Comparative phytochemical profiling and bioactivities of wild Thymus species from Mount Ida (Kazdağı)

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Abstract

Wild Thymus species growing in the Kazdağları region of Türkiye possess rich profiles and biological effects that have not yet been fully characterized. This study presents the first integrated phytochemical characterization and multiple biological activity assessment of four of these species. In the analyses conducted for this purpose, Thymus citriodorus showed the highest Total Antioxidant Capacity values. Rosmarinic acid was the most dominant phenolic acid measured by HPLC in all samples (5.89–9.23mg/g). A total of 143 volatile compounds were identified by GC-MS, the main one being Thymol (highest 31.12mg/kg). Using the disk diffusion method, Thymus bornmuelleri and Thymus sibthorpii showed the largest inhibition sites against S. aureus, while Thymus longicaulis showed the largest inhibition site against P. aeruginosa. In the WST-1 cytotoxicity test against the HT29 human colorectal adenocarcinoma cell line, all four extracts reduced cell viability in a concentration-dependent manner for 72h. (up to 10.15% at 2000µg/mL).

1. Introduction

Some plants have been studied as natural antioxidant sources because they are rich in natural products such as polyphenols, vitamins, polysaccharides, and minerals [1, 2]. Various studies have shown that the consumption of natural antioxidant compounds protects cells against damage from reactive oxygen species such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite [3]. Numerous studies have shown that some plants and plant extracts are as effective as synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which can have carcinogenic effects on living organisms [4, 5]. In particular, thyme extracts appear to possess very beneficial antioxidant properties, associated with their volatile oil and phenolic compound content [6–8].

Garden thyme (Thymus vulgaris L.) and wild thyme (Thymus serpyllum) belong to the Lamiaceae family and are among the most important herbs for their aromatic and medicinal properties, known to the ancient Egyptians, Greeks, and Romans [9]. This spice is noteworthy for its natural antioxidant content, beneficial physiological functions, and antimicrobial activities. Often used as a seasoning, thyme enriches the flavor of oily and heavy dishes and facilitates digestion [10]. The plant parts of Thymus species are aromatic plants known as "Kekik" in Türkiye [11]. Due to its semi-shrubby nature and attractive flowers, it is used as an ornamental plant, and due to its active ingredients, it is used in food preservation and in the fight against bee diseases. Furthermore, thanks to its insect repellent or lethal properties, it is effective in controlling warehouse pests and nematodes [12].

In terms of phytochemicals, volatile compounds are one of the most important characteristics of thyme species and can greatly influence consumer acceptance of thyme and related food products. Wild thyme (Thymus serpyllum) is considered a source of volatile compounds containing sesquiterpenes, alcohols, aldehydes, ketones, esters, and acids [13, 14]. Kulisic et al. [15] reported the presence of eight phenolic phytochemicals in wild thyme extract, with rosmarinic acid being the main compound among the identified phenolics.

Thyme is important to the food and pharmaceutical industries due to its health benefits related to antioxidant, antimicrobial, antispasmodic, and antihypertensive effects; it is also beneficial in the treatment of respiratory and gastrointestinal disorders [16–18]. The most important compounds synthesized by this plant are carvacrol and thymol, which not only possess antioxidant properties but also inhibit microorganisms by reducing vital intracellular substances, disrupting bacterial enzyme systems, and increasing cell membrane permeability [19]. Ertürk et al. [20] investigated the antimicrobial effect of commercially available thyme essential oils on clinically significant bacteria and yeasts and found that thyme oil showed strong antimicrobial activity against all tested microorganisms except Pseudomonas aeruginosa.

In this study, the total antioxidant capacity, total phenolic content, volatile components, and phenolic content of wild thyme species locally known as “Mountain thyme” (Thymus longicaulis C.), “Lemon thyme” (Thymus citriodorus), “Purple thyme” (Thymus bornmuelleri Velen.), and “Ball thyme” (Thymus sibthorpii Benth.), growing in the Kazdağları Mountains in Balıkesir Province, Turkey, an important natural habitat, were determined, and the plants were characterized and their

phytochemicals were identified. The obtained results were statistically analyzed, and the explanatory dominance of the phytochemicals in the composition on the plant characteristics was theoretically calculated. In addition, the antimicrobial ability of the plants was measured against some bacteria and a yeast against antibiotic drugs, and finally, their cytotoxic effect on colon cancer cells (HT29) was investigated, and their effects on health were discussed. With this approach, an original study was conducted, aiming to contribute to the literature.

2. Materials and Methods

2.1. Materials, chemicals and instruments

Plant materials (dried aerial parts of *Thymus bornmuelleri* Velen./TBV, *Thymus citriodorus*/TC, *Thymus longicaulis C.* Presl/TLC, and *Thymus sibthorpii* Benth./TSB) were purchased from a commercial vendor at a local open-air market at Hasan Boğuldu Pond in Edremit, Balıkesir, Türkiye, in August 2024. A photograph of the market stall was taken and archived by the authors to document the commercial origin of the plant specimen and ensure traceability. Due to the dried and processed condition of the commercial materials, the definitive botanical identification was performed by Dr. Cahit ÇEÇEN, Lecturer in Medical Services and Techniques, Mardin Artuklu University. The verified specimens were then packaged and stored at room temperature until used in extraction experiments.

Total phenolic content and DPPH radical-scavenging capacity were determined spectrophotometrically using a Biochrom Libra S70 Dual UV spectrophotometer. Phenolic compound analysis was performed on a Waters Alliance e2695 HPLC system equipped with a reverse-phase C18 column (5 µm, 4.6 × 250 mm; GL Sciences, Tokyo, Japan), with detection achieved using a Waters 2996 photodiode array detector (Milford, MA, USA). Volatile compound analysis was conducted using a Shimadzu GCMS-QP2020 instrument coupled to an Agilent J&W DB-HeavyWAX polyethylene glycol polar capillary column (60 m × 0.25 mm × 0.25 µm) and a Supelco SPME fibre assembly (50/30 µm DVB/CAR/PDMS). Samples were stored under refrigerated conditions in a Beko ProSmart Inverter refrigerator prior to analysis, and extraction-related centrifugation procedures were carried out using a Hettich Universal 320 R refrigerated centrifuge. Culture media, agar, standard reference materials, and analytical-grade reagents, including DPPH (2,2-diphenyl-1-picrylhydrazyl) and sodium carbonate, were purchased from the Merck Millipore and Merck EMSURE series. Solvents and supplementary chemicals, including Folin–Ciocalteu reagent, ethanol, methanol, and acetonitrile, were obtained from J.T. Baker and ISOLAB.

2.2. Methods

2.2.1. Extraction

Thyme samples were extracted for analysis of total antioxidant capacity, total phenolic content, individual phenolic compounds, antimicrobial, and cytotoxicity studies. For this purpose, the method used by Vitali et al. [21] was used with slight modifications. Briefly, 2 g of thyme sample was weighed and ground, placed in an Erlenmeyer flask, 20 mL of dilute methanol (80%) was added, and it was left to stand in a shaker at room temperature for 2 hours.

