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Antimicrobial and Antioxidant Activity of the Essential Oil and Methanol Extracts of *Thymus pectinatus* Fisch. et Mey. Var. *pectinatus* (Lamiaceae)

GÜLHAN VARDAR-ÜNLÜ,*,† FERDA CANDAN,‡ ATALAY SÖKMEN,§ DIMITRA DAFERERA,⊥ MOSCHOS POLISSIOU,⊥ MÜNEVVER SÖKMEN,‡ EROL DÖNMEZ,§ AND BEKTAŞ TEPE§

Department of Microbiology, Faculty of Medicine, and Departments of Chemistry and Biology, Faculty of Science and Literature, Cumhuriyet University, Sivas, 58140 Turkey, and Laboratory of General Chemistry, Agricultural University of Athens, Athens, Greece

The essential oil, obtained by using a Clevenger distillation apparatus, and water-soluble (polar) and water-insoluble (nonpolar) subfractions of the methanol extract of *Thymus pectinatus* Fisch. et Mey. var. *pectinatus* were assayed for their antimicrobial and antioxidant properties. No (or slight) antimicrobial activity was observed when the subfractions were tested, whereas the essential oil showed strong antimicrobial activity against all microorganisms tested. Antioxidant activities of the polar subfraction and the essential oil were evaluated using 2,2-diphenyl-1-picrylhydrazyl, hydroxyl radical, superoxide radical scavenging, and lipid peroxidation assays. The essential oil, in particular, and the polar subfraction of the methanol extract showed antioxidant activity. The essential oil was analyzed by GC/MS, and 24 compounds, representing 99.6% of the essential oil, were identified: thymol, γ-terpinene, *p*-cymene, carvacrol, and borneol were the main components. An antimicrobial activity test carried out with fractions of the essential oil showed that the activity was mainly observed in those fractions containing thymol, in particular, and carvacrol. The activity was, therefore, attributed to the presence of these compounds. Other constituents of the essential oil, such as borneol, γ-terpinene, and *p*-cymene, could be also taken into account for their possible synergistic or antagonistic effects. On the other hand, thymol and carvacrol were individually found to possess weaker antioxidant activity than the crude oil itself, indicating that other constituents of the essential oil may contribute to the antioxidant activity observed. In conclusion, the results presented here show that *T. pectinatus* essential oil could be considered as a natural antimicrobial and antioxidant source.

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

INTRODUCTION

The essential oils and extracts of many plant species have become popular in recent years, and attempts to characterize their bioactive principles have recently gained momentum in many pharmaceutical and food-processing applications (*1*). The genus *Thymus* (Lamiaceae) consists of over 300 evergreen species of herbaceous perennials and subshrubs, native to Southern Europe and Asia (*2*). This genus is represented by 38 species and altogether 64 taxa, 24 of which are endemic in Turkey and the East Aegean Islands (*3*, *4*). Members of this genus are called "kekik" in Turkish and are used as herbal tea and condiments. *Thymus pectinatus* Fisch. et Mey. var. *pectinatus* is a dwarf shrub, up to 12 cm high, endemic in Inner Anatolia, growing wild in open steppe on gypsous or calcerous fields (*3*).

The in vitro antimicrobial and antioxidant activities of the essential oils and extracts of several *Thymus* species have recently been reported (*5*−*8*). The essential oil composition of *T. pectinatus* has been studied (*9*). However, antimicrobial and antioxidant properties seem not to have been reported before.

In the present study, the antimicrobial and antioxidant activities of the essential oil and extracts from *T. pectinatus* were investigated. The antimicrobial activities were determined by using agar well diffusion, agar disk diffusion, and broth microdilution methods. The antioxidant activities were determined by using four complementary in vitro assays: inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and the oxygen radicals such as lipid peroxides, superoxides, and hydroxyl

* To whom correspondence should be addressed. E-mail: unlum@yahoo.com. Telephone and fax: +90 (346) 219 1606.
† Department of Microbiology, Cumhuriyet University.
‡ Department of Chemistry, Cumhuriyet University.
§ Department of Biology, Cumhuriyet University.
⊥ Agricultural University of Athens.

