

In Vitro Antimicrobial and Antioxidant Activities of the Essential Oils and Various Extracts of *Thymus eigii*

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This study was designed to examine the in vitro antimicrobial and antioxidant activities of the essential oil and various extracts obtained from aerial parts of *Thymus eigii*. The essential oil was particularly found to possess stronger antimicrobial activity, whereas other nonpolar extracts and subfractions showed moderate activity and polar extracts remained almost inactive. GC-MS analysis of the oil resulted in the identification of 39 compounds, representing 93.7% of the oil; thymol (30.6%), carvacrol (26.1%), and *p*-cymene (13.0%) were the main components. The samples were also subjected to a screening for their possible antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β -carotene–linoleic acid assays. In the former case, the polar subfraction of the methanol extract was found to be superior to all extracts tested, only 16.8 $\mu\text{g}/\text{mL}$ of which provided 50% inhibition, whereas all extracts, particularly the polar ones, seem to inhibit the oxidation of linoleic acid in the latter case. These data were further supported by total phenolics analysis, indicating that the antioxidative potential of the extracts was closely related to their phenolic constituents.

KEYWORDS: Antimicrobial activity; antioxidant activity; essential oil; methanol extract; *Thymus eigii*

INTRODUCTION

Thymus eigii (syn. *T. syriacus* subsp. *eigii*) (Lamiaceae) belongs to a genus, *Thymus*, of over 300 species of hardy perennial herbaceous plants and subshrubs, which are native to southern Europe and Asia (1). Chief among them, *Thymus vulgaris* L. (thyme or garden thyme), a well-known aromatic and medicinal plant, has an increasing importance in horticulture (2). This species was noted to be used by the Sumerians as long ago as 3500 B.C. and by the ancient Egyptians in embalming. *Thymus* oils were used, along with clove, lemon, and chamomile essential oils, as a disinfectant and antiseptic in hospitals until the beginning of the 20th century (3). This genus has also a great reputation for many other pharmacological properties, such as spasmolytic, expectorant, and mammalian age delaying (4, 5). At present, the essential oils of many *Thymus* species are widely used as flavoring agents in food processing and many pharmacological preparations, and particularly thyme oil is still among the world's top 10 essential oils (4). Reports concerning antimicrobial and antioxidant properties of many *Thymus* members have been numerous in the literature, and the

essential oils have frequently remained in the center of many studies (5–12).

Like other *Thymus* species available in the Turkish flora, 38 species and altogether 64 taxa, 24 of which are endemic (13, 14), *T. eigii* is called “kekik” and from our verbal communication with the local people from the collection area, herbal parts of this plant are used as tea and condiment. This plant is a shrublet, up to 12–20 cm, and narrowly distributed in southern Anatolia (13). To the best of our knowledge, the essential oil composition of *T. eigii* was reported previously (15), but no information was available concerning the in vitro antimicrobial and antioxidant activities of the essential oil and extracts of this plant species. The aims of this work were (i) to evaluate the in vitro antimicrobial and antioxidant properties of the essential oil, obtained by using a Clevenger distillation apparatus, as well as the various extracts, obtained by using several solvents of varying polarity in a Soxhlet extractor and (ii) to determine the chemical composition of *T. eigii* essential oil. For these, the in vitro antimicrobial activities were determined by using agar well diffusion, agar disk diffusion, and broth microdilution methods. The in vitro antioxidant activities were determined by using two complementary assays, namely, inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and the β -carotene–linoleic acid systems. The chemical composition of the essential oil was evaluated by using gas chromatography–mass spectroscopy (GC-MS).

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EXPERIMENTAL PROCEDURES

Plant Material. *T. eigi* plants were collected from the Söğütülgöl plateau (1000 m), Düziçi-Osmaniye, Turkey, when flowering (late July 2001). The voucher specimen was identified by a senior plant taxonomist, Dr. Erol Dönmez, at the Department of Biology, Cumhuriyet University, Sivas, Turkey, and has been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas, Turkey (CUFH Voucher ED 6364).

Preparation of the Extracts. Air-dried herbal parts of *T. eigi* were subjected to the different extraction procedures given below.

