298	Carvacrol	t	RI, MS, ST
12	1352	1346	α -Terpinyl acetate	96.151 \pm 0.198	RI, MS
13	1372	1374	α -Copaene	t	RI, MS
14	1379	1387	β -Bourbonene	0.623 \pm 0.001	RI, MS
15	1388	1389	β -Elemene	t	RI, MS
16	1414	1417	(-)-Caryophyllene	1.322 \pm 0.003	RI, MS
17	1432	1432	<i>trans</i> - α -Bergamotene	t	RI, MS
18	1440	1439	Aromadendrene	t	RI, MS
19	1450	1452	α -Humulene	t	RI, MS
20	1455	1458	<i>allo</i> -Aromadendrene	0.051 \pm 0.000	RI, MS
21	1472	1478	γ -Muuroolene	0.052 \pm 0.000	RI, MS
22	1477	1484	Germacrene-D	0.522 \pm 0.001	RI, MS
23	1496	1500	α -Muuroolene	t	RI, MS
24	1507	1505	β -Bisabolene	t	RI, MS
25	1517	1513	γ -Cadinene	0.221 \pm 0.001	RI, MS
26	1524	1521	β -Sesquiphellandrene	t	RI, MS
27	1578	1582	β -Caryophyllene oxide	0.051 \pm 0.000	RI, MS, ST
28	1640	1638	τ -Cadinol	t	RI, MS
29	1643	1644	τ -Muurolol	t	RI, MS
30	1655	1652	α -Cadinol	t	RI, MS
Monoterpene hydrocarbons (1, 2, 4)				t	
Oxygenated monoterpenes (3, 5-12)				97.153	
Sesquiterpene hydrocarbons (13-26)				2.791	
Oxygenated sesquiterpene (27-30)				0.051	
Total area sum				99.990	

t = trace; ^aRetention index calculated; ^bRetention index of reference³⁴; ^cGC-MS/FID analyses were replicated three times; *Ultra Kit WRK 105 terpene mixture was used in co-injection experiments. ST: Standard co injection, Eucalyptol, thymol and caryophyllene oxide were isolated by our group from plant species, previously

Different percentages of α -terpinyl acetate have been found in various *Thymus* species. α -Terpinyl acetate, a secondary metabolite, is a monoterpene ester, that can be found in some *Thymus* species such as *T. praecox* ssp. *skorpilii* var. *skorpilii*⁶, *T. migricus*⁷, *T. pulegioides*²¹, *T. munbyanus* subsp. *ciliatus*, *T. zygoides*, *T. willkommii*²¹, *T. serpyllum*²², *T. zygis*²³, *T. praecox* subsp. *jankae*²⁴, *T. munbyanus* subsp. *abylaeus*²⁵, *T. glabrescens*, *T. moesiacus*, *T. pannonicus*, *T. tosevii* ssp. *tosevii*, *T. longicaulis*, *T. macedonicus* spp. *macedonicus*²⁶. α -Terpinyl acetate is a commercially important fragrance compound with a soft, herbal, floral, and lavender scent. It is widely used as a fragrance component in the production of shampoos, soaps, antiperspirants, and creams. Another use of α -terpinyl acetate is as a flavor additive in food products, including beverages, berry ice creams, gelatin, baked goods, puddings, and chewing gum. Therefore, the search for new natural sources of this compound is highly important.

Chemical polymorphism is typical of *Thymus* species, where different individuals of the same species can synthesize oils with varying chemical compositions. *Thymus* species use glandular trichomes to biosynthesize and store their essential oils. As biosynthetically active site of various significant natural compounds across multiple plant species, these glandular trichomes can synthesize, store, and excrete a diversity of natural compounds. For example, glandular trichomes on the leaf and stem surfaces of *T. pulegioides* were identified as capitate and peltate types, which are consistent with the glandular trichomes of *T. cherlerioides*².