2.2.2. Total phenolic content analysis (TPC)

The TPC values of the extracts were measured using the Folin-Ciocalteu method with a UV spectrophotometer [22]. Briefly, 100 µL of phenolic extract (1 mg/mL, in methanol) was added to a mixture of 900 µL of pure water and 5 mL of Folin-Ciocalteu reagent (0.2 N). After waiting 8 minutes in the dark, 5 mL of Na_2CO_3 was added to the mixture and vortexed. It was left in the dark for two hours, and absorbance values were read at 765 nm using a UV-vis spectrophotometer. TPC was calculated from the calibration curve obtained with solutions at different concentrations of gallic acid standard and expressed as gallic acid equivalent (GAE).

2.2.3. Radical scavenging activity (RSA)

Extracts were subjected to DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging activity analysis, similar to the method applied by Osei et al., and absorbances were measured using a UV spectrophotometer [23]. For this purpose, 2.7 mL of methanolic DPPH solution (60 µM) was added to 0.3 mL of phenolic extract (1 mg/mL, in methanol). This mixture was left in the dark at room temperature for 30 minutes, and absorbances were measured at 517 nm using a UV-vis spectrophotometer. Methanol was used as a reference, and the methanol:water (80:20 v/v) solution value was read for the control absorbance. Trolox was used as the standard substance, and the results were calculated as trolox equivalents.

2.2.4. Phenolic compounds

Phenolic analysis was performed using an HPLC instrument similar to the method of Veneziani et al. [24]. For this purpose, the extracts were filtered via 0.45 µm PDVF, and 20 µL was injected into a C-18 column (5 µm, 4.6 x 250 mm, GL Sciences, Tokyo, Japan) of a Waters Allance E2695 HPLC instrument for the detection of some minor components. The mobile phase, prepared from ultrapure water (solvent A, pH = 2 and phosphoric acid) and methanol/acetonitrile (solvent B, 9:1 by volume), was used at a flow rate of 0.8 mL/min. The device was operated at 30°C, and an elution gradient was created with 95 – 5% A-B solvent for 2 minutes, 75 – 25% for 8 minutes, 60 – 40% for 10 minutes, 50–50% for 16 minutes, and 0-100% for 14 minutes. The system was held stationary for 10 minutes and returned to the starting point after 13 minutes. For quantitative determination, calibration curves were plotted for rosmarinic acid at 330 nm and for other reference compounds at 280 nm. Results were determined using a PDA detector and evaluated in the Empower 3 application of the device.

2.2.5. Volatile component analysis

Volatile compounds in the samples were determined using gas chromatography-mass spectrometry (GC-MS, Shimadzu GCMS-QP2020) by upper cavity-solid-phase microextraction (HS-SPME) method [25]. For this purpose, 2 g of plant sample was weighed into an SPME vial, the cap was tightly closed, and an SPME fiber (2 cm) was immersed in the vial and kept at 40°C for 30 minutes to allow the volatile compounds to be adsorbed onto the fiber. Following the extraction process, the SPME fiber was placed in the injection port of the GC-MS instrument to perform the desorption of volatile compounds and kept at 250°C for 5 minutes. Helium gas was used as the carrier gas at a flow rate of 1.05 mL min^{-1}. The oven temperature was initially maintained at 40°C for 2 minutes, then increased at a rate of 3°C per minute to 80°C and held at this temperature for 1 minute. Subsequently, the temperature was increased at a rate of 5°C per minute to 240°C and maintained at this temperature for 6 minutes. Volatile compounds were identified by comparing mass spectra in the instrument software library (Wiley 9 and NIST05). The RI values of the compounds were calculated according to the method of Dool and Kratz [26] by injecting the n-alkane series (C10–C26) into the GC-MS system under the same chromatographic conditions. The concentrations of the components were calculated relative to the peak area of 2.5 µg isobutylacetate (in methanol) added to each sample vial as an internal standard (%RSD 5.78).

2.2.6. Antimicrobial activity

To determine antimicrobial activity, Gram-negative (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027), Gram-positive (Staphylococcus aureus ATCC 25923) bacteria and a standard yeast strain (Candida albicans ATCC 10231) were used. Nutrient Broth and Nutrient Agar were used as liquid and solid media for growing the test bacteria used to determine antibacterial activity. Sabouraud Dextrose Broth and Sabouraud Dextrose Agar were used as liquid and solid media for growing the yeast used to determine antifungal activity.

The disk diffusion method was used to determine antimicrobial activity [27]. Bacteria taken from fresh cultures with a loop were incubated in Nutrient Broth medium at 37°C, and yeast in Sabouraud Dextrose Broth medium at 30°C until a turbidity equal to 0.5 McFarland was achieved. Turbidity control was performed by spectrophotometric measurement, adjusted to a range of 0.08–0.10 at a wavelength of 625 nm. 0.1 mL of bacterial cultures with appropriate turbidity were spread onto Nutrient Agar medium, and 0.1 mL of yeast culture were spread onto Sabouraud Dextrose Agar medium. Then, 6 mm sterile blank paper discs (Bioanalyse, Ankara, Turkey) were placed on the inoculated petri dishes. 10 µL of the prepared

extracts (in DMSO) were impregnated onto paper discs. After incubating the petri dishes containing bacteria at 37°C for 24 hours and the petri dishes containing yeasts at 30°C for 48 hours, the diameters of the inhibition zones formed were measured to determine antimicrobial activity. The same procedure was performed for positive and negative controls (solvent). Each test was performed in three parallels, and the results are given as the means (mm) ± standard deviation of the inhibition zones. Erythromycin (15 µg disc-1), Gentamicin (10 µg disc-1), Ciprofloxacin (1 µg disc-1), and Nystatin (100 µg disc-1) antibiotic discs were used as positive controls.

2.2.7. In vitro cell viability assessment (The WST-1 Assay)

The human colorectal cancer cell line HT-29 was kindly provided by Dr. Mehmet Kadir Erdoğan (Cancer Research Laboratory, Bingöl University) and maintained in DMEM (Sigma, #D6429) supplemented with 10% fetal calf serum (Biowest, #S191H), penicillin (100 U/mL), and streptomycin (100 µg/mL) under standard culture conditions (37°C, 5% CO_2). Experiments were performed using exponentially growing, mycoplasma-free cells. Cytotoxic effects of the extracts were assessed by WST-1 assay as described [28]. Briefly, 5 × 10^3 cells per well were seeded into 96-well plates, allowed to adhere overnight, and treated with different extract concentrations for 72 h. WST-1 reagent (10 µL) (Roche) was then added to each well, and absorbance was measured at 450 nm after 4 h using a Thermo Multiskan microplate reader. Cell viability was calculated relative to the untreated DMSO control. All experiments were performed with at least three biological replicates.