Extraction of the Essential Oil (EO). The air-dried and ground aerial parts of the plants were submitted for 3 h to water distillation using a Clevenger-type apparatus (yield = 1.02%, v/w). The obtained EO was dried over anhydrous sodium sulfate and, after filtration, stored at 4 °C until tested and analyzed.

Preparation of the Deodorized Hot Water Extract (DeHW). After completion of hydrodistillation, the liquid retentate was collected and lyophilized. This procedure gave DeHW in a yield of 10.58% (w/w) (16).

Preparation of the Deodorized Methanol Extract (DeMeOH). The solid retentate of the hydrodistillation was dried and re-extracted with methanol. The resulting extract (DeMeOH) (8.19%, w/w) was fractionated with water and chloroform (CHCl₃) to obtain deodorized water-soluble (DeMW) (4.22%, w/w) and water-insoluble (deodorized chloroformic) (DeMC) (3.97%, w/w) subfractions (16).

Preparation of the Hexane (HE), Dichloromethane (DCM), and Methanol (MeOH) Extracts. A portion (100 g) of dried plant material was extracted with HE (3.75%, w/w), followed by DCM (3.14%, w/w) and MeOH (7.86%, w/w) in a Soxhlet apparatus (6 h for each solvent). The latter extract was suspended in water and partitioned with chloroform to obtain water-soluble (polar) (MW) (6.12%, w/w) and water-insoluble (chloroformic, nonpolar) (MC) (1.46%, w/w) subfractions (17). All extracts obtained were lyophilized and kept in the dark at 4 °C until use.

GC Analysis Conditions of the Essential Oil. The essential oil was analyzed using a Hewlett-Packard 5890 II GC equipped with an FID detector and an HP-5 ms capillary column (30 m × 0.25 mm, film thickness = 0.25 μm). Injector and detector temperatures were set at 220 and 290 °C, respectively. GC oven temperature was kept at 50 °C for 3 min, then programmed to 180 °C at a rate of 3 °C/min, finally raised to 240 °C at 6 °C/min, and held for 5 min. Helium was the carrier gas, at a flow rate of 1 mL/min. Diluted samples (1:100 in acetone, v/v) of 1.0 μL were injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

GC-MS Analysis Conditions of the Essential Oil. The analysis of the essential oil was performed under the same conditions with GC, using a Hewlett-Packard 5890 II gas chromatograph equipped with a Hewlett-Packard 5972 mass selective detector in the electron impact mode (70 eV). Identification of the components was based on comparisons of their relative retention times and mass spectra with those obtained from standards and/or the NBS75K library data and the literature (18). All standards were purchased from the Sigma-Aldrich Co. Alkanes were used as reference points in the calculation of relative retention indices (RRI).

DPPH Assay. The hydrogen atoms or electrons' donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple methanol solution of DPPH. As reported elsewhere (19, 20), this spectrophotometric assay uses the stable radical DPPH as a reagent.

DPPH Assay on TLC. This procedure was applied only for the isolated essential oil. Five microliters of a 1:10 (v/v) dilution of the essential oil in toluene was applied to the TLC plate and a toluene/ethyl acetate (93:7) mixture was used as developer. The plate was sprayed with a 0.2% DPPH reagent in methanol and left at room temperature for 30 min. As explained above, yellow spots formed from bleaching the purple color of the DPPH reagent were evaluated as positive antioxidant activity.

β-Carotene—Linoleic Acid Assay. In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile

organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. This assay was carried out following a method given elsewhere (16).

Assay for Total Phenolics. Assays of the total phenolic constituents of the aforesaid extracts of *T. eigi* were performed employing the literature methods involving Folin—Ciocalteu reagent and gallic acid as standard (21, 22).

Microbial Strains. The essential oil and extracts were individually tested against a panel of microorganisms including *Staphylococcus aureus* ATCC 25923 and ATCC 29213 [for minimum inhibitory concentration (MIC) test], *Streptococcus pneumoniae* ATCC 49619, *Moraxella catarrhalis* ATCC 49143, *Bacillus cereus* ATCC 11778, *Acinetobacter lwoffii* ATCC 19002, *Enterobacter aerogenes* ATCC 13043, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, *Pseudomonas aeruginosa* ATCC 27853, *Clostridium perfringens* KUKENS-Turkey, mycobacterium, *Mycobacterium smegmatis* CMM 2067, *Candida albicans* ATCC 10239, and *Candida krusei* ATCC 6258. Bacterial strains were cultured overnight at 37 °C in Mueller Hinton agar (MHA), with the exception of *S. pneumoniae* (MHA containing 50 mL of citrate blood/L) and *C. perfringens* (in anaerobic conditions). Yeasts were cultured overnight at 30 °C in Sabouraud dextrose agar.