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radicals. The chemical composition of the essential oil and its fractions was evaluated by using GC/MS analysis.

MATERIALS AND METHODS

Plant Material. *T. pectinatus* plants were collected from Tecer Mountain, Sivas, Turkey, when flowering (July 2001). The voucher specimens have been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas, Turkey (CUFH Voucher No. ED 6330).

Preparation of the Methanolic Extracts. The air-dried and finely ground sample was extracted by using the method described previously (*10*). The resulting extract was suspended in water and partitioned with chloroform ($CHCl_3$) to obtain water-soluble (polar) (8.7%) and water-insoluble (nonpolar, chloroformic) subfractions (1.9%), which were then lyophilized and kept in the dark at +4 °C until tested.

Extraction of the Essential Oil. The air-dried and ground aerial parts of plants collected were submitted for 3 h to water distillation using a Clevenger-type apparatus (yield 1.8% v/w). The obtained essential oil was dried over anhydrous sodium sulfate and, after filtration, stored at +4 °C until tested and analyzed.

Fractionation of the Essential Oil. A 10-mL portion of the essential oil was chromatographed on silica gel as packing material (20 g, Silicagel 60G, Merck 7734, column size 10 × 300 mm). A gradient of ether in petroleum ether (30−40°C) was employed as eluent (each eluent was prepared as 100 mL). The whole process was monitored by TLC. Ether content was gradually increased from 1% to 50% (v/v) through the fractionation, thereby altering the polarity. The composition of each fraction was examined on silica gel TLC plates with petroleum ether/ether (3:1 v/v) as the developing reagent. The plates were sprayed with a mixture of 5% sulfuric acid and 1% vanillin (in ethanol) and heated at 120 °C for the visualization of the components in each fraction. Fractions showing similar patterns were combined; altogether, four fractions were obtained. The solvents of each fraction were removed by flushing through nitrogen.

GC/MS Analysis Conditions. The analysis of the essential oil was performed using a Hewlett-Packard 5890 II GC, equipped with a HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm) and a HP 5972 mass-selective detector. For GC/MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium was the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. Column temperature was initially at 50 °C, and then gradually increased to 150 °C at a 3 °C/min rate, held for 10 min, and finally raised to 250 °C at 10 °C/min. Diluted samples (1:100 v/v, in acetone) of 1.0 μL were injected manually and in the splitless mode. The components were identified on the basis of comparison of their relative retention time and mass spectra with those of standards, NBS75K library data of the GC/MS system, and literature data (*11*). The results were also confirmed by the comparison of the compounds' elution order with their relative retention indices on nonpolar phases reported in the literature (*11*).

Microbial Strains. The methanolic extracts (both polar and nonpolar subfractions) and the essential oil and its fractions of *T. pectinatus* were individually tested against a panel of microorganisms, including Gram-positive cocci, *Staphylococcus aureus* ATCC 25923 and, for minimum inhibitory concentration (MIC) test, ATCC 29213, S*treptococcus pneumoniae* ATCC 49619; Gram-negative coccus, *Moraxella catarrhalis* ATCC 49143; Gram-positive bacillus, *Bacillus cereus* ATCC 11778; Gram-negative bacilli, *Acinetobacter lwoffii* ATCC 19002, *Enterobacter aerogenes* ATCC 13043, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, and *Pseudomonas aeruginosa* ATCC 27853; anaerobic bacterium, *Clostridium perfringens* KUKENS-Turkey; mycobacterium, *Mycobacterium smegmatis* CMM 2067; and the yeasts, *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: the agar well diffusion method for the methanol extracts and the disk diffusion method for the essential oil and its fractions (*13*). The MICs of the essential oil against the test organisms were determined by the broth microdilution method (*12*). The MICs of amikacin, clindamycine, and ciprofloxacin were also determined in parallel experiments in order to control the sensitivity of the standard test organisms (*12*). All tests were performed in duplicate.

