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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ESSENTIAL OIL CONTENTS AND BIOLOGICAL ACTIVITIES OF *THYMUS CANOVIRIDIS* JALAS AND *THYMUS SIPYLEUS* BOISS.

THYMUS CANOVIRIDIS JALAS VE THYMUS SIPYLEUS BOISS. TÜRLERİNİN UÇUCU YAĞ İÇERİKLERİ VE BİYOLOJİK AKTİVİTELERİ

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ABSTRACT

Objective: *Members of the Lamiaceae family are considered to be major sources of bioactive therapeutic agents. Many of them are important medicinal and aromatic plants used in traditional and modern medicine and in the food, cosmetic and pharmaceutical industries. The aim of this study was to investigate in detail the biological activities and chemical composition of the essential oils of Thymus canoviridis Jalas and Thymus sipyleus Boiss. species belonging to the genus Thymus, one of the most important genera of the Lamiaceae family.*

Material and Method: *The essential oil content of the species was determined by GC-MS. Antioxidant activities of the essential oils were determined using lipid peroxidation, DPPH free radical, ABTS cation radical and CUPRAC methods. In addition, cytotoxic activities against breast cancer (MCF-7) and colon cancer (HT-29) cell lines and anticholinesterase (against AChE and BChE enzymes), urease, tyrosinase, elastase, collagenase and angiotensin converting enzyme inhibition activities were determined.*

Result and Discussion: *When the essential oil composition of T. sipyleus was analyzed, the major compounds were 1,8-cineole (eucalyptol) (18.16%), camphor (15.08%) and endo-borneol (11.63%), while T. canoviridis was found to be rich in carvacrol (72.88%). T. canoviridis showed high antioxidant activity in lipid peroxidation (IC_{50}: 45.72±0.12 µg/ml), ABTS (IC_{50}: 6.12±0.03 µg/ml) and CUPRAC (IC_{50}: 5.31±0.01 µg/ml) methods. The selectivities of T. canoviridis and T. sipyleus species against MCF-7 cell line were 4.39 and 6.81, respectively. In the enzyme inhibition studies, both Thymus species showed moderate inhibition activity against BChE enzyme (Inhibition%: 57.88±1.14, 39.21±0.89, respectively). In addition, T. sipyleus showed moderate inhibition of elastase enzyme (Inhibition%: 25.33±0.79). When the results are evaluated in general, it can be said that T. canoviridis essential oil with its rich carvacrol content and high cytotoxic and antioxidant activity can be preferred as a safer and natural option instead of synthetic preservatives in food, pharmaceutical and cosmetic industries to extend shelf life and ensure food safety.*

Keywords: *Antioxidant activites, carvacrol, cytotoxic, GC-MS, T. canoviridis, T. sipyleus*

ÖZ

Amaç: *Lamiaceae ailesinin üyeleri, biyoaktif terapötik ajanların başlıca kaynakları olarak kabul edilir. Birçoğu geleneksel ve modern tıpta ve gıda, kozmetik ve ilaç endüstrilerinde kullanılan önemli tıbbi ve aromatik bitkilerdir. Bu çalışmada Lamiaceae familyasına mensup en önemli cinslerden biri olan Thymus cinsine ait Thymus canoviridis Jalas ve Thymus sipyleus Boiss.*

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türlerinin uçucu yağlarının biyolojik aktiviteleri ve kimyasal bileşiminin detaylı bir şekilde incelenmesi amaçlanmıştır.

Gereç ve Yöntem: *Türlerin uçucu yağ içeriği GC-MS cihazı ile belirlenmiştir. Ayrıca uçucu yağların lipit peroksidasyon, DPPH serbest radikal, ABTS katyon radikali ve CUPRAC metotları kullanılarak antioksidan aktiviteleri, meme kanseri (MCF-7) ve kolon kanseri (HT-29) hücre dizilerine karşı sitotoksik aktiviteleri ve antikolinesteraz (AChE ve BChE enzimlerine karşı), üreaz, tirozinaz, elastaz, kolajenaz ve anjiyotensin dönüştürücü enzim inhibisyon aktiviteleri belirlenmiştir.*