Antimicrobial Screening. Two different methods were employed for the determination of antimicrobial activities: an agar well diffusion method for the extracts and a disk diffusion method for the essential oil (23). The MICs of the essential oil against the test microorganisms were determined by broth microdilution method (24). The MICs of netilmicin and amphotericin B were also determined in parallel experiments in order to control the sensitivity of the test microorganisms. All tests were performed in duplicate.

Agar well diffusion and disk diffusion assays and determination of MICs were performed according to a previous paper (12).

RESULTS

Chemical Composition of the Essential Oil. The overall activity test results from our experiments indicated the superiority of the essential oil to the other extracts studied. Therefore, priority has been given to the oil produced from the water-distilled herbal parts of *T. eigi* (yield = 1.02% v/w). GC-MS analysis of the crude oil resulted in the identification of 39 compounds representing 93.7% of the oil. Thymol (30.6%), carvacrol (26.1%), and *p*-cymene (13.0%) were the main components comprising 69.7% of the oil (Table 1).

Antioxidant Activity. DPPH Assay. Free radical scavenging activities of the extracts were measured in the DPPH assay, and the reaction followed a concentration-dependent pattern. Inhibition ratio (percent) against increasing *T. eigi* essential oil concentration is shown in Figure 1. As can be seen from the figure, free radical inhibition of the oil is correlated with its concentration, because it reaches at higher value in the presence of the highest oil concentration. All extracts were subjected to the same procedure, and extract concentrations providing 50% inhibition were given in Table 2. The free radical scavenging activity of the aqueous methanol extract (MW) was superior to those of all other extracts (IC₅₀ = 16.8 μg/mL). Moreover, polar extracts generally exhibited stronger activity than the nonpolar extracts. When compared to butylated hydroxytoluene (BHT), the MW was the most effective radical scavenger. The observed antioxidant activity could be attributed to the presence of hydrophilic phenolic substances, such as phenolic acids.

DPPH Assay on TLC. The oil was applied on silica gel TLC plates, and nine zones were determined. With DPPH reagent at least three spots appeared immediately after the TLC plate had been sprayed. These spots were identified as carvacrol, thymol, and α-terpineol when compared to the standard test compounds and GC-MS analysis. Therefore, the antioxidative potential of the essential oil is considered to be related to high contents of

Table 1. Composition of *T. eigii* Essential Oil (Percent)

	compound ^a	RRI ^b	<i>T. eigii</i>
1	α -thujene ^e	1130	1.2
2	α -pinene ^d	1146	1.2
3	camphene ^e	1173	1.0
4	β -pinene ^d	1233	0.2
5	1-octen-3-ol ^e	1256	1.5
6	β -myrcene ^d	1268	0.3
7	3-octanol ^e	1287	0.4
8	α -terpinene ^d	1313	0.7
9	<i>p</i> -cymene ^d	1330	13.0
10	<i>cis</i> -sabinene hydrate ^e	1404	0.9
11	terpinolene ^e	1432	0.1
12	linalool ^d	1451	0.8
13	camphor ^d	1505	0.4
14	borneol ^d	1539	1.9
15	terpinen-4-ol ^e	1550	0.4
16	<i>p</i> -cymen-8-ol ^e	1562	0.2
17	α -terpineol ^d	1565	1.5
18	methyl chavicol ^e	1573	0.4
19	α -terpineol isomer ^e	1576	1.0
20	thymol methyl ether ^e	1618	tr ^c
21	thymoquinone ^e	1624	1.4
22	anethole (E) ^e	1658	1.1
23	thymol ^d	1669	30.6
24	carvacrol ^d	1681	26.1
25	eugenol ^d	1719	0.1
26	α -copaene ^e	1729	tr
27	β -bourbonene ^e	1735	0.2
28	β -caryophyllene ^d	1759	3.6
29	β -copaene ^e	1766	0.1
30	α -caryophyllene ^e	1781	0.1
31	γ -muurolene ^e	1795	0.2
32	germacrene D ^e	1798	0.3
33	γ -amorphene ^e	1805	0.2
34	bicyclogermacrene ^e	1808	tr
35	γ -cadinene ^e	1818	0.2
36	δ -cadinene ^e	1822	0.3
37	thymohydroquinone ^e	1848	0.3
38	caryophyllene oxide ^e	1852	1.4
39	cadinol (epi- α) ^e	1882	0.4
	total		93.7