2.2.8. Statistical studies

Graphs, standard errors, and R^2 calculations were performed using Microsoft Office LTSC Standard for Mac 2024 (16.101). Significant differences in antioxidant analyses were calculated using the "post-hoc/Tukey" function in the "analysis/compare means/one-way ANOVA" function in IBM SPSS Statistics Ver.27. Significant differences in cytotoxicity analyses were calculated using the One-way/ANOVA method with GraphPad Prism 8.3. Principal component analysis was performed using the determinant, KMO and Bartlett test, varimax rotation, and loading plot in the "analysis/dimension reduction/factor" function in IBM SPSS Statistics Ver.27 and sorted according to dimension characteristics. Other default settings were not changed. Factor analysis was performed at the following standards: determinant > 0.00001, KMO > 0.5, and Bartlett test significance level < 0.05. The matrix was analyzed using varimax matrix rotation, and test results that yielded very similar values (< 0.100) across different components were excluded from the analysis.

3. Results and Discussion

3.1. Total Antioxidant Capacity (TAC)

For the total phenolic content (TAC) analysis of the samples, DPPH radical scavenging activity (RSA) and total phenolic content (TPC) methods were studied. The results obtained for each sample are given in Table 1.

Table 1
The RSA (mg TE/g DW), IC50 for DPPH (mg/L), and TPC (mg GAE/g DW) values of the samples were calculated

RSA	TBV	TC	TLC	TSB
	333.57±11.03	**440.05±2.09**	**283.91±1.04**	**311.42±1.43**
IC50	53.54±1.80	40.56±0.19	62.86±0.23	57.31±0.26
TPC	49.82±0.58	94,08±0.29	63.99±0.87	70.64±1.16

All thyme variances showed significant differences in both RSA ($p < 0.05$) and TPC ($p < 0.001$) values

All thyme varieties differed significantly in terms of RSA and TPC. However, it remains difficult to conclude that all varieties were significantly lower than one another in a pairwise sense. Among the tested groups, TC exhibited the most favorable values for both RSA and TPC. The lowest RSA value was observed in TLC, whereas the lowest TPC value was recorded in TBV. Köksal et al. [29] reported TPC values of 256.0 mg GAE/g for the aqueous extract and 158.0 mg GAE/g for the ethanol extract of *Thymus vulgaris* L. In the same study, DPPH radical-scavenging activity yielded IC_{50} values ranging from 12.1 to 13.4 mg/L. Similarly, Mahrye et al. (2022) reported a TPC value of 123.60 ± 1.51 mg GAE/g DW for an 80% methanolic extract of *T. vulgaris* [30]. In another study, Mokhtari et al. (2023) found a DPPH IC_{50} value of 69.39 ± 3.01 for a thyme sample extracted with 70% methanol [31]. Based on these comparisons, although the TPC values of the samples analysed in the present study appear to be somewhat lower than those reported for similar species, their notable DPPH radical-scavenging activity nevertheless indicates that they possess considerable antioxidant potential.

3.2. Phenolic Compounds

The most dominant phenolic acid in thyme is rosmarinic acid. In the samples we studied, rosmarinic acid values were also measured to be the highest and were at very high levels relative to dry plant weight (Table 2).

Table 2
Phenolic components and their quantities determined by HPLC method. (mg/g DW)

TBV	Catechin	Caffeic acid	Vanillic acid	p-Coumaric acid	Gallic acid	Rosmarinic acid
	0.276	**0.026**	**0.054**	**0.010**	**0.021**	**5.888**
TC	0.408	0.005	0.030	0.026	0.021	9.229
TLC	0.276	0.003	0.041	0.039	0.025	7.117
TSB	0.424	0.008	0.063	0.064	0.016	6.918

The highest rosmarinic acid value was detected in the TC variation, while the highest value for catechin was found in the TSB variation. For other phenolic acids, TBV showed the highest results for caffeic acid, TSB for vanillic acid, TSB for p-Quomaric acid, and TLC for gallic acid. The results indicate that all thyme samples studied contain significant amounts and characteristics of phenolic compounds, and their differing characteristics suggest that different thyme variations may be prioritized depending on the specific objective.

Rosmarinic acid and some of its derivatives have become an important phytochemical in human health, exhibiting anticancer, antimicrobial, antidiabetic, neurodegenerative disorder-preventing, cardiovascular protective, and anti-inflammatory properties, as well as a property that prevents photodamage caused by ultraviolet radiation [32–34]. The amounts of rosmarinic acid in this plant ranged from 0.49 to 55.8 mg/g in different extracts in previous studies [35–37]. Thyme can be used as an important source for this compound, which was measured in our samples at rates as high as 9.23 parts per thousand by mass.

After rosmarinic acid, the component measured in the highest amounts was catechin. Catechin (flavan-3-ol) is a type of phenol that is the most abundant and important component [38]. Catechin exhibits protective effects on the functions and integrity of the liver, kidneys, heart, and brain. It has also been shown to possess a wide range of pharmacological activities, including antimicrobial, anticancer, antihypertensive, anticoagulant, and anti-ulcer effects [39]. Previous studies on Thymus vulgaris have also reported the detection of 28.00 ppm catechin [40].

3.3. Voletile components

The volatile components of the samples are quite important for their phytochemical character. A total of 26 alcohols, 12 aldehydes, 15 ketones, 2 acids, 22 esters, 57 terpenes, and 9 miscellaneous compounds were identified in the samples studied (Table 3).

Table 3
Phytochemicals and their quantities determined by GC-MS analysis of thyme samples. (mg/kg DW)

Volatile compound	R.I.	TBV	TC	TLC	TSB
Alcohols					
3-Hexen-1-ol, (Z)-	1391	0.270	0.563	0.119	-
3-Octanol (CAS)	1398	25.030	13.391	0.120	0.335
1-Octen-3-ol (CAS)	1414	1.451	-	0.122	0.326
1-Octen-3-ol	1456	44.377	10.894	0.129	9.916
1-Heptanol	1459	-	0.527	-	-
3-Cyclohexene-1-methanol	1476	-	2.181	-	-
1-Nonen-3-ol (CAS)	1521	-	-	0.138	0.567
1,6-Octadien-3-ol, 3,7-dimethyl-	1552	1.092	47.044	-	314.833
1-Nonanol (CAS)	1660	-	3.081	-	-
2,7-Octadien-4-ol, 2-methyl-6-methylene-, (S)-	1681	-	-	-	0.466
1,8-menthadien-4-ol	1696	1.136	-	-	1.358
.alpha.-Terpineol	1704	4.796	-	0.162	5.136
endo-Borneol	1714	15.533	51.581	0.163	42.045
trans-3(10)-Caren-2-ol	1719	-	2.187	-	-
Benzenemethanol. 4-(1-methylethyl)- (CAS)	1857	-	4.877	-	-
3,7-Octadiene-2,6-diol, 2,6-dimethyl-	1940	-	-	-	5.016
2-Ethyl-5-n-propylphenol	1951	-	1.980	0.188	0.724
(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-methanol	1960	-	-	-	0.500
1,5-Hexadiene-3,4-diol, 2,5-dimethyl-	1978	-	1.342	-	-
.alpha.-Cedrol	1803	-	11.226	-	-
trans-Carveol	1842	0.289	0.501	0.177	0.759
Geraniol	1848	-	53.106	-	-
p-Cymen-7-ol	2107	-	1.494	-	-
1,4-dihydroxy-p-menth-2-ene	2095	7.554	5.759	0.403	3.466
1,7-Octadiene-3,6-diol, 2,6-dimethyl-	2116	-	-	-	4.841
Thymol [Phenol, 5-methyl-2-(1-methylethyl)- (CAS)]	2157	26.427	2.586	0.419	31.118
Aldehydes					
Propanal	865	-	-	-	0.220
Methacrolein	942	-	-	-	1.024
2-Butenal	1080	-	0.529	-	-
2-Hexenal, (E)-	1239	-	0.678	-	-