The chemical profile of the essential oil of *T. cherlerioides* does not match that of some *Thymus* species reported in the literature. For example, the phenolic monoterpenes - thymol and carvacrol were determined as the main chemical classes of *T. capitatus*⁵, *T. vulgaris*, *T. serpyllum*²⁷, *T. haussknechtii*²⁸, *T. longicaulis* subsp. *chaubardii* var. *chaubardii*, *T. zygoides* var. *lycaonicus* and *T. longicaulis* subsp. *longicaulis* var. *subisophyllus*²⁹ essential oils. When we examined the previous studies of the species of the genus, we found that the thymol content was reported between 30.0% and 91.8%, while the carvacrol content was 2.1-62.7% in *T. vulgaris* and *T. serpyllum*²⁶. By contrast, the *T. haussknechtii* and *T. longicaulis* subsp. *longicaulis* var. *subisophyllus* were reported to contain thymol (52.2%) and carvacrol (60.0%), respectively^{28,29}.

In addition, the content of thymol in *T. zygis* subsp. *gracilis* and *T. vulgaris* amounts to 22.3-43.3% and 38.1%, respectively³⁰. In another study, the main component identified in the essential oil of *T. broussonnetii* was carvacrol (61.22%)³¹. The main components of *T. pectinatus* was reported to be thymol (52.2%)³². Monoterpenes of *Thymus* species are divided into two groups: phenolic (thymol and carvacrol) and nonphenolic (linalool, *p*-cymene, and α -terpinenol). It has been found that the generation of phenolic compounds is higher in warmer and drier climates, while nonphenolic compounds are generally produced in higher amounts in colder areas³³. Tümen et al.⁶ reported that essential oils of *Thymus* species contain high concentrations of carvacrol and thymol, which are isomeric phenolic terpenes⁶. Linalool is the third most important compound for the genus *Thymus*. In the oils of 56 taxa, it is present at percentages exceeding 10 percent^{6,33}.

Although it is normal for multiple environmental factors such as soil composition, altitude, growing conditions, seasonal effects, and temperature to cause variations in the quantity and quality of secondary metabolites produced by *Thymus* species. Chemical composition analysis reveals a significant dominance of α -terpinyl acetate, distinguishing *T. cherlerioides* collected from Kaz Dağı as a potentially new chemotype within the genus *Thymus*. While the dominant components in most of the previously reported *Thymus* species were generally reported as thymol, carvacrol, *p*-cymene or γ -terpinene, almost all of the essential oil obtained from *T. cherlerioides* species (96.1%) was determined as α -terpinyl acetate²⁷⁻³³. Therefore, the identification of α -terpinyl acetate as the major compound suggests that this species represents a distinct chemical profile that may reflect both genetic differentiation and ecological adaptation specific to its native high-altitude habitat.

Pharmacologically, α -terpinyl acetate exhibits anti-inflammatory, analgesic, and sedative properties, and there are additional reports of antifungal and antibacterial activities¹⁷⁻²¹. Therefore, the dominance of α -terpinyl acetate in *T. cherlerioides* may offer new opportunities to researchers and traditional medicine practitioners in their efforts to develop natural preservatives, therapeutic essential oil blends, or environmentally friendly fragrance raw materials.

The antioxidant capacities of the essential oil of *T. cherlerioides* were determined using Trolox Equivalent Antioxidant Capacity (TEAC) values, as

measured by the CUPRAC method which utilizes Cu^{2+} -neocuproine reagent as a chromogenic oxidizing reactant. For CUPRAC values, a higher value indicates potent antioxidant activity. In this case, the Cu^{2+} reducing capacity of the essential oil of *T. cherlerioides* ($1.32 \text{ mmol TR g}^{-1}$) was found to be lower than that of curcumin ($2.44 \text{ mmol TR g}^{-1}$). In the CUPRAC method, the essential oil of *T. cherlerioides* showed moderate antioxidant activity compared to the standard compound as well. Consequently, the DPPH free radical scavenging activity of the essential oil of *T. cherlerioides* was found to be significantly higher than the Cu^{2+} reducing capacity (Table 2) with the IC_{50} $13.26 \pm 4.53 \text{ mg/mL}$ which is comparable with the curcumin data ($14.21 \pm 6.46 \text{ } \mu\text{g/mL}$). This relatively low IC_{50} value confirms that the oil has a potent ability to donate hydrogen atoms or electrons to neutralize DPPH radicals.