^a Compounds listed in order of elution from an HP-5 ms column. ^b Relative retention indices. ^c Trace ($\leq 0.05\%$). ^d Identification of components based on standard compounds. ^e Tentative identification.

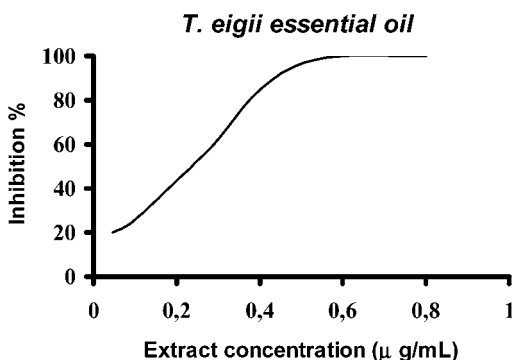


Figure 1. Inhibition ratio (percent) against increasing *T. eigii* extract concentration in DPPH method. Results are means of three different experiments. Data points are without bars because the standard deviation was too small to show.

these components in the oil. In the DPPH system, antioxidant activities of thymol and carvacrol were previously reported (12, 20, 25), whereas the latter was concluded as having weaker activity (25).

β -Carotene–Linoleic Acid Assay. As far as the percentage of the relative antioxidative activity is concerned, the inhibition

Table 2. Effects of *T. eigii* Essential Oil, Extracts, and Positive Controls on in Vitro Free Radical (DPPH and Hydroxyl) Generation: IC₅₀ Values Are Given as Micrograms per Milliliter^a

sample	DPPH	sample	DPPH
HE	144.60 \pm 1.20	DeMW	39.60 \pm 1.20
DCM	99.00 \pm 0.70	HW	142.50 \pm 3.60
MC	93.10 \pm 0.95	EO	225.00 \pm 5.25
MW	16.80 \pm 0.50	BHT	19.80 \pm 1.10
DeMC	128.9 \pm 2.10		

^a Results are given as mean \pm standard deviation of three different experiments.

Table 3. Amounts of Total Phenolic Compounds in *T. eigii* Extracts^a

extract	gallic acid equivalent (μ g/mg)	extract	gallic acid equivalent (μ g/mg)
control		MC	146.00 \pm 3.50
oil (EO)		DeMW	215.00 \pm 4.00
HE	165.00 \pm 2.00	DeMC	34.00 \pm 1.00
DCM	49.50 \pm 1.50	DeHW	70.00 \pm 1.50
MW	110.00 \pm 2.50		

^a Results are given as mean \pm standard deviation of three different experiments.

was higher in the polar extracts and or/subfractions, for example, polar subfractions of methanol extracts of deodorized material and deodorized hot water extract, with relative antioxidative activities at 87.1 and 83.1%, respectively (**Figure 2b**), whereas those of the nonpolar extracts and/or subfractions ranged from 58.2 to 78.7% (**Figure 2a**).

Assay for Total Phenolics. In the preparation of *T. eigii* extracts, a number of solvents with various polarities have been employed using a Soxhlet extractor. On the basis of the absorbance values of the various extract solutions, reacted with Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents as described above, results of the colorimetric analysis of total phenolics are given in **Table 3**. The amount of total phenolics was highest in the polar subfraction of methanol extracts from deodorized material (DeMW) (21.5%), followed by hexane (HE) extract (16.5%) and nonpolar and polar subfractions of methanol extract (14.6 and 11.0%, respectively), and the lowest was in the chloroform subfraction of the methanol extract of deodorized material (3.4%).