Volatile compound	R.I.	TBV	TC	TLC	TSB
Octanal	1304	-	0.773	-	-
Nonanal	1404	-	3.717	0.121	-
2-Furancarboxaldehyde (CAS)	1478	-	-	-	0.294
.alpha.-Campholenal	1504	-	-	0.136	2.552
3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-	1524	-	2.843	-	-
2-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	1534	-	-	-	0.494
Benzaldehyde	1539	-	0.445	0.141	-
2,6-Octadienal, 3,7-dimethyl-, (Z)-	1693	-	9.815	-	-
Ketones					
Acetone	891	0.313	0.669	0.027	0.662
3-Buten-2-one (CAS)	1004	-	-	-	0.305
3-Heptanone, 6-methyl-	1232	0.504	-	-	-
3-Octanone	1273	2.500	1.647	-	-
1-Octen-3-one	1316	-	-	-	0.381
6-Methyl-5-hepten-2-one	1352	-	0.702	-	-
3-Isopropylidene-5-methyl-hex-4-en-2-one	1524	2.269	-	-	-
Ethanone, 1-(3-methylenecyclopentyl)-	1527	-	-	0.139	-
1-(3-Isopropenyl-2,2-dimethylcyclopropyl)-2-methylpropan-1-one	1527	2.571	-	-	-
(+)-2-Bornanone	1534	0.581	-	0.1400	-
Pinocarvone	1587	-	-	0.147	-
Umbellunone	1658	-	-	0.156	0.934
1-(2-Ethyl-1,3-dimethyl-cyclopent-2-enyl)-ethanone	1659	0.443	-	-	-
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)-	1697	-	-	0.161	12.713
7-Oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl)-	1751	10.797	-	-	-
Acids					
Acetic acid (CAS)	1462	1.791	2.136	0.130	4.156
Nonanoic acid	2163	1.656	1.119	-	-
Esters					
Acetic acid, methyl ester (CAS)	905	-	-	0.028	0.295
Butanoic acid, 2-methyl-, methyl ester	1053	0.446	-	0.049	-
Methyl isovalerate	1062	3.593	-	-	-
2-Butenoic acid, 3-methyl-, methyl ester	1194	0.750	0.504	-	-
Hexanoic acid, methyl ester (CAS)	1211	0.883	-	-	-

Volatile compound	R.I.	TBV	TC	TLC	TSB
3-Hexenoic acid, methyl ester, (Z)-	1279	1.434	-	0.098	-
isopentyl 2-methylbutanoate	1294	-	3.224	-	-
3-Hexen-1-ol, acetate, (E)- (CAS)	1334	-	0.815	-	-
HEXYL 2-METHYL BUTYRATE	1438	-	0.450	-	-
(-)-CARVYL ACETATE	1439	0.374	-	-	-
Z-3-hexenyl 2-methylbutanoate	1484	-	3.464	-	-
LINALYL ACETATE	1567	-	-	0.145	6.099
1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	1567	-	0.555	-	-
Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	1598	-	-	0.149	1.500
Benzoic acid, methyl ester (CAS)	1641	0.777	2.029	-	1.207
Benzoic acid, ethyl ester (CAS)	1684	-	0.840	-	-
4-Terpinenyl acetate	1721	0.688	-	0.164	1.100
Methyl nicotinate	1795	-	-	0.172	0.644
Geranyl acetate	1766	-	10.584	0.169	-
Benzoic acid, 2-hydroxy-, methyl ester (CAS)	1802	-	-	-	2.620
Sabinyl acetate	1933	0.499	-	-	-
Acetic acid, 4a-methyldecahydronaphthalen-1-yl ester	1889	-	-	-	27.064
Terpenes					
.ALPHA.-PINENE, (-)-	1058	38.666	20.219	0.050	11.206
.alpha.-Thujene	1063	30.897	27.894	0.051	11.091
Camphene (CAS)	1098	6.278	6.590	0.058	5.143
L-beta-Pinene	1133	10.367	5.673	0.066	2.855
.beta.-Phellandrene	1226	31.852	1.253	0.157	12.833
.DELTA.3-Carene	1171	10.175	3.202	0.075	3.525
.beta.-Myrcene	1186	187.166	108.179	0.078	90.216
8-p-Menthadiene	1190	1.450	-	0.079	-
.ALPHA. TERPINENE	1199	139.290	115.459	0.081	28.450
D-Limonene	1217	32.962	28.762	0.085	12.299
Eucalyptol	1227	-	57.729	-	-
trans-.beta.-Ocimene	1254	-	284.038	0.093	0.829
.gamma.-Terpinene	1264	786.009	702.352	0.095	273.832
β-Ocimene, (Z)-	1268	9.509	119.717	0.096	8.711
o-Cymene	1288	545.719	267.791	0.100	136.546

Volatile compound	R.I.	TBV	TC	TLC	TSB
2-Carene	1296	17.785	6.586	0.102	9.068
allo-Ocimene	1383	-	15.203	-	-
Nopol	1402	0.769	-	-	-
Cosmene	1443	-	1.801	-	-
Sativene, (+)-	1452	-	1.204	-	-
.alpha.-Cubebene	1469	-	4.489	-	-
Isoledene	1481	1.766	4.528	0.133	1.249
Ylangene	1495	-	7.798	-	-
.alfa.-Copaene	1505	1.375	16.146	-	-
.BETA. BOURBONENE	1534	-	9.198	-	-
.alpha.-Gurjunene (CAS)	1546	1.120	-	0.142	0.485
trans Sabinene hydrate	1556	31.353	66.262	0.273	27.163
.beta.-ylangene	1590	-	4.860	-	-
alpha- Bergamotene	1597	-	5.967	-	-
.beta.-elemene	1604	-	1.206	-	-
GERMACRENE-D	1608	-	7.126	-	-
Caryophyllene	1617	287.374	84.264	0.151	93.283
Gamma.gurjunene	1621	0.266	9.064	0.158	3.857
Selina-3,7(11)-diene	1623	4.336	-	-	-
(+)-Aromadendrene	1627	29.081	44.348	0.302	25.408
Sativene, (+)-	1635	2.426	-	0.153	2.611
Dihydrocarvone	1645	4.584	2.409	0.155	1.037
Alloaromadendrene	1666	2.171	4.380	0.157	1.337
trans-Pinocarveole	1669	0.392	-	0.157	1.436
cis-.beta.-Farnesene	1672	-	13.274	-	-
.alpha.-Humulene (CAS)	1690	11.241	5.800	0.160	4.755
GERMACRENE-D	1730	1.821	17.788	0.165	1.077
.alpha.-Amorphene	1734	-	10.776	-	-
Carvenone	1735	3.122	-	0.165	-
.beta.-Bisabolene	1740	1.526	297.673	0.166	15.818
(-)-Carvone	1754	-	-	0.167	-
Bicyclogermacrene	1755	-	26.352	-	-
.delta.-Cadinene (CAS)	1774	2.442	30.874	0.170	1.603