Overall, the antioxidant data indicate that *T. cherlerioides* essential oil, despite lacking phenolic monoterpenes, demonstrates competitive antioxidant performance relative to synthetic standards and curcumin. It is well known that antioxidant capacity depends on chemical composition, so good antioxidant activity of the essential oil of *T. cherlerioides* is related to the content of α -terpinyl acetate (96.1%). Compared to previous studies on the essential oil of some species within the same genus, the essential oil of aerial parts of *T. cherlerioides* exhibits a lower DPPH free radical scavenging capacity. For instance, *T. vulgaris* essential oil has over 50% inhibition on DPPH free radical at higher than 3 mg/mL doses³⁵. Also, another study reported an inhibition activity of 87.69-94.15% for *T. longicaulis* subsp. *longicaulis* var. *longicaulis*³⁶. This chemotype, rich in oxygenated monoterpenes, may represent a valuable natural alternative for antioxidant applications in food preservation, pharmaceuticals, and cosmetics. In the ABTS free radical scavenging assay, the essential oil of *Elettaria cardamomum*, which is rich in α -terpinyl acetate, exhibited higher antioxidant potential ($\text{IC}_{50} = 19.87 \text{ } \mu\text{g/mL}$) than the other two varieties¹⁷. Similarly, in the DPPH assay, the

essential oil showed strong antioxidant activity ($\text{IC}_{50} = 13.26 \pm 4.53 \text{ } \mu\text{g/mL}$), further highlighting that the high α -terpinyl acetate content contributes significantly to the overall antioxidant potential of this chemotype.

In the literature, synthetic antioxidants such as BHA and BHT have been reported to exhibit DPPH IC_{50} values of $4.87\text{-}30.62 \text{ } \mu\text{g/mL}$ ³⁷, while curcumin shows an IC_{50} of $11.8 \text{ } \mu\text{M}$ ³⁸. Among thymol- and carvacrol-rich *Thymus* species, *T. vulgaris* from Morocco (thymol, 50 %) was reported with a DPPH IC_{50} of $7.12 \pm 0.21 \text{ } \mu\text{g/mL}$ ³⁹, and *T. canoviridis* showed an IC_{50} of $6.12 \pm 0.03 \text{ } \mu\text{g/mL}$ ⁴⁰. In comparison, the α -terpinyl acetate-rich *T. cherloides* essential oil in this study exhibited a DPPH IC_{50} value of $13.26 \pm 4.53 \text{ } \mu\text{g/mL}$, indicating moderate antioxidant activity relative to these reported reference values and thymol and carvacrol rich essential oils.

CONCLUSIONS

This study provides the first detailed chemical and antioxidant analysis of the essential oil from *T. cherlerioides* aerial parts collected from Kazdağı in Türkiye. GC-MS and GC-FID analyses identified α -terpinyl acetate as the main component, with a high proportion of oxygenated monoterpenes (97.0%). The essential oil of *T. cherlerioides* displayed remarkable antioxidant capacity with DPPH and CUPRAC assays. These findings suggest that *T. cherlerioides* essential oil has significant promise for use as a natural antioxidant agent in food, pharmaceutical, and cosmetic industries. Moreover, α -terpinyl acetate has been reported to possess a wide range of biological activities, including antimicrobial, antioxidant, antibacterial, antifungal, insecticidal, anti-Alzheimer's, enzymatic, and phytotoxicity properties. Additionally, the significant presence of α -terpinyl acetate in the essential oil further supports the potential applications of these extracts in medicine, fragrance, and flavouring. The chemical results of this research could be useful for chemotaxonomy, cultivation, and potential applications of *Thymus* taxa. Summarizing the

Table 2. Antioxidant activity of the *T. cherlerioides* essential oil, BHA, BHT and curcumin

	Essential oil	BHA	BHT	Curcumin
DPPH IC_{50} ($\mu\text{g/mL}$)	13.26 ± 4.53	23.52 ± 11.56	43.94 ± 11.25	14.21 ± 6.46
TEAC _{CUPRAC}	1.32	-	-	2.44

IC_{50} values are mean \pm SD (n = 3)

antioxidant results as moderate relative to standards and thymol/carvacrol-rich oils.