Antimicrobial Activity. As far as the in vitro antimicrobial activity results are concerned, the essential oil possessed extremely strong activity, followed by nonpolar extracts and hexane (HE), dichloromethane (DCM), and chloroformic subfractions (DeMC and MC) of the methanol extracts from deodorized and nondeodorized materials. Also, all nonpolar extracts exhibited similar activity patterns, whereas all polar subfractions (MW and DeMW) and deodorized hot water (DeHW) extracts did not exert any antimicrobial activity (**Table 4**).

In the presence of the oil, the strongest activity was observed against *B. catarrhalis* and *C. perfringens* with an MIC at 0.06 mg/mL (w/v), followed by *B. cereus* with an MIC at 0.14 mg/mL (w/v), and *S. aureus*, *S. pneumoniae*, and *M. smegmatis* with MICs at 0.06 mg/mL (w/v). The weakest activity was observed against *P. aeruginosa* (MIC = 18.00 mg/mL). The growth inhibitions of test microorganisms were also evaluated by using the oil and its main constituents (carvacrol, thymol, and *p*-cymene) individually in broth microdilution method. The lowest MIC value was found in the presence of carvacrol against the following microorganisms: *C. perfringens* (MIC = 0.06 mg/mL, w/v), *B. cereus* and *M. smegmatis* at 0.13 mg/mL (w/

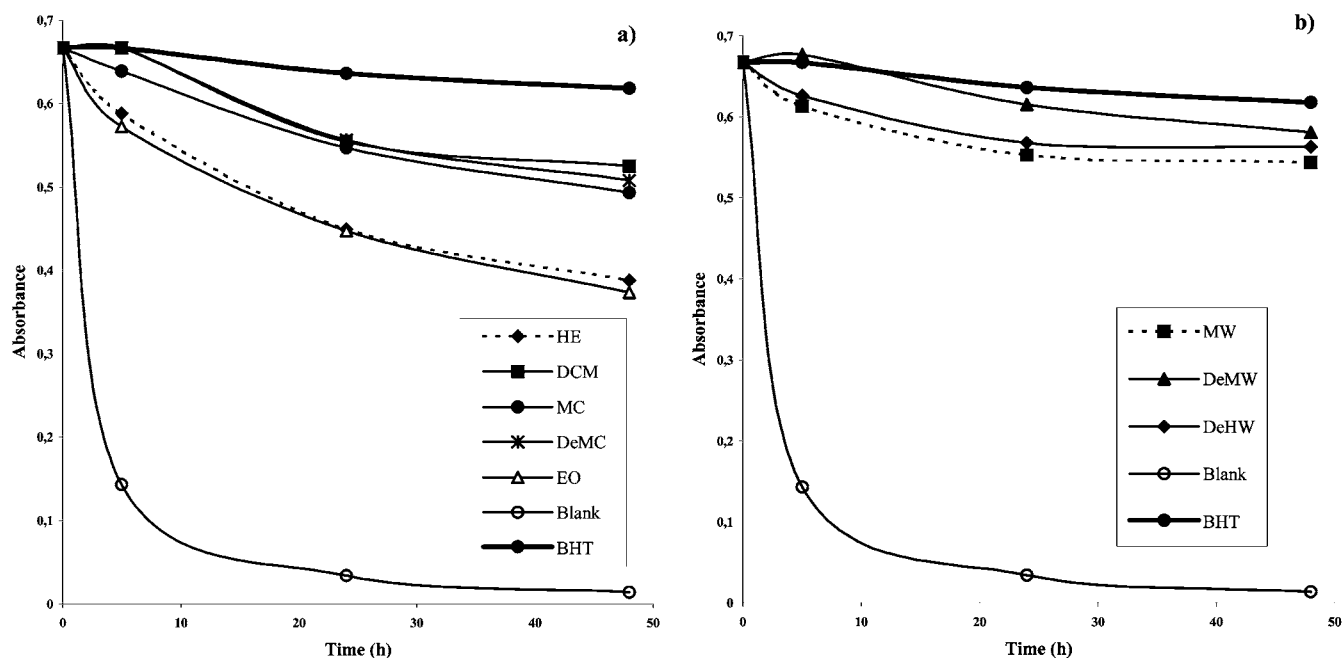


Figure 2. Absorbance change of β -carotene at 490 nm in the presence of *T. eigi* extracts: (a) nonpolar extracts; (b) polar extracts, control and positive control BHT. Results are means of three different experiments. Data points are without bars because the standard deviation was too small to show.