Sonuç ve Tartışma: *T. sipyleus uçucu yağ bileşimi incelendiğinde majör bileşikler 1,8-sineol ökaliptol (%18.16), camphor (%15.08) ve endo-borneol (%11.63) olarak tespit edilmiş, T. canoviridis ise karvakrol (%72.88) bakımından oldukça zengin bulunmuştur. T. canoviridis lipid peroksidasyon (IC_{50}: 45.72±0.12 µg/ml), ABTS (IC_{50}: 6.12±0.03 µg/ml) ve CUPRAC (IC_{50}: 5.31±0.01 µg/ml) yönteminde yüksek antioksidan aktivite göstermiştir. T. canoviridis ve T. sipyleus türlerinin MCF-7 hücre serisine karşı sağlıklı hücre serine göre selektivitelerinin sırasıyla 4.39 ve 6.81 olduğu belirlenmiştir. Yapılan enzim inhibisyon çalışmalarında Thymus türlerinin her ikisi de BChE enzimine karşı orta inhibisyon aktivitesi (% İnhibisyon: 57.88±1.14, 39.21±0.89, sırasıyla) göstermiştir. Ayrıca T. sipyleus'un, orta düzeyde elastaz enzim inhibisyonu (% İnhibisyon: 25.33±0.79) gösterdiği saptanmıştır. Sonuçlar genel olarak değerlendirildiğinde T. canoviridis uçucu yağının zengin karvakrol içeriği, yüksek sitotoksik ve antioksidan aktivitesi ile gıda, ilaç ve kozmetik endüstrisinde raf ömrünü uzatmak ve gıda güvenliğini sağlamak için sentetik koruyucular yerine daha güvenli ve doğal bir seçenek olarak tercih edilebileceği söylenebilir.*

Anahtar Kelimeler: *Antioksidan aktivite, GC-MS, karvakrol, sitotoksik, T. canoviridis, T. sipyleus*

INTRODUCTION

Among the 236 genera in the Lamiaceae family, the genus *Thymus* is among the eight most significant ones in terms of the total number of species it contains. Thyme is traditionally used both to identify the genus and to name *Thymus vulgaris* L., the most commercially used species [1]. The Lamiaceae family is represented by 45 genera, 546 species and 730 taxa in Türkiye, with an endemism rate of 44.2% [2]. Although their distribution in the world is mostly typical for the Mediterranean region, they are distributed especially in the Iberian Peninsula, Europe, Asia, North and West Africa, and North America. It has been observed that they are distributed in many places due to their ability to live in different habitats and grow at high altitudes [3]. The genus *Thymus* includes 40 species and 42 taxa in Türkiye, 16 of which are endemic [4].

Because of their attractive flavor, nutritional value, and medicinal properties, *Thymus* spp. are widely utilized in the food and pharmaceutical industries as spices, sauces, perfumes, and cosmetics [5]. In traditional medicine, infusion and decoction of the leaves and flowering parts have been used in the prevention and treatment of numerous complaints such as diabetes, colds, coughs, flu, indigestion, nausea and dysentery [6].

Numerous studies have been conducted on *Thymus* spp. to determine their chemical composition. The genus *Thymus* was found to be rich in essential oils characterized by considerable variability in chemical composition. The oil chemotypes include monoterpenes, sesquiterpenes and their oxygenated and hydrocarbon derivatives [7]. The main compounds in most *Thymus* essential oils are thymol and carvacrol, which are phenolic monoterpenes. This causes *Thymus* spp. to typically have a strong, spicy flavor. Other monoterpenes commonly detected in essential oils are linalool and, in lower percentages, borneol, camphor, limonene, β-pinene, α-terpineol and terpinen-4-ol [1]. *Thymus* spp. are also rich in phenolic compounds, flavonoids, phenolic acids, terpenic compounds (di and triterpenes) responsible for their therapeutic effects such as antioxidant, antibacterial, cytotoxic, anticancer etc. [8]. A recent study revealed that *Thymus* spp. rich in thymol and carvacrol can be used in food preservation due to their antibacterial activity against many bacterial species [9].