Volatile compound	R.I.	TBV	TC	TLC	TSB
.gamma.-Muurolene	1780	1.239	39.176	0.170	3.567
Gamma.cadinene	1781	-	18.108	-	-
CIS-.ALPHA.-BISABOLENE	1785	-	17.447	-	-
.beta.-Bisabolene (CAS)	1789	-	-	0.171	0.340
Ascaridole	1889	59.660	32.586	0.523	9.757
.alpha.-Calacorene	1942	-	0.847	-	-
cis-Caryophyllene	2075	1.284	-	0.201	0.503
Spathulenol	2137	0.524	2.3628	-	-
(+)-Carvotanacetone	2206	-	503.012	0.214	392.986
Miscellaneous					
Benzene, (methoxymethyl)-	1403	-	-	-	0.801
Benzene, (2-methyl-1-propenyl)-	1448	6.879	3.374	0.128	1.608
Cyclopropane, 2-(1,1-dimethyl-2-pentenyl)-1,1-dimethyl- (CAS)	1560	1.165	-	0.144	1.771
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	1571	1.0294	0.651	0.145	1.197
Cyclohexene, 3-pentyl-	1578	-	-	0.146	1.432
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (CAS)	1613	-	-	0.151	23.250
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	1735	-	-	-	1.626
Tetracyclo[3.3.1.1(1,8).0(2,4)]decane	1795	-	1.878	-	-
Caryophyllene oxide	2019	1.821	0.781	0.195	2.118

“-“ indicates that the component could not be detected

The study identified several important phytochemicals that can exhibit antioxidant, antimicrobial, and anticancer properties. Thymol, a key characteristic component of thyme, was detected in all samples, particularly in high amounts in TBV and TSB samples. The significant antimicrobial and anticancer effects of the thymol compound are discussed in detail later. Beta-caryophyllene, measured in high amounts in the samples, is a major sesquiterpene in essential oils and is known to have antioxidant, anticancer, antimicrobial, and anti-inflammatory properties, as well as protective and strengthening effects on many systems in the human body [41]. A study determined that aromadendrene is effective against Staphylococcus aureus resistant to antibiotics such as methicillin and enterococci resistant to vancomycin [42]. In particular, carvotanacetone, which was present in significant amounts in our samples, has been reported to inhibit the proliferation of colon cancer cells and interfere with the ubiquitin-proteasome pathway [43].

In addition, isoledene has significant antifungal properties [44], ylangene has antibacterial properties [45], and alpha-humulene has anti-inflammatory properties [46]. Endoborneol [47] has been used as a messenger drug for some medications to facilitate drug compliance and delivery [48]. Besides, some of these phytochemicals, such as alpha-terpineol, are used in the perfume/cosmetic fields and stand out as important aromatics in some teas [49].

Principal component analysis (PCA) statistics were performed to highlight the volatile components that most commonly encompassed the characteristics of the studied thyme plants, and an attempt was made to identify components that would be more descriptive of the differences. This provides theoretical information not about which components are

important phytochemically, but about which components are more explanatory in the studied plants. Accordingly, among the 143 volatile components identified in total, 11 compounds, in order of importance, consisting of alpha.pinene, o.cymene, alpha.humulene, benzenemethylpropenyl, L-beta.pinene, dihydrocarvone, delta-3-carene, caryophyllene, beta.myrcene, 1-octen-3-ol, and 2-carene, account for > 50% of the variance explained in the studied thyme samples (Fig. 2).

3.4. Antimicrobial activity

The antimicrobial activities of methanolic extracts obtained from four *Thymus* species—*Thymus bornmuelleri* Velen (TBV), *Thymus citriodorus* (TC), *Thymus longicaulis* C. (TLC), and *Thymus sibthorpii* Benth. (TSB)—were evaluated against *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, and *Candida albicans* ATCC 10231 at four concentrations (10–100 mg/mL). Overall, all extracts produced measurable inhibition at higher concentrations, and the general activity levels remained relatively close among the samples. However, species-dependent differences were evident, and a consistent increase in inhibition zone diameters with rising extract concentrations confirmed a dose-dependent antimicrobial response. Notably, at lower concentrations, some extracts failed to produce detectable inhibition against certain test microorganisms.

The antimicrobial activities of the methanolic extracts showed clear species-dependent differences at the highest concentration tested (100 mg/mL). Against *S. aureus*, the strongest inhibition was observed for *T. bornmuelleri* (10.2 mm) and *T. sibthorpii* (10.0 mm), while *T. citriodorus* and *T. longicaulis* exhibited smaller zones (8.5 and 9.0 mm, respectively). In the case of *P. aeruginosa*, inhibition levels were generally lower (consistent with the intrinsic resistance of Gram-negative bacteria) but *T. longicaulis* demonstrated the greatest activity (10.2 mm), followed by *T. bornmuelleri* and *T. citriodorus* (8.7 mm), whereas *T. sibthorpii* showed a slightly weaker effect (8.2 mm). Antifungal activity against *C. albicans* was moderate, with *T. sibthorpii* producing the highest inhibition (8.9 mm), followed by *T. citriodorus* (8.1 mm), while *T. bornmuelleri* (7.1 mm) and *T. longicaulis* (7.2 mm) exhibited lower activity. The results are presented in Table 4.

Table 4
Antimicrobial activity (inhibition zone diameters, mm) of methanol extracts of thyme samples (mean ± SD) and positive controls against tested microorganisms.

Sample	***S.aureus*** **ATCC 25923**	***P.aeroginosa*** **ATCC 9027**	***C.albicans*** **ATCC 10231**
TBV (80 µg/mL)	10,2 ± 0,08	8,7 ± 0,03	7,1 ± 0,02
TBV (40 µg/mL)	9,6 ± 0,03	8,5 ± 0,05	6,9 ± 0,02
TBV (20 µg/mL)	8,5 ± 0,05	8,5 ± 0,04	---
TBV (10 µg/mL)	---	8,5 ± 0,05	---
TSB (80 µg/mL)	10,0 ± 0,09	8,2 ± 0,07	8,9 ± 0,02
TSB (40 µg/mL)	7,8 ± 0,03	8,2 ± 0,03	7,0 ± 0,05
TSB (20 µg/mL)	7,1 ± 0,01	8,2 ± 0,03	6,7 ± 0,03
TSB (10 µg/mL)	---	8,1 ± 0,01	---
TC (80 µg/mL)	8,5 ± 0,05	8,7 ± 0,06	8,1 ± 0,03
TC (40 µg/mL)	7,1 ± 0,03	8,2 ± 0,03	7,9 ± 0,01
TC (20 µg/mL)	---	8,0 ± 0,02	---
TC (10 µg/mL)	---	8,0 ± 0,05	---
TLC (80 µg/mL)	9,0 ± 0,02	10,2 ± 0,08	7,2 ± 0,03
TLC (40 µg/mL)	8,0 ± 0,05	9,5 ± 0,05	---
TLC (20 µg/mL)	---	8,6 ± 0,04	---
TLC (10 µg/mL)	---	7,8 ± 0,03	---
Nystatin (100 µg/disk)	nt	nt	27 ± 0,71
Erythromycin (15 µg/disk)	27 ± 0,84	16 ± 0,15	nt
Gentamicin (10 µg/disk)	nt	20 ± 0,55	nt

"---" indicates no inhibition, "nt" indicates not tested.