Table 4. Antimicrobial Activities of the Essential Oils and Various Extracts of *T. eigi*

microorganism	EO ^b	extracts ^a						
		HE	DCM	MeOH		DeHW	DeMeOH	
				MW	MC		DeMW	DeMC
<i>Staphylococcus aureus</i>	30.00 ± 0.00	NA ^c	NA	NA	13.00 ± 0.00	NA	NA	NA
<i>Streptococcus pneumoniae</i>	>60.00	18.00 ± 1.40	19.00 ± 1.40	NA	17.00 ± 1.40	NA	NA	16.50 ± 2.10
<i>Moraxella catarrhalis</i>	23.00 ± 1.10	NA	NA	NA	NA	NA	NA	NA
<i>Bacillus cereus</i>	28.25 ± 2.36	14.50 ± 2.10	11.50 ± 2.10	NA	12.00 ± 0.00	NA	NA	11.00 ± 1.40
<i>Acinetobacter lwoffii</i>	38.75 ± 1.50	10.00 ± 0.00	11.50 ± 0.70	NA	12.00 ± 1.40	NA	NA	14.00 ± 1.40
<i>Enterobacter aerogenes</i>	25.00 ± 0.00	NA	NA	NA	NA	NA	NA	NA
<i>Escherichia coli</i>	26.25 ± 2.62	NA	NA	NA	NA	NA	NA	NA
<i>Klebsiella pneumoniae</i>	11.50 ± 0.50	NA	NA	NA	NA	NA	NA	11.00 ± 1.40
<i>Proteus mirabilis</i>	21.50 ± 1.00	NA	NA	NA	NA	NA	NA	NA
<i>Pseudomonas aeruginosa</i>	8.75 ± 1.50	NA	NA	NA	NA	NA	NA	NA
<i>Clostridium perfringens</i>	37.50 ± 2.80	12.00 ± 0.00	9.50 ± 0.70	NA	15.50 ± 0.70	NA	NA	13.50 ± 0.70
<i>Mycobacterium smegmatis</i>	>60.00	NA	NA	NA	NA	NA	NA	NA
<i>Candida albicans</i>	>60.00	13.00 ± 0.00	12.00 ± 1.40	NA	12.50 ± 0.70	NA	NA	12.00 ± 0.00
<i>Candida krusei</i>	>60.00	NA	NA	NA	NA	NA	NA	NA

^a Diameter of inhibition zone including well diameter of 8 mm. ^b Diameter of inhibition zone including disk diameter of 6 mm. ^c Not active.

v), followed by *S. aureus*, *S. pneumoniae*, *M. catarrhalis*, *A. lwoffii*, and the yeasts *C. albicans* and *C. krusei* with MIC values at 0.28 mg/mL (w/v), individually. Again, *P. aeruginosa* was the most resistant microorganism with an MIC at 4.50 mg/mL (w/v) (Table 5).

DISCUSSION

Chemical Composition of the Essential Oil. Our findings support a rule with an indication that thymol and carvacrol are present in the essential oils of some Lamiaceae species, such as *Thymus*, *Satureja*, and *Origanum*, in almost equal amounts. Also, our GC-MS results differ from those of a previous study, in which carvacrol (64.61%) was the predominant constituent of *T. eigi* oil (15). This finding indicates that the composition of any plant essential oil studied is influenced by the presence of several factors, such as local, climatic, seasonal, and experimental conditions (26, 27), which alter the biological activities studied (12).

Antioxidant Activity. DPPH radical scavenging activities of the extracts and the oil led us to make a general conclusion;