Agar Well Diffusion Method. The polar subfraction of the water-soluble extract was weighed and dissolved in phosphate-buffered saline (PBS; pH 7.0−7.2), 10 mg/mL. The nonpolar one was dissolved in dimethylsulfoxide (DMSO), 10 mg/mL. Both subfractions were filter-sterilized using a 0.45-μm membrane filter. Each microorganism was suspended in sterile saline and diluted at ca. 10^6 colony-forming unit (cfu)/mL. They were "flood-inoculated" onto the surface of MHA. The wells (8 mm in diameter) were cut from the agar, and 0.06 mL of extract solution was delivered into them. After incubation for 24 h at 37 °C, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimeters.

Disk Diffusion Method. The agar disk diffusion method was employed for the determination of antimicrobial activities of the essential oil and its fractions in question (*13*). Briefly, a suspension of the tested microorganism (0.1 mL of 10^8 cells/mL) was spread on the solid media plates. Filter paper disks (6 mm in diameter) were soaked with 15 μL of the oil and placed on the inoculated plates. After being kept at 4 °C for 2 h, they were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. The diameters of the inhibition zones were measured in millimeters.

Determination of Minimum Inhibitory Concentration (MIC). A broth microdilution broth susceptibility assay was used, as recommended by NCCLS, for the determination of the MIC (*12*). All tests were performed in Mueller Hinton broth (MHB; BBL) supplemented with Tween 80 detergent (final concentration of 0.5% v/v), with the exception of the yeasts [Sabouraud dextrose broth (SDB) + Tween 80]. Bacterial strains were cultured overnight at 37 °C in MHA, and the yeasts were cultured overnight at 30 °C in SDB. Test strains were suspended in MHB to give a final density of 5 × 10^5 cfu/mL, and these were confirmed by viable counts. Geometric dilutions ranging from 0.036 to 72.00 mg/mL of the essential oil were prepared in a 96-well microtiter plate, including one growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil). Plates were incubated under normal atmospheric conditions at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom.

Antioxidative Capacity. The antioxidative capacity of *T. pectinatus* extracts was examined by comparing to the activity of known antioxidants such as BHT, curcumin, and ascorbic acid by the following four complementary in vitro assays: inhibition of DPPH radical and the oxygen radicals such as lipid peroxides, superoxides, and hydroxyl radicals. Since the nonpolar subfraction of methanol extract is partly soluble in aqueous test media and its color interfered with the spectroscopic measurements, only the polar subfraction and essential oil could be tested for their antioxidative capacity.

Hydroxyl Radical Scavenging. Hydroxyl radical scavenging was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The attack of the hydroxyl radical to deoxyribose leads to thiobarbituric acid reactive substances (TBARS) formation (*14*). Various concentrations of the extract were added to the reaction mixture containing 3.0 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2, and 20 mM phosphate buffer (pH 7.4), making up a final volume of 3.0 mL. The reaction mixture was incubated at 37 °C for 1 h. The formed TBARS were measured by the method of Ohkawa et al. (*15*). One milliliter of thiobarbituric acid (TBA, 1%) and 1.0 mL of trichloroacetic acid (TCA, 2.8%) were added to test tubes and incubated at 100 °C for 20 min. After the mixtures cooled, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate.

Inhibition (I) of deoxyribose degradation in percent was calculated in the following way:

$$I = (A_0 - A_1/A_0) \times 100$$

where A_0 is the absorbance of the control reaction (containing all reagents except the test compound) and A_1 is the absorbance of the test compound.

DPPH Assay. The DPPH assay was carried out as described by Cuendet et al. (*16*). Fifty-microliter aliquots of various concentrations of the samples were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30-min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The assay was carried out in triplicate.

Inhibition of Superoxide Radicals. Superoxide radical generated by the xanthine/xanthine oxidase system was determined spectrophotometrically by monitoring the product of nitroblue tetrazolium (NBT) (*17*). Various concentrations of the extract were added to the reaction mixture containing 100 μM xanthine, 600 μM NBT, 0.05 U/mL xanthine oxidase, and 0.1 M phosphate buffer (pH 7.4), making up a final volume of 2.0 mL. After incubation of the mixture at 25 °C for 10 min, the absorbance was read at 560 nm and compared with the control samples in which the enzyme, xanthine oxidase, was not included. The percent scavenging of superoxide was calculated from the optical density of the treated and control samples.