These findings are consistent with previous reports on methanolic *Thymus* extracts. Gortzi et al. [50] documented that methanolic extracts of *Thymus longicaulis* and *Thymus ocheus* produced inhibition zones of approximately 10 mm against *S. aureus*, 10–11 mm against *P. aeruginosa*, and 8 mm against *C. albicans*, which closely align with the results of the present study. Similarly, earlier studies on *Thymus daenensis* methanolic extracts reported clear antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Streptococcus pyogenes*), whereas no inhibitory effect was observed against Gram-negative species [51]. The relatively low antifungal activity observed here is also consistent with previous reports showing that methanol extracts of *Thymus* species exert limited or no inhibitory effects on yeasts; for example, both the polar and non-polar fractions of the methanol extract of *Thymus spathulifolius* lacked antifungal activity [52]. Similarly, Gortzi et al. [50] reported weak antifungal activity of

methanolic *Thymus* extracts against *Candida albicans*, with inhibition zones of approximately 8 mm for both *Thymus longicaulis* and *Thymus ocheus*. These values closely parallel the limited antifungal activity observed in the present study.

These literature findings support the present results, indicating that methanolic *Thymus* extracts consistently exhibit moderate but reproducible antimicrobial effects across different species and experimental conditions. To the best of our knowledge, no previous studies have reported the antimicrobial activity of methanolic extracts of *T. bornmuelleri*, *T. citriodorus*, or *T. sibthorpii*. This highlights the novelty of the present work and provides new insight into the biological properties of these lesser-studied species.

The quantitative analysis of phenolic compounds revealed clear interspecific variation among the *Thymus* taxa. Rosmarinic acid exhibited its highest concentration in *Thymus citriodorus* (9.23), followed by *Thymus longicaulis* C. (7.12), while lower amounts were detected in the extracts of *Thymus sibthorpii* Benth. (6.91) and *Thymus bornmuelleri* Velen (5.88). Catechin reached its maximum level in *Thymus sibthorpii* Benth. Among the other phenolic acids, the peak value of caffeic acid was detected in *Thymus bornmuelleri* Velen; vanillic acid and p-coumaric acid were both most abundant in *Thymus sibthorpii* Benth.; and gallic acid showed its highest accumulation in *Thymus longicaulis* C. Previous studies have demonstrated that rosmarinic acid, catechin, vanillic acid, p-coumaric acid, gallic acid, and caffeic acid exhibit antimicrobial properties against various bacterial and fungal microorganisms [53–58].

The antimicrobial activity of the thyme extracts was found to be strongly correlated with the phenolic and volatile compound profiles revealed by HPLC and GC–MS analyses. The strongest antibacterial effect against *S. aureus* was demonstrated by the *T. bornmuelleri* and *T. sibthorpii* extracts, suggesting that phenolics alone may not fully account for the observed activity. GC–MS results supported this finding, revealing that these extracts contained significantly higher levels of thymol (listed as “Phenol, 5-methyl-2-(1-methylethyl)-”), a monoterpene phenol widely known for its potent antimicrobial effects [59–61]. The *T. sibthorpii* extract had the highest thymol content (31.12%), followed by *T. bornmuelleri* (26.43%), which closely aligned with their superior inhibition zones against *S. aureus*. Conversely, the *T. citriodorus* and *T. longicaulis* extracts, despite being richer in rosmarinic acid, contained substantially lower thymol levels and exhibited weaker antibacterial activity, suggesting that thymol is a key determinant of antimicrobial potential, likely acting synergistically with phenolic acids.

Furthermore, the relatively weak inhibition observed against *P. aeruginosa* and *C. albicans* is consistent with their well-recognized intrinsic resistance and tolerance mechanisms. In *P. aeruginosa*, intrinsic resistance mechanisms include reduced membrane permeability, strong efflux pump activity, and chromosomally encoded periplasmic β-lactamases that degrade antimicrobials and limit access to target cells [62, 63]. In *Candida albicans*, reduced susceptibility to antifungal agents is mainly associated with intrinsic tolerance mechanisms, including the upregulation of efflux pumps of the ABC and major facilitator families and the activation of stress-response pathways such as calcineurin and MAP kinase signaling, all of which enhance survival during antifungal exposure [64–66]. Overall, these findings indicate that the antimicrobial activity of thyme extracts is primarily determined by the abundance of thymol and other bioactive monoterpenes, while phenolic acids contribute additively but are not the primary drivers of activity.

3.5. Cytotoxic Activity Against HT29 Human Colorectal Adenocarcinoma Cells

The cytotoxic potential of the four *Thymus* species methanolic extracts — *T. bornmuelleri* (TBV), *T. citriodorus* (TC), *T. longicaulis* (TLC), and *T. sibthorpii* (TSB) — was systematically evaluated against the human colorectal adenocarcinoma cell line HT29 employing the WST-1 cell viability assay. Colorectal cancer (CRC) remains a formidable global health burden, ranking third in incidence and second in cancer-related mortality worldwide, with approximately 1.93 million new cases and over 904,000 deaths recorded in 2022 alone [67]. The identification of plant-derived bioactive compounds capable of selectively targeting CRC cells therefore constitutes a research priority of considerable pharmaceutical and clinical relevance, rendering *Thymus* species — characterized by their high thymol, rosmarinic acid, and β-caryophyllene content —

a scientifically well-motivated subject of cytotoxic investigation. Cells were exposed to a broad concentration gradient spanning seven dose levels (31.25–2000 µg/mL) over a 72-hour incubation period, and cell viability results are expressed as percentage relative to the DMSO-treated vehicle control (Table 5; Fig. 3).

Table 5
Effects of TBV, TC, TLC, and TSB extracts on HT29 cell viability as determined by the WST-1 assay after 72 h of treatment.

TBV	**31,25 µg/mL**	**62,5 µg/mL**	**125 µg/mL**	**250 µg/mL**	**500 µg/mL**	**1000 µg/mL**	**2000 µg/mL**
	84,41±2.26	**79,84±4.23***	**47,41±15.74***	**38,43±9.63***	**25,49±10.40***	**16,97±7.10***	**11,37±6.12***
TC	87,03±5.40	84,89±5.72	49,64±30.27	38,61±10.22*	32,11±6.48*	25,96±4.80*	10,15±5.32*
TLC	98,49±0.31	93,82±2.32	97,57±0.50	92,54±1.53	44,57±5.13*	35,22±4.78*	20,60±3.08*
TSB	72,06±7.53	75,59±8.53	68,19±13.38	60,88±8.34	43,85±7.15*	36,94±10.01*	18,99±4.11*

Cells were exposed to increasing concentrations of each extract (31.25–2000 µg/mL), and cell viability was expressed as a percentage relative to the untreated control group. Data are presented as mean ± standard deviation (SD) from three independent experiments.