ACKNOWLEDGMENTS

This work was supported by the Balıkesir University Scientific Research Projects Unit (BAP 2023/139).

AUTHOR CONTRIBUTION STATEMENT

Z.Ö.: Isolation of essential oil, DPPH experiments and writing the draft of the manuscript. S.C.: GC-MS and CUPRAC tests. T.K.: Collection of plant species. S.S.: Collection of plant species and identification of the species. A.C.G.: Planning the study, GC-MS measurements, data evaluation, manuscript writing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding this article.

REFERENCES

1. Selvi, S., Polat, R., Çakılcıoğlu, U., Celep, F., Dirmenci, T. and Ertuğ, Z.F. (2022). An ethnobotanical review on medicinal plants of the Lamiaceae family in Türkiye. *Turk. J. Bot.* 46(4): 283-332.
2. Selvi, S., Açar, M. and Satıl, F. (2013). Comparative micromorphological and anatomical investigations on *Thymus pulvinatus* and *T. cherlerioides* (Lamiaceae) growing in Kazdağı (Edremit-Balıkesir/Türkiye). *Bio. Di. Con.* 6: 12-20.
3. Yıldız, B. (2024). *Thymus*. In *Bizimbitkiler 2013*. Retrieved September 23, from <http://www.bizimbitkiler.org.tr>.
4. Tohidi, B., Rahimmalek, M. and Trindade, H. (2019). Review on essential oil, extracts composition, molecular and phytochemical properties of *Thymus* species in Iran. *Ind. Crop. Prod.* 134(2019): 89-99.
5. Mavis, M., Ali, M.S.B., Hanoglu, A., Ozalp, Y., Yavuz, D.O., Baser, K.H.C. and Serakinci, N. (2023). Evaluation of therapeutic role of *Thymus capitatus* (L.) Hoffm. & Link, *Origanum dubium* Boiss. essential oils and their major constituents as enhancers in cancer therapy. *Rec. Nat. Prod.* 17(4): 715-720.
6. Tümen, G., Kirimer, N. and Baser, K. (1995). Composition of the essential oils of *Thymus* species growing in Türkiye. *Chem. Nat. Compd.* 31(1): 55-60.
7. Küçükbay, F.Z., Kuyumcu, E., Çelen, S., Azaz, A.D. and Arabacı, T. (2014). Chemical composition of the essential oils of three *Thymus* taxa from Türkiye with antimicrobial and antioxidant activities. *Rec. Nat. Prod.* 8(2): 110-120.
8. Karaca, M., Elmasulu, S., Kürkçüoğlu, M., Ince, A.G., Çınar, A., Onus, A. and Turgut, K. (2011). Major volatile compounds of 50 *Thymus* taxa naturally grown in Antalya region of Türkiye. *Planta. Med.* 77(12): PE12.
9. Özek, T., Tabanca, N., Demirci, F., Wedge, D.E. and Baser, K.H.C. (2010). Enantiomeric distribution of some linalool containing essential oils and their biological activities. *Rec. Nat. Prod.* 4(4): 180-192.
10. Karaca, M., Ince, A.G., Aydın, A., Elmasulu, S.Y. and Turgut, K. (2015). Microsatellites for genetic and taxonomic research on thyme (*Thymus* L.). *Turk. J. Biol.* 39(1): 147-159.
11. Goren, A.C., Topcu, G., Bilsel, G., Bilsel, M., Wilkinson, J.M. and Cavanagh, H. (2004). Analysis of essential oil of *Satureja thymbra* by hydrodistillation, thermal desorber, and headspace GC/MS techniques and its antimicrobial activity. *Nat. Prod. Res.* 18(2): 189-95.
12. Altun, M. and Goren, A.C. (2007). Essential oil composition of *Satureja cuneifolia* by simultaneous distillation-extraction and thermal desorption GC-MS techniques. *J. Essent. Oil-Bear. Plants.* 10(2): 139-144.
13. Altun, M., Ünal, M., Kocagöz, T. and Gören, A.C. (2007). Essential oil compositions and antimicrobial activity of *Salvia* species. *J. Essent. Oil-Bear. Plants.* 10(3): 251-258.
14. Karageçili, H., Polat, T., Yılmaz, M.A., Fidan, M., Karaismailoğlu, M.C. and Gülçin, İ. (2024). Evaluation of the antioxidant, antidiabetic and anti-alzheimer effects of *Capsella bursa-pastoris*-polyphenolic profiling by LC-MS/MS. *Rec. Nat. Prod.* 18(6): 643-662.
15. Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature.* 181(4617): 1199-1200.
16. Apak, R., Güçlü, K., Özyürek, M. and Karademir, S.E. (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchim. Acta.* 160(4): 413-419.
17. Alam, A., Majumdar, R.S. and Alam, P. (2019). Development of HPTLC method for determination of α -terpinyl acetate, and evaluation of antioxidant properties of essential oils in *Elettaria cardamomum*. *Trop. J. Pharm. Res.* 18(10): 2139-2145.
18. Singh, V., Agrawal, G.P. and Singh, B. (2024). GC-MS Analysis of bio-Active compound α -Terpinyl acetate extract of bay leaf having antimicrobial and antifungal properties. *Flora and Fauna.* 35(1): 22-30.
19. Song, H.J., Yong, S.H., Kim, H.G., Kim, D.H., Park, K.B., Shin, K.C. and Choi, M.S. (2022). Insecticidal activity against *Myzus persicae* of terpinyl acetate and bornyl acetate in *Thuja occidentalis* essential oil. *Horticulturae.* 8(10): 969.
20. Chowdhury, S. and Kumar, S. (2020). Alpha-terpinyl acetate: A natural monoterpenoid from *Elettaria cardamomum* as multi-target directed ligand