Inhibition of Lipid Peroxide Formation. The reaction mixture contained 0.1 mL of 25% (w/v) rat liver homogenate in 40 mM Tris-HCl buffer, pH 7.0, 30 mM KCl, 0.16 mM ferrous iron, various concentrations of the extract and positive controls, and 0.06 mM ascorbic acid in a final volume of 0.5 mL. It should be noted that extract and positive controls have their own control reactions (containing all related reagents except the test compounds). The mixture was then incubated at 37 °C for 1 h (*18*). The lipid peroxide formation was measured by the method of Ohkawa et al. (*15*). For this, 0.4 mL of the reaction mixture was treated with 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 0.8% TBA, and 1.5 mL of 20% acetic acid solution adjusted to pH 3.5 with NaOH. The total volume was then made up to 4 mL by adding distilled water, and the mixture wasx kept in a water bath at 95 °C for 1 h. After the mixture cooled, 1 mL of distilled water and 5 mL of the mixture of *n*-butanol and pyridine (15:1 v/v) were added, and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken, and its absorbance at 532 nm was measured. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compounds with those of controls not treated with the extracts. Calculations were done as mentioned in the hydroxyl radical scavenging method.

RESULTS

Chemical Composition of the Essential Oil and Its Fractions. GC/MS analysis of the crude oil resulted in the identification of 24 compounds representing 99.6% of the essential oil. Thymol (49.8%), γ-terpinene (16.1%), *p*-cymene (14.8%), carvacrol (3.7%), and borneol (2.7%) were the main components, comprising 87.1% of the oil. The fractionation of the essential oil gave four fractions. The composition of each fraction is given in **Table 1**. Each fraction was found to be rich in some of the main components detected in the whole oil. Fraction I was rich in *p*-cymene (38.8%) and γ-terpinene (41.2%), fraction II in thymol (95.5%), fraction III in thymol (80.7%) and carvacrol (10.9%), and fraction IV in borneol (62.3%).

Antimicrobial Activity. The results of bioassays showed that the polar subfraction of the methanolic extract of *T. pectinatus* did not exhibit antimicrobial activity, whereas the nonpolar one was moderately active against *St. pneumoniae*, *C. albicans*, and *C. krusei*. The crude essential oil exhibited a broad-spectrum strong antimicrobial activity against all test organisms. The strongest activity was observed against the yeasts, *C. albicans* and *C. krusei*, followed by *S. aureus*, *Cl. perfringens*, and *A. lwoffii*, respectively. The weakest activity was observsd against *Ps. aeruginosa.* The growth inhibitions of test microorganisms were also evaluated by the broth microdilution method, and the lowest MIC value was determined against *Cl. perfringens* at 0.30 mg/mL (w/v), followed by *S. aureus*, *St. pneumoniae*, *A. lwoffii*, and *E. coli*, with MIC values of 1.12 mg/mL (w/v) (**Table 2**).

Table 1. Composition of *Thymus pectinatus* Essential Oil and Its Fractions (%)