Literature studies on *Thymus canoviridis* Jalas (TC-E) and *Thymus sipyleus* Boiss. (TS-E) belonging to the genus *Thymus*, which are widely used in the public and industry, were reviewed. In a study investigating the antioxidant and antimicrobial activities of essential oils of *Thymus* spp., thymol was found as the major compound in TC-E essential oil and it was determined that it showed good antimicrobial activity against *Proteus vulgaris* and *Staphylococcus aureus* bacteria. It has also been reported that TC-E and other *Thymus* spp. can be used as a natural preservative component in food and medical industries [6]. Due to the popular use of *Thymus* spp., TC-E was examined for its effect on rhinosinusitis pathogens. The major component was found to be thymol, and the volatile components of the oil showed antibacterial and anti-inflammatory potential supporting the traditional use [10]. In this study, similar to previous studies, the chemical composition of the essential oils of *T. canoviridis* and *T. sipyleus* was evaluated by GC-MS. The present study, in contrast to others in the literature, aimed to comprehensively investigate the angiotensin converting enzyme activities, anticholinesterase, urease, tyrosinase, elastase, cholinase, and antioxidant activities of the species' essential oils.

MATERIAL AND METHOD

Plant Material

Thymus canoviridis Jalas (TC-E) was collected from Ezurum (19. km from Hınıs to Pasinler, steppe, 1921m, 39°32′35″ N, 41°43′43″ E) in August and *Thymus sipyleus* Boiss. (TS-E) was collected and identified by Dr. Mehmet Fırat from Erzincan (40. km from Kemaliye to Çemişkesek, dry steppe, 1500m, 39°11′34″ N, 38°38′14″ E) in July. Herbarium specimens were dried for storage in Van Yüzüncü Yıl University Faculty of Science herbarium. Herbarium numbers are M. Fırat 32626 and 32474 (VANF), respectively. The current name of the plant was written according to International Plant Names Index and The Plant List.

GC-FID and GC-MS Conditions for Essential Oils

The essential oils obtained by hydrodistillation method using Clevenger apparatus were determined by Agilent brand 7890A Model GC-FID gas chromatograph flame ionization detector combined with Agilent 5977B model mass spectrometry (MS) device in Dicle University Faculty of Pharmacy [11].

Antioxidant Activity

β-Carotene lipid peroxidation, ABTS cation radical, DPPH free radical and CUPRAC (Copper (II) ion reducing antioxidant capacity) methods were used to determine the antioxidant properties of the samples [12-14]. In these four antioxidant test methods, α-tocopherol and BHT (Butylated hydroxytoluene) compounds were used as standard reference. IC_{50} calculations were performed using samples with concentrations of 100, 50, 25, 10 and 5 µg/ml [36].

Toxic-Cytotoxic Activity

In order to determine toxic and cytotoxic effects of the essential oils, the method developed by Mojarraba et al. (2013) was used with minor modifications [15,16].

Anti-cholinesterase Inhibitory Activity

The spectrophotometric method based on AChE and BChE inhibition developed by Ellman et al. (1961) was used to determine anticholinesterase enzyme activity. In this test method, galantamine was used as a standard reference substance [17].

Anti-urease Inhibitory Activity

The method developed by Hina et al. (2015) was used to determine the urease inhibition activity of the samples. Thiourea was used as a standard reference for the anti-urease activity test method [18].

Anti-Aging Inhibitory Activity

Tyrosinase, elastase and collagenase inhibitory activity methods were used to determine the anti-aging potential of the samples. For tyrosinase inhibitory activity, the method developed by Hearing and Jimenez (1987) was applied with minor modifications. Elastase inhibition activity was performed using the method developed by Kraunsoe et al. (1996) with minor modifications. Collagenase activity was performed using the protocol developed by Thring et al. (2009) with minor modifications [19-21].

$$\text{Enzim inhibition (\%)} = 100\text{-(OD test well /OD control)} \times 100$$

Antihypertensive Activity (Angiotensin I-Converting Enzyme, ACE, Inhibition)

The method developed by Kwon et al. (2006) was used with minor modifications. Lisinopril was used as a standard. ACE inhibition was calculated using peak areas with the following equation [22].

Inhibition% = [Areacontrol – (Areasample – Areasample blank)]/(Areacontrol – Areablank) x 100

Statistical Analysis

The results of the activity assays were shown as means ± standard error meaning. The results were evaluated using an unpaired and one-way analysis of variance ANOVA. The differences were regarded as statistically significant at $p < 0.05$.