- in Alzheimer's disease. *J. Funct. Foods.* 68(2020): 103892.
21. **Vaičiulytė, V., Ložienė, K., Švedienė, J., Raudonienė, V. and Paškevičius, A. (2021).** α -Terpinyl acetate: Occurrence in essential oils bearing *Thymus pulegioides*, phytotoxicity, and antimicrobial effects. *Molecules.* 26(4): 1065.
 22. **Nikolić, B., Matović, M., Mladenović, K., Todosijević, M., Stanković, J., Đorđević, I., Marin, P.D. and Tešević, V. (2019).** Volatiles of *Thymus serpyllum* obtained by three different methods. *Nat. Prod. Commun.* 14(6): 1-3
 23. **Pérez-Sánchez, R., Ubera, J.L., Lafont, F. and Gálvez, C. (2008).** Composition and variability of the essential oil in *Thymus zygis* from Southern Spain. *J. Essent. Oil Res.* 20(3): 192-200.
 24. **Stojanović, G., Jovanović, O., Petrović, G., Mitić, V., Jovanović, V. S. and Jovanović, S. (2014).** Composition of headspace volatiles and essential oils of three *Thymus* species. *Nat. Prod. Commun.* 9(11): 1609-1612.
 25. **Benomari, F.Z., Djabou, N., Moumani, M., Hassani, F., Muselli, A. and Costa, J. (2020).** Chemical variability of essential oils of three subspecies of *Thymus munbyanus* Boiss. & Reut. from Western Algeria. *J. Essent. Oil Res.* 32(5): 474-484.
 26. **Čavar Zeljković, S. and Maksimović, M. (2015).** Chemical composition and bioactivity of essential oil from *Thymus* species in Balkan Peninsula. *Phytochem. Rev.* 14(3): 335-352.
 27. **Kulisic, T., Radonic, A. and Milos, M. (2005).** Antioxidant properties of thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) essential oils. *Ital. J. Food Sci.* 17(3): 315-324.
 28. **Sevindik, H.G., Özek, T., Yerdelen, K.Ö., Önal, M., Özbek, H., Güvenalp, Z. and Demirezer, L.Ö. (2016).** Chemical composition, antioxidant capacity, acetyl- and butyrylcholinesterase inhibitory activities of the essential oil of *Thymus haussknechtii* Velen. *Rec. Nat. Prod.* 10(4): 503-507.
 29. **Azaz, A.D., Irtem, H.A., Kurkcuoğlu, M. and Baser, K.H.C. (2004).** Composition and the in vitro antimicrobial activities of the essential oils of some *Thymus* species. *Z. Naturforsch. C.* 59(1-2): 75-80.
 30. **Nieto, G. (2020).** A review on applications and uses of *Thymus* in the food industry. *Plants.* 9(8): 961.