				fraction			
	compound[a]	K.I.[b]	crude	I	II	III	IV
1	thujene	930	1.3	2.9			
2	α-pinene	939	1.4	2.9			
3	camphene	954	0.9	1.6			
4	β-pinene	979	0.4	0.7			0.6
5	1-octen-3-ol	979	0.2	0.2			1.0
6	3-octanone	984				0.3	
7	β-myrcene	991	1.4	4.0			
8	3-octanol	991					0.2
9	α-phellandrene	1003	0.2	0.4			
10	α-terpinene	1017	1.8	3.3			0.4
11	*p*-cymene	1025	14.8	38.8			
12	β-phellandrene	1030	0.4				0.8
13	eucalyptol	1031	1.1			1.7	
14	γ-terpinene	1060	16.1	41.2			0.7
15	*cis*-sabinene hydrate	1070	0.8				12.0
16	terpinolene	1089	0.2	0.3			0.4
17	*trans*-sabinene hydrate	1089					0.3
18	linalool	1097					0.4
19	*trans*-menth-2-en-1-ol	1141					0.5
20	borneol	1169	2.7				62.3
21	terpinen-4-ol	1177	0.7			0.2	2.6
22	α-terpineol	1189	0.1				4.0
23	*cis*-dihydrocarvone	1193				0.1	
24	linalyl propanoate		0.1				
25	myrtenol	1196					0.3
26	*trans*-dihydrocarvone	1201				0.1	
27	thymol methyl ether	1235				0.1	
28	thymol	1290	49.8	0.2	95.5	80.7	
29	thymol + cuminol	1291[c]					2.6
30	carvacrol	1299	3.7		1.0	10.9	
31	thymol acetate	1352	0.8		3.5	3.9	
32	carvacrol acetate	1373	tr[d]			0.2	
33	caryophyllene	1419	0.6	2.7			
34	γ-muurolene	1480	0.1	0.5			
35	caryophyllene oxide	1583				0.2	
	total		99.6	99.7	100.0	98.4	89.1

[a] Compounds listed in order of elution from an HP-5 MS column. [b] Kovats index on a DB-5 column in reference to *n*-alkanes (*11*). [c] K.I. for cuminol. [d] Trace (<0.05%).

Antioxidative Capacity. *Free Radical Scavenging Activity.* As shown in **Tables 3** and **4**, respectively, the polar subfraction of the methanol extract, the essential oil, and the reference chemicals were all able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The strongest effect was observed for the essential oil, with an IC_{50} of 0.36 ± 0.10 μg/mL. IC_{50} is the concentration of sample required to scavenge 50% of the free radicals.

The above-mentioned extract was also found to scavenge the superoxide radicals generated from the xanthine/xanthine oxidase method. The IC_{50} of the extract was 104.70 ± 3.80 μg/mL, whereas those of ascorbic acid and curcumin were 1372.00 ± 3.60 μg/mL and 9.14 ± 0.12 mg/mL, respectively.

When the essential oil or its main components were incubated with the reaction mixture used in the deoxyribose degradation assay, they removed hydroxyl radicals from the sugar and prevented its degradation. Concentrations resulting in 50% inhibition were estimated as 1.40 ± 0.03, 351.30 ± 5.60, 1.30 ± 0.01, and 0.90 ± 0.05 μg/mL for the essential oil, the extract, carvacrol, and thymol, respectively (**Tables 3** and **4**).

Table 2. Antimicrobial Activity of the Essential Oil and the Methanolic Extracts of *Thymus pectinatus* Using Agar Disk Diffusion, Minimum Inhibitory Concentration and Agar Diffusion Method

	essential oil						MeOH[c]		MIC of antibiotics[d]		
	crude		fraction[a]								
microorganisms	DD[a]	MIC[b]	I	II	III	IV	H_2O	$CHCl_3$	AK	CF	CM
Staphylococcus aureus	37	1.12	na[e]	21	33	24	na	na	2.00	0.25	nt[f]
Streptococcus pneumoniae	23	1.12	8	22	29	19	na	16	nt	nt	0.125
Moraxella catarrhalis	26	2.25	9	32	34	25	na	na	nt	nt	nt
Bacillus cereus	20	2.25	9	18	24	11	na	na	nt	nt	nt
Acinetobacter lwoffii	30	1.12	11	28	40	40	na	na	nt	nt	nt
Enterobacter aerogenes	16	9.00	7	14	27	na	na	na	nt	nt	nt
Escherichia coli	23	1.12	9	25	28	17	na	na	2.00	0.015	nt
Klebsiella pneumoniae	22	4.50	na	25	31	11	na	na	nt	nt	nt
Proteus mirabilis	24	4.50	na	10	26	na	na	na	nt	nt	nt
Pseudomonas aeruginosa	10	36.00	na	9	14	na	na	na	1.00	0.25	nt
Clostridium perfringens	36	0.30	15	20	43	20	na	na	nt	nt	0.25
Mycobacterium smegmatis	26	2.25	8	22	>50	40	na	na	nt	nt	nt
Candida albicans	>50	2.25	12	>50	>50	>50	na	13	nt	nt	nt
Candida krusei	>50	2.25	18	32	>50	22	na	13	nt	nt	nt