RESULT AND DISCUSSION

Essential Oil Contents

The essential oil content of the species was determined by GC-FID and GC-MS (Table 1 and Figure 1). When the results were evaluated, 97.31% and 32 compounds of TS-E and 96.78% and 30 compounds of TC-E were determined. Oxygenated monoterpenes (69.14%) and oxygenated sesquiterpenes (14.13%) constituted the major part of TS-E composition. When the composition of TS-E was analyzed, the major compounds were oxygenated monoterpenes 1,8-cineole (eucalyptol) (18.16%), camphor (15.08%) and *endo*-borneol (11.63%). The composition of TC-E was also found to contain mostly oxygenated monoterpenes (80.1%) and was rich in carvacrol (72.88%), an oxygenated monoterpene. In a study conducted with TS-E, the main compounds were carvacrol (18.2%) and 1,8-cineole (11.6%), in addition to significant amounts of *p*-cymene (9.2%), camphor (8.3%), camphene (7.2%), 3-octanol (5.8%), *β*-caryophyllene (5.0%), borneol (4.9%) [23]. In another study, the main components of the species were identified as borneol (11.2%), *α*-muurolol (9.2%), *β*-caryophyllene (7.6%), geranial (7.3%) and neral (5.4%) [24]. In a study conducted with TC-E, it was found that the essential oil of the species contained mainly thymol (60.44-64.79%) and *β*-caryophyllene (8.49-6.58%) [6]. Partially concurrently with this investigation, Baser et al. (1998) found that the principal constituents of the species' essential oil were carvacrol (29.51%), geraniol (13.25%), and thymol (9.9%). It can be generally stated that the chemical composition of the essential oils of *Thymus* spp. and subspecies of various origins varies greatly and is highly diverse because of several factors such as soil and climate, the vegetative cycle, seasonal change, etc [25-27].

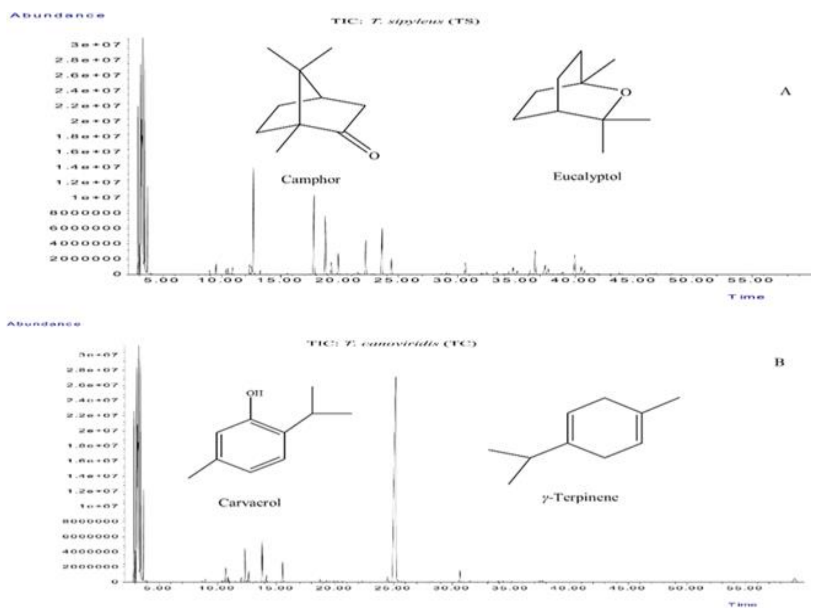

Figure 1. GC-MS chromatograms, **A**: GC-MS chromatogram of essential oil of *T. sipyleus*, **B**: GC-MS chromatogram of essential oil of *T. canoviridi*

Table 1. Chemical composition of the essential oil of *Thymus* species

No	RI[a]	Constituents[b]	TS-E	TC-E	Identification Methods
1	928	3-Thujene	tr	0.20	Co-GC, MS, RI
2	935	α-Pinene	0.56	0.30	Co-GC, MS, RI
3	951	Camphene	1.41	tr	Co-GC, MS, RI
4	976	Sabinene	0.72	tr	Co-GC, MS, RI
5	980	β-Pinene	0.81	tr	Co-GC, MS, RI
6	985	3-Octanone	tr	1.51	Co-GC, MS, RI
7	991	β-Myrcene	0.80	0.60	Co-GC, MS, RI
8	995	3-Octanol	tr	0.11	Co-GC, MS, RI
9	1019	α-Terpinene	tr	0.55	Co-GC, MS, RI
10	1027	*o*-Cymene	1.40	4.06	Co-GC, MS, RI
11	1031	Limonene	1.23	0.40	Co-GC, MS, RI
12	1034	1,8-Cineole (Eucalyptol)	18.16	1.41	Co-GC, MS, RI
13	1047	*cis*-β-Ocimene	0.60	0.13	Co-GC, MS, RI
14	1060	γ-Terpinene	tr	5.00	Co-GC, MS, RI
15	1069	Sabinene hydrate	tr	0.90	Co-GC, MS, RI
16	1101	Linalool	0.30	2.77	Co-GC, MS, RI
17	1149	Camphor	15.08	tr	Co-GC, MS, RI
18	1170	*endo*-Borneol	11.63	0.51	Co-GC, MS, RI
19	1181	4-Terpineol	2.06	0.18	Co-GC, MS, RI
20	1195	β-Fenchyl alcohol	4.00	0.19	Co-GC, MS, RI
21	1243	*cis*-Citral	6.00	tr	Co-GC, MS, RI
22	1246	Carvacrol methyl ether	tr	0.10	Co-GC, MS, RI
23	1255	*trans*-Geraniol	0.11	tr	Co-GC, MS, RI
24	1273	*trans*-Citral	8.56	tr	Co-GC, MS, RI
25	1290	Bornyl acetate	3.24	tr	Co-GC, MS, RI
26	1297	Thymol	tr	1.16	Co-GC, MS, RI
27	1303	Carvacrol	tr	72.88	Co-GC, MS, RI
28	1393	β-Bourbonene	0.36	0.07	Co-GC, MS, RI
29	1429	Caryophyllene	2.10	1.89	Co-GC, MS, RI
30	1448	Aromandendrene	tr	0.26	Co-GC, MS, RI
31	1463	α-Humulene	0.27	0.08	Co-GC, MS, RI
32	1470	Alloaromadendrene	0.46	tr	Co-GC, MS, RI
33	1484	γ-Muurolene	1.60	0.14	Co-GC, MS, RI
34	1490	Germacrene D	0.55	tr	Co-GC, MS, RI
35	1504	Varidiflorene	tr	0.30	Co-GC, MS, RI
36	1514	β-Bisabolene	0.61	0.22	Co-GC, MS, RI
37	1523	γ-Cadinene	tr	0.10	Co-GC, MS, RI
38	1531	δ-Cadinene	0.56	0.17	Co-GC, MS, RI
39	1567	Nerolidol	3.95	tr	Co-GC, MS, RI
40	1588	Spathulenol	2.04	0.29	Co-GC, MS, RI
41	1595	Caryophyllene oxide	1.03	0.30	Co-GC, MS, RI
42	1625	Epicubenol	0.50	tr	Co-GC, MS, RI
43	1650	*T*-Cadinol	5.00	tr	Co-GC, MS, RI
44	1664	α-Cadinol	1.61	tr	Co-GC, MS, RI
Hydrocarbons			-	1.62	
Monoterpenes hydrocarbons			7.53	11.24	
Oxygenated monoterpenes			69.14	80.1	
Sesquiterpenes hydrocarbons			6.51	3.23	
Oxygenated sesquiterpenes			14.13	0.59	
	Total identified (%)		97.31	96.78	

[a]Kovats index on HP–5MS fused silica column, [b]A nonpolar Agilent HP-5MS fused silica column, [c]Percentage concentration, [Co-GC]Co-injection with authentic compounds, [RI]Retention Index literature comparison, [tr] trace: %<0.05

Results of Biological Activities

Antioxidant and Toxic-Cytotoxic Activities

The antioxidant activity of the essential oils of the species was determined by lipid peroxidation, DPPH free radical, ABTS cation radical and CUPRAC methods (Table 2). In lipid peroxidation and DPPH methods, TC-E showed good antioxidant activity (IC_{50}: 45.72±0.12 and 60.60±1.68 µg/ml, respectively) and TS-E showed low antioxidant activity (IC_{50}: 650.12±2.41 and 1000 µg/ml, respectively). High antioxidant activity was observed for TC-E in ABTS and CUPRAC methods. In ABTS method, TC-E showed higher antioxidant activity (IC_{50}: 6.12±0.03 µg/ml) than BHT and α-TOC used as reference (IC_{50}: 13.67±0.33 µg/ml and IC_{50}: 10.43±0.44 µg/ml, respectively). In the CUPRAC method, it was determined that TC-E showed higher antioxidant activity (IC_{50}: 5.31±0.01 µg/ml) compared to the references (IC_{50}: 8.24±0.05 µg/ml and IC_{50}: 18.57±0.27 µg/ml, respectively), supporting the ABTS results. In a study conducted with 4 different *Thymus* spp., including *T. canoviridis* species, antioxidant activity was examined using only DPPH method and it was found to show good antioxidant activity. In addition, it was determined that thymol and carvacrol detected in the species were the active components of essential oils [6]. It is available in the literature that oxygenated monoterpenes, especially thymol and carvacrol, two well-known phenolic compounds, are mainly responsible for the antioxidant potential of essential oils containing them [28,29]. In this context, it can be said that the antioxidant capacity of *T. canoviridis* essential oil is related to carvacrol (72.88%), which is the major compound in this study. In another study in which the antioxidant activity of TS-E extract was compared with a different *Thymus* spp., the antioxidant activity of TS-E was found to be low similar to this study. The reason for the low activity was attributed to the low amounts of both monoterpene hydrocarbons and oxygenated monoterpenes [24].

The toxic effects of the samples were determined by MTT method on healthy cell line (PDF) and cytotoxic effects on cancerous MCF-7 (breast cancer) and HT-29 (colon cancer) (Table 2). In general, the essential oil of the two species showed low toxicity on healthy human cell line (PDF). TC-E and TS-E samples showed moderate cytotoxicity (43.12±0.99 and 65.92±1.32 % viability against HT-29 cells, 200 µg/ml) on colon cancer. The two species showed very high cytotoxic activity (16.84±0.06 and 10.72±0.03 % viability, 200 µg/ml, respectively) against MCF-7 cells in breast cancer.

There are no studies in the literature on the cytotoxic activity of essential oils of *T. sipyleus* and *T. canoviridis*. In a study comparing the cytotoxic activity of methanol extracts of *T. sipyleus* and *Thymus leucostomus* Hausskn. et Velen, the cytotoxic effects of the extracts of these species on cervical (HeLa) and ovarian cancer (Skov-3) cells were investigated using colorimetric assay. The results showed that the methanol extract of *T. sipyleus* was moderately active against Skov-3 cell line (IC_{50}: 60 µg/ml) compared to *T. leucostomus* (IC_{50}: 360 µg/ml). Against HeLa cell line, *T. sipyleus* showed no effect after 72 hours (IC_{50}: 907 mu g/ml) [30]. In another study investigating the anticancer effects of *Thymus* spp., the effect of *Thymus* vulgaris on breast cancer was examined. Thymol (55.88%), linalool (13.71%), carvacrol (8.36%) and p-cymene (6.00%) were found as the main components in the essential oil and the anticancer activity results revealed that treatment of MCF-7 cells with 100 µg/ml of essential *T. vulgaris* oil promoted apoptosis and induced DNA damage on the cells [31]. In a study investigating the selective cytotoxic and antiproliferative properties of *Thymus* caramanicus Jalas extract, MCF-7 human breast cancer cells were used and cytotoxicity was determined using MTT and neutral red assays. Vincristine was used as an anticancer control drug in the extract combination treatment. As a result, it was reported that *Thymus* extract has a potential antiproliferative property against human breast cancer cells and its combination with the chemotherapeutic agent vincristine can effectively induce cell death and that the most active components of *T. caramanicus*, carvacrol (51.0%) and thymol (20.84%) may be responsible for the anticancer effect observed in this study [32].

Enzyme Inhibitory Activities

Anticholinesterase (acetylcholinesterase and butyrylcholinesterase), urease, tyrosinase, elastase, collagenase, and angiotensin converting enzyme (ACE) inhibition activities of essential oils of TC-E and TS-E species were determined *in vitro* (Table 2). When the results were evaluated, it was determined that the two studied species showed moderate inhibition activity against BChE enzyme (Inhibition%:

57.88±1.14, 39.21±0.89, respectively). In addition, TS-E showed moderate inhibition of elastase enzyme (Inhibition%: 25.33±0.79) while TC-E showed no inhibition of elastase enzyme. In the AChE, urease, tyrosinase, tyrosinase, collagenase, ACE inhibition activities of both *Thymus* spp., the essential oils showed low or no activity. In another study conducted with *T. canoviridis, Thymus pubescens* Boiss. et Kotschy ex Celak. var. *pubescens* and *Thymus leucotrichus* Hal. var. *leucotrichus*, the biological activities of the essential oils of the species were investigated. IC_{50} values were calculated as 34.99 µg/ml, 44.15 µg/ml and 36.75 µg/ml for anticholinesterase enzyme, respectively. *T. canoviridis*, which is very rich in carvacrol (52.87%), was found to have higher antioxidant, antimicrobial and anticholinesterase activity than other essential oils [33]. In addition, studies have reported that carvacrol has antioxidant, antimicrobial, antidiabetes, antiparkinsonian, antialzheimer and antiglaucoma effects [34].

Table 2. Biological activities of the *Thymus* species[1]

	Antioxidant activity				**Toxic-cytotoxic activity**		
	IC_{50} (µg/ml)			**$A_{0.5}$ (µg/ml)**	**(%viability 200 µg/ml)**		
Samples	**Lipid peroxidation**	**DPPH**	**ABTS**	**CUPRAC**	**PDF**	**HT-29**	**MCF-7**
TS-E	650.12±2.41^{a}	>1000^{a}	312.45±3.21^{a}	217.50±5.12^{a}	73.00±1.23^{a}	65.92±1.32^{a}	10.72±0.03^{a}
TC-E	45.72±0.12^{b}	60.60±1.68^{b}	6.12±0.03^{b}	5.31±0.01^{b}	74.06±1.02^{a}	43.12±0.99^{b}	16.84±0.06^{b}
Butyl hydroxytoluene[2]	10.38±0.11^{c}	55.12±1.03^{c}	13.67±0.33^{b}	8.24±0.05^{c}	-	-	-
α- Tocopherol[2]	16.23±0.12^{d}	16.43±0.34^{d}	10.43±0.44^{d}	18.57±0.27^{d}	-	-	-
Doxorubicin (20 µg/ml)	-	-	-	-	-	23.26±0.86^{c}	-
Cisplatin (20 µg/ml)	-	-	-	-	-	-	48.12±0.99^{c}
Enzyme activity (inhibition %, at 100 µg/ml)							
Samples	**AChE**	**BChE**	**Urease**	**Tyrosinase**	**Elastase**	**Collagenase**	**ACE**
TS-E	NA	39.21±0.89^{a}	3.75±0.02^{a}	7.16±0.08^{a}	25.33±0.79^{a}	12.19±0.12^{a}	15.10±0.23^{a}
TC-E	4.19±0.01^{a}	57.88±1.14^{b}	11.94±0.11^{b}	NA	NA	3.17±0.03^{b}	11.19±0.18^{b}
Galantamine[2]	88.16±1.43^{b}	80.13±1.02^{c}	-	-	-	-	-
Thiourea[2]	-	-	95.89±1.36^{c}	-	-	-	-
Kojic acid[2]	-	-	-	92.07±1.21^{b}	-	-	-
Oleanolic acid[2]	-	-	-	-	43.75±1.38^{b}	-	-
Epicatechin gallate[2]	-	-	-	-	-	86.84±1.68^{c}	-
Lisinopril[2]							98.8±1.23^{c}

[1]Values expressed are means ± S.D. of three parallel measurements and values were calculated according to negative control. Values with different letters in the same column were significantly different ($p < 0.05$). [2]Standard compound. NA: Not active

In a study investigating the antioxidant and anticholinesterase activities of *Cyclotrichium origanifolium* (Labill.) Manden & Scheng (CO) and *T. sipyleus* (TS), the AChE and BChE activities of CO and TS were determined at a concentration of 200 µg/ml using galantamine as a standard compound. The best inhibition values against AChE and BChE enzymes were determined for CO (Inhibition%: 58.40 and 60.73 respectively) and TS (Inhibition%: 56.65 and 48.76 respectively) compared to galantamine [35]. The BChE enzyme inhibition activity of *T. sipyleus* was found to be moderate, similar to our study.

The chemical composition of the essential oils of *T. canoviridis* and *T. sipyleus* species were analyzed by GC-MS. There are no studies in the literature on the toxic-cytotoxic activities of the essential oils of the species and this study is the first in this sense. In addition, the enzyme inhibition (anticholinesterase, urease, tyrosinase, elastase, cholinase and angiotensin converting enzyme activities) of both species were examined in detail in this study. Especially *T. canoviridis* species showed high antioxidant activity. Both species showed high cytotoxic effect on breast cancer cells. When the results are evaluated, it can be said that *T. canoviridis* essential oil with its rich carvacrol content and high cytotoxic and antioxidant activity can be preferred as a safer and natural option instead of synthetic preservatives in the food, pharmaceutical and cosmetic industries to extend shelf life and ensure food safety.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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