 31. **El-Bouzidi, L., Ouchtiti, H., Ben-Bakrim, W., Benbnidak, K., Mounir, A., Khadra, A., El-Abbassi, A. and Bekkouche, K. (2025).** Cytogenotoxicity assessment of the essential oils from aerial parts of *Thymus pallidus* Batt, *Thymus satureioides* Cosson, and *Thymus broussonnetii* Boiss. *J. Essent. Oil-Bear. Plants.* 28(3): 579-590.
 32. **Aksit, H., Bayar, Y., Simsek, S. and Ulutas, Y. (2022).** Chemical composition and antifungal activities of the essential oils of *Thymus* species (*Thymus pectinatus*, *Thymus convolutus*, *Thymus vulgaris*) against plant pathogens. *J. Essent. Oil-Bear. Plants.* 25(1): 200-207.
 33. **Stahl-Biskup, E. (2002).** Essential oil chemistry of the genus *Thymus*-a global view. In *Thyme* (pp. 89-138). CRC Press.
 34. **Adams, R.P. (2017).** Identification of essential oil components by gas chromatography/mass spectrometry (ed. 4.1). Allured Publ Crop Carol Steam, IL.
 35. **Grigore, A., Paraschiv, I.N.A., Colceru-Mihul, S., Bubueanu, C., Draghici, E. and Ichim, M. (2010).** Chemical composition and antioxidant activity of *Thymus vulgaris* L. volatile oil obtained by two different methods. *Rom Biotech Lett.* 15(4): 5436-5443.
 36. **Sarikurkcu, C., Ozer, M.S., Eskici, M., Tepe, B., Can, Ş. and Mete, E. (2010).** Essential oil composition and antioxidant activity of *Thymus longicaulis* C. Presl subsp. *longicaulis* var. *longicaulis*. *Food Chem. Toxicol.* 48(7): 1801-1805.
 37. **Çınar, E. and Topal, G. (2022).** Syntheses of some hydrazones derived from 2-(aryloyloxy) benzaldehydes and 2, 4-dinitrophenylhydrazine and evaluation of their anticholinesterase and antioxidant activities. *Org. Commun.* 15(4): 324-332.
 38. **Girst, G., Ötvös, S.B., Fülöp, F., Balogh, G.T. and Hunyadi, A. (2021).** Pharmacokinetics-driven evaluation of the antioxidant activity of curcuminoids and their major reduced metabolites- A medicinal chemistry approach. *Molecules.* 26(12): 3542.
 39. **Chahboun, N., Barrahi, A., Koursaoui, L., Mortada, S., Fahsi, K., Flouchi, R., Karrouchi, K., Faouzi, M.E.A., El Faydy, M., Abuelizz, H.A. and Thakur, A. (2024).** Chemical composition, biological activities, and anti-corrosion performance of Moroccan essential oil *Thymus vulgaris* from the Oued Amlil region, Taza. *Int. J. Electrochem. Sci.* 19(12): 100859.
 40. **Yiğitkan, S. and Fırat, M. (2024).** Essential oil contents and biological activities of *Thymus canoviridis* jalas and *Thymus sipyleus* Boiss. *J. Fac. Pharm. Ankara.* 48(3): 1068-1077.