Table 5. MICs^a of the Essential Oil, Its Commercially Available Major Components, and Control Antibiotics

microorganism	commercially available chemicals				antibiotics	
	oil	thymol	carvacrol	<i>p</i> -cymene	NET ^b	AMP B ^c
<i>S. aureus</i>	0.28	1.12	0.28	≥72.00	8 × 10 ⁻³	
<i>S. pneumoniae</i>	0.28	0.28	0.28	≥72.00		
<i>M. catarrhalis</i>	0.06	0.56	0.28	36.00		
<i>B. cereus</i>	0.14	0.56	0.14	72.00		
<i>A. lwoffii</i>	0.56	0.28	0.28	36.00		
<i>E. aerogenes</i>	2.25	0.56	1.12	≥72.00		
<i>E. coli</i>	1.12	1.12	0.56	≥72.00	1 × 10 ⁻²	
<i>K. pneumoniae</i>	4.50	1.12	2.25	≥72.00	1 × 10 ⁻²	
<i>P. mirabilis</i>	2.25	1.12	1.12	≥72.00		
<i>P. aeruginosa</i>	18.00	9.00	4.50	≥72.00	1 × 10 ⁻²	
<i>C. perfringens</i>	0.06	0.56	0.06	18.00		
<i>M. smegmatis</i>	0.28	0.28	0.14	36.00		
<i>C. albicans</i>	0.56	0.28	0.28	18.00		1 × 10 ⁻³
<i>C. krusei</i>	0.56	0.28	0.28	18.00		1 × 10 ⁻³

the activity, seen for the different extracts of *T. eigi*, is closely related to the content of phenolic compounds. Indeed, when the results given in Table 3 and Figure 2b were compared, it

was seen that the amount of phenolic content was high in polar extracts. The hexane extract was found to have high phenolic constituents exhibiting weaker radical scavenging activity. It seems clear that the presence of polar phenolics is another fact concerning the evaluation of the activity in free radical scavenging. Besides, the moderate activity seen in the polar subfraction of methanol extract from deodorized retentate (DeMW), which is often spoiled, reflects the thermostable nature of these polar phenolics. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (28, 29). Moreover, radical scavenging activity is one of various mechanisms to contribute overall activity, thereby creating a synergistic effect.

In the β -carotene–linoleic acid system, all extracts seem to inhibit the oxidation of linoleic acid, which is an important issue in food processing and preservation (Figure 2). Antioxidants minimize the oxidation of lipid components in cell membranes or inhibit the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation that are known to be carcinogenic. Especially, polar extracts exhibited stronger activity than nonpolar ones, which is in accordance with free radical scavenging activity results. The presence of polyphenols, particularly methoxylated flavonoids in *Thymus* species, was reported previously (30). Therefore, any type of extraction procedure is suitable to obtain *T. eigii* active components inhibiting lipid oxidation. The plant has excellent protective activity.

Antimicrobial Activity. The antimicrobial nature of the essential oils isolated from several Lamiaceae species has been attributed to the presence of various substances, mainly the phenolic monoterpenes thymol and carvacrol. In a previous paper, the individual oil components were tested, and thymol was found to be the most active substance with the widest spectrum, followed by carvacrol, α -terpineol, and terpinen-4-ol, whereas *p*-cymene was reported to be the least active (31). Our results are in agreement with the latter case (Table 5). However, the most active component was found to be carvacrol, rather than thymol, and these findings are in agreement with other previous studies (9, 32–34). Synergistic effects of thymol and carvacrol and an antagonistic effect of *p*-cymene are quite possible, and these facts would be considered when the antimicrobial activity of the oil from any particular plant was studied. Similar and moderate activities of nonpolar extracts can be attributed to the presence of several types of compounds belonging to different classes, such as oleoresins in the hexane extract (16); sterols and their derivatives, flavones, and flavonoids in the dichloromethane extract (DCM) (35); and more polar thermolabile and/or thermostable phenolics in the subfractions of methanol extracts from both deodorized and nondeodorized materials, respectively (17). Due to their complex nature, more detailed studies employing bioassay-guided fractionation would be worthwhile to explore the nature of the active extracts prepared.

Antioxidative and antimicrobial properties of the essential oils and various extracts from many plants have recently been of great interest in both academia and the food industry, because their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants with natural ones. Owing to strong antibacterial and excellent protective features exhibited in antioxidant activity tests, the essential oil and extracts from the herbal parts of *T. eigii* could be considered a natural source that can be freely used in the food industry as a culinary herb. Our study may be considered to be the first report on the antimicrobial and antioxidant properties of the essential

oil and various extracts of *T. eigii*. We hope that our results will provide a starting point for investigations to exploit new natural antimicrobial and antioxidant substances present in the extracts of the plant species studied.

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