[a] DD, disk diffusion method. Diameter of zone of inhibition (mm) including disk diameter of 6 mm. [b] MIC, minimum inhibitory concentration; values given as milligrams per milliliter for the essential oils[1] and as micrograms per milliliter (for antibiotics).[2] [c] MeOH, methanolic extracts; diameter of zone of inhibition (mm) including well diameter of 8 mm. [d] AK, amikacin; CF, ciprofloxacine; CM, clindamycine. [e] na, not active. [f] nt, not tested.

Table 3. Effects of *Thymus pectinatus* Essential Oil and Positive Controls on the In Vitro Free Radical (DPPH and Hydroxyl) and Lipid Peroxidation Generation IC_{50} (μg/mL)

sample	DPPH	hydroxyl	lipid peroxidation
the crude oil	0.36	1.4 ± 0.03	9.5 ± 0.02
carvacrol	245.0	1.3 ± 0.01	5.2 ± 0.02
thymol	161.7	0.90 ± 0.05	6.5 ± 0.01
borneol	na	na[a]	na
p-cymene	na	na	na
BHT	19.8	nt[b]	7.8 + 0.04

[a] na, not active. [b] nt, not tested.

Table 4. Effects of Methanolic Extracts (Water-Soluble Part) of *Thymus pectinatus* and Positive Controls on the In Vitro Free Radical (DPPH, Superoxide, and Hydroxyl) and Lipid Peroxidation Generation IC_{50} (μg/mL)

sample	DPPH	superoxide	hydroxyl	lipid peroxidation
extract	14.7 ± 0.2	104.7 ± 3.8	351.3 ± 5.6	905.2 ± 13.5
curcumin	7.8 ± 0.3	9.14 ± 0.12	13.21± 0.07	38.40 ± 0.1
ascorbic acid	3.76 ± 0.1	1372 ± 3.6	nt[a]	nt

[a] nt, not tested.

Lipid Peroxidation. As demonstrated in **Table 3**, the essential oil of *T. pectinatus* and its main components inhibit lipid damage caused by hydroxyl radicals. The IC_{50} for essential oil was 9.50 ± 0.02 μg/mL. The concentration of *T. pectinatus* extract needed for 50% inhibition was 905.2 ± 13.5 μg/mL.

DISCUSSION

Chemical Composition of the Essential Oil. GC/MS analysis of *T. pectinatus* essential oil revealed an abundance of monoterpene hydrocarbons and phenolic monoterpenes in the essential oil. Thymol was the predominant compound, followed by γ-terpinene, *p*-cymene, carvacrol, and borneol. This finding is in agreement with the previous report, in which thymol content was reported as ranging between 47.8% and 61.7%, depending on the flowering season (*9*).

Antimicrobial Activity. As shown in **Table 2**, the polar subfraction of the methanol extract did not exert any activity, whereas the nonpolar (chloroformic) one was found to be effective against the yeasts and *St. pneumoniae*. Although no generalization can be made for this, the nonpolar phase or subfraction exhibits, in many cases, greater activity than the polar one (*10*). The activity observed in the polar one can be attributed to the presence of the phenolic compounds (*19*). On the other hand, the essential oil particularly exhibited strong, broad-spectrum antimicrobial activity. The antimicrobial nature of the essential oils from several *Thymus* species is apparently related to their high phenolic contents, particularly thymol and carvacrol (*6*, *20*). This claim is further supported by our findings (**Table 2**), indicating thus high content of thymol in fractions II and III. Addition of carvacrol in fraction III made them most effective against the microorganisms. Therefore, the synergistic effect of these chemicals could also be accounted for by the activity (*21*). The findings presented here also suggest that other constituents of the essential oil, such as borneol in fraction IV, have an antimicrobial action, as reported previously (*22*). Each may also contribute to the antimicrobial effect of thymol and carvacrol (*23*). Preliminary screening of both standard and isolated compounds on the same