Significant differences compared with the control in the test results are indicated by "*" ($p < 0.05$).

All four methanolic extracts elicited a statistically significant ($p < 0.05$), concentration-dependent attenuation of HT29 cell viability across the entirety of the tested concentration range. The threshold concentration at which viability first declined significantly below control values was notably extract-dependent: TBV demonstrated significant cytosuppressive activity from 62.5 µg/mL onward, TC and TSB from 250 µg/mL, and TLC only from 500 µg/mL, indicating marked inter-species variation in cytotoxic potency. The overall ranking of cytotoxic efficacy was established as: TBV ≈ TC > TSB > TLC.

TBV exhibited the most pronounced concentration-dependent cytotoxicity among all evaluated extracts, with cell viability declining precipitously to 47.41 ± 15.74% at 125 µg/mL and reaching a nadir of 11.37 ± 6.12% at the maximum tested concentration of 2000 µg/mL — representing an 88.6% reduction relative to the vehicle control. TC demonstrated a comparably potent cytotoxic response, with viability reducing to 49.64 ± 30.27% at 125 µg/mL and 10.15 ± 5.32% at 2000 µg/mL, albeit with markedly elevated variability at intermediate concentrations, suggestive of extract-specific heterogeneity in phytochemical solubility or differential cellular uptake kinetics. Collectively, both TBV and TC reduced HT29 viability below 50% at concentrations ≥ 125 µg/mL, indicative of moderate-to-strong cytotoxic potency at this dose threshold. TSB produced an intermediate cytotoxic profile, with viability remaining at 68.19 ± 13.38% at 125 µg/mL before declining progressively to 18.99 ± 4.11% at 2000 µg/mL. TLC was unequivocally the least cytotoxic of the four extracts: cell viability remained above 90% throughout the concentration range of 31.25–250 µg/mL (92.54 ± 1.53%), with a statistically significant reduction only emerging at concentrations ≥ 500 µg/mL (44.57 ± 5.13%), and residual viability of 20.60 ± 3.08% persisting even at the highest concentration tested.

The differential cytotoxic potencies observed across the four extracts are mechanistically interpretable through their respective phytochemical compositions, as determined by GC-MS and HPLC analyses. The superior cytotoxicity of TBV is principally attributable to its dominant volatile constituent, thymol (26.43 mg/kg DW), a well-characterized monoterpene phenol with documented antiproliferative activity in CRC models. Thymol suppresses CRC cell proliferation and metastatic potential through multiple convergent mechanisms, including induction of G1/S cell cycle arrest and apoptosis via the BAX/Bcl-2 pathway, inhibition of invasion and migration through blockade of ERK2/p38/Akt phosphorylation in HT29 cells, and suppression of the Wnt/β-catenin signalling pathway in HCT116 and LoVo cell lines both *in vitro* and *in vivo* [68]. Corroborating this broad anti-CRC activity spectrum, a thymol derivative — acetic acid thymol ester — has further been demonstrated to exert concurrent cytotoxic and genotoxic effects in HT-29 and HCT-116 cells [69]. Additionally, β-caryophyllene, detected at notably elevated levels in TBV (287.37 mg/kg DW), constitutes a mechanistically significant

secondary contributor; this CB2 receptor agonist suppresses CRC cell proliferation and tumour angiogenesis in both ectopic and orthotopic xenograft models by inhibiting VEGF secretion and blocking VEGFR2 signalling [70], whilst simultaneously potentiating the cytotoxic efficacy of paclitaxel and cisplatin through interference with ABC transporter-mediated drug efflux mechanisms [71].

The comparably potent cytotoxicity of TC, despite its substantially lower thymol content relative to TBV, is explicable through the convergence of multiple complementary bioactive constituents. Rosmarinic acid, present at particularly high concentrations in TC (9.229 mg/g DW), represents a major mechanistic contributor: Liu et al. (2025) demonstrated that rosmarinic acid exerts IC_{50} values of 100–200 µg/mL against HT29 and SW480 CRC cells at 24 hours, operating through NF-κB pathway inhibition, downregulation of cyclin D1, cyclin A1, MYC, and p-AKT, induction of caspase-3 cleavage, and suppression of Bcl-2 expression. Furthermore, rosmarinic acid has been shown to inhibit CRC metastasis via AMPK activation and suppression of epithelial-mesenchymal transition markers [72], potentiate cisplatin-induced apoptosis and ferroptosis in DLD-1 and LoVo CRC cells [73], and attenuate colitis-associated CRC progression through the TLR4-mediated NF-κB–STAT3 signalling axis *in vivo* [74]. Additionally, carvotanacetone — detected at substantial concentrations in TC (503.01 mg/kg DW) — has been reported to inhibit colon cancer cell proliferation through disruption of the ubiquitin-proteasome pathway [43], and may plausibly account for a significant proportion of the robust cytotoxicity observed in TC at concentrations ≥ 250 µg/mL. The high levels of β-bisabolene and trans-β-ocimene further enrich TC's bioactive terpene profile, potentially contributing to its overall antiproliferative activity through additive or synergistic interactions.

The intermediate cytotoxic activity observed for TSB is consistent with its phytochemical profile. Although TSB contained the highest proportion of thymol among all extracts (31.12%), its absolute levels of β-caryophyllene (93.28 mg/kg DW) and carvotanacetone (392.99 mg/kg DW) were notably lower than those detected in TBV and TC, respectively, which may have limited its overall cytotoxic potency. In addition, TSB exhibited the highest catechin content (0.424 mg/g DW) among the four extracts, which may have contributed modestly to its antiproliferative effect, given the reported pro-apoptotic and anti-proliferative properties of catechin in colorectal cancer models.

The markedly reduced cytotoxicity of TLC across the entire concentration range tested is consistent with its distinct phytochemical composition. GC–MS analysis showed that TLC had the lowest thymol content among the four extracts, together with substantially lower levels of total volatile bioactive monoterpenes. Although TLC contained the highest gallic acid concentration (0.025 mg/g DW) and a relatively high rosmarinic acid level (7.117 mg/g DW), these phenolic constituents alone appear insufficient to elicit pronounced antiproliferative activity in HT29 cells under the present experimental conditions. Taken together, these findings support the view that cytotoxic activity is unlikely to be driven by a single compound, but rather by the synergistic interaction of multiple constituents within the overall phytochemical matrix.

4. Conclusion

This study represents the first integrated phytochemical and biochemical activity characterization of four different wild thyme species in the biodiverse Mount Ida region. While each species has undeniable effects on human health, some species stand out more than others. T. citriodorus has the highest total phenolic content (94.08 mg GAE/g DW) and radical scavenging activity (440.05 mg TE/g DW). This ability is thought to be due to its high rosmarinic acid content (9.229 mg/g DW). T. bornmuelleri and T. sibthorpii had higher thymol content (26.427 and 31.118 mg/kg DW, respectively) compared to other species, and consistently proved to be the most potent antibacterial agents. T. longicaulis stood out, showing the strongest inhibition against P. aeruginosa. In terms of cytotoxic properties, T. bornmuelleri and T. citriodorus species showed a more dominant presence on HT29 cells due to the possible synergistic effects of rosmarinic acid, carvotanacetone, thymol, and β-caryophyllene. These findings reveal that the Thymus taxa studied are valuable phytochemical resources with pharmaceutical, food preservation, and nutraceutical potential.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Ümmügülsüm Tükenmez Emre: Formal analysis, Conceptualization, Data curation, Resources, Investigation, Validation, Visualization, Writing – original draft. **Muhammed Güngören:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Ramazan Gündoğdu:** Formal analysis, Conceptualization, Data curation, Resources, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mustafa Yunus Emre:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Resources.

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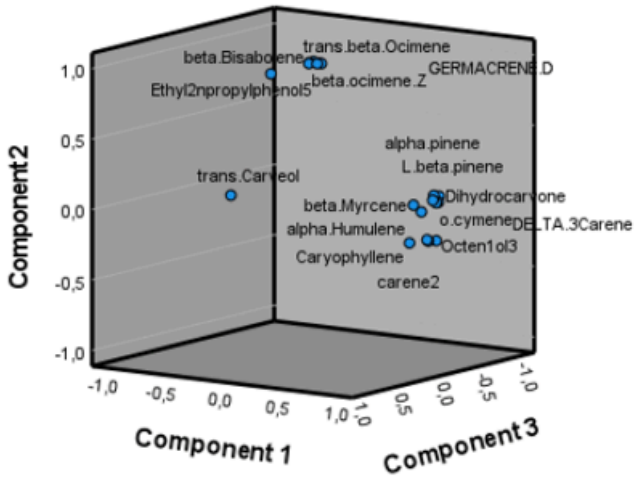
Figures

Figure 1

The wild thyme samples were obtained from the Mount Ida-Kazdağları region (red point)



	Component		
	1	2	3
alpha.Pinene	0,995		
o.Cymene	0,995		
alpha.Humulene	0,993		
Benzene.Methyl.Propenyl	0,992		
L-beta.Pinene	0,991		
Dihydrocarvone	0,986		
delta.-3-carene	0,983		
Caryophyllene	0,981		
beta.Myrcene	0,979		
1-Octen-3-ol	0,974		
2-Carene	0,960		
gamma.Muurolene		0,999	
beta.Bisabolene		0,998	
beta.Ocimene.Z		0,995	
trans-beta.Ocimene		0,994	
delta.Cadinene		0,993	
Germacrene.D		0,992	
2-Ethyl-5-n-propylphenol		0,969	
trans-Carveol			0,958
Eigenvalues	10,706	7,153	1,141
Variance explained (%)	56,349	37,646	6,005
Cumulative (%)	56,349	93,995	100,000

Figure 2

PCA results of volatile components detected in thyme samples.

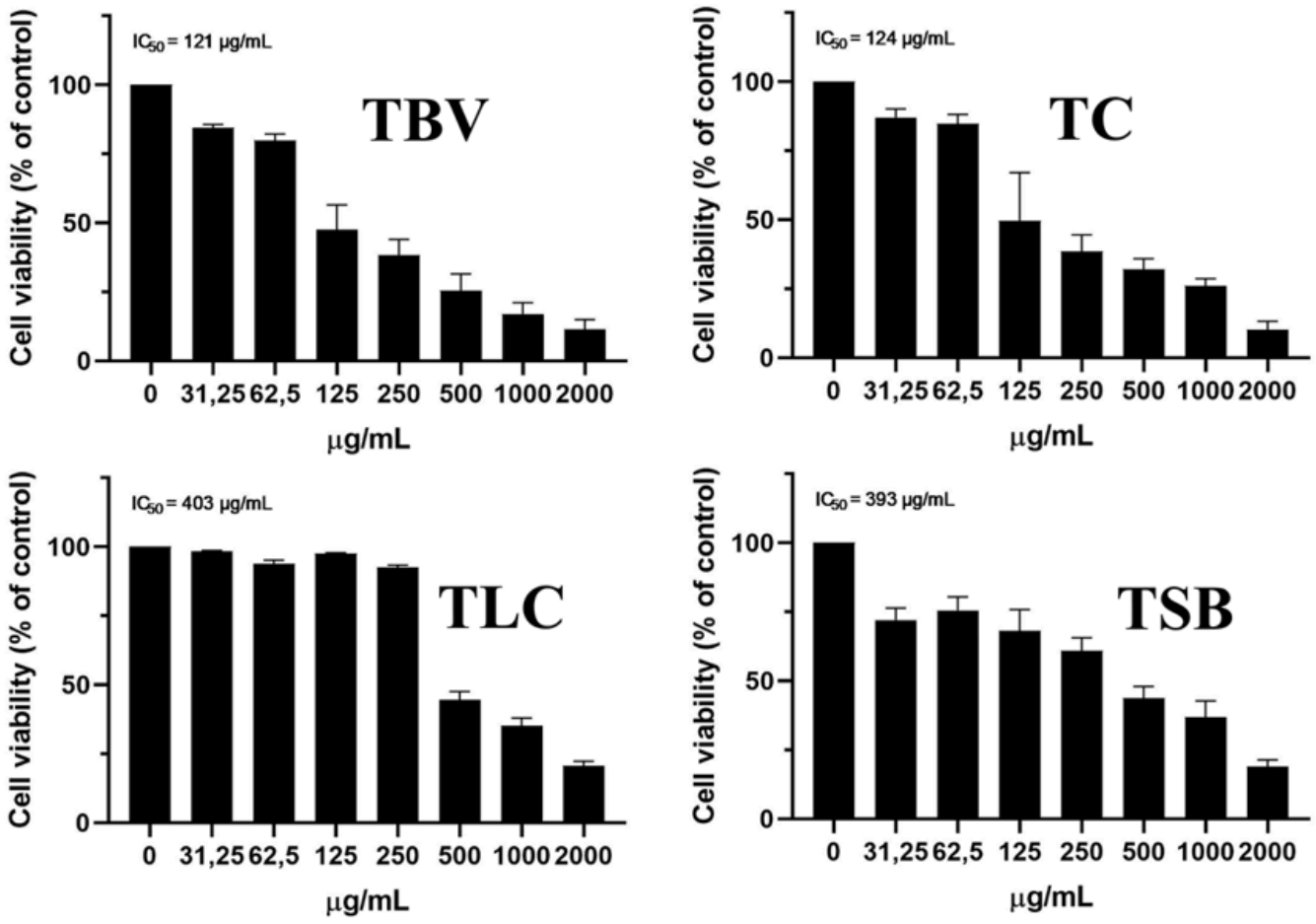


Figure 3

Effects of TBV, TC, TLC, and TSB extracts on HT29 cell viability as determined by the WST-1 assay after 72 h of treatment. Cells were exposed to increasing concentrations of each extract (31.25–2000 µg/mL), and cell viability was expressed as a percentage relative to the untreated control group. Data are presented as mean ± standard deviation (SD) from three independent experiments. IC_{50} values were calculated from the dose–response curves and are indicated within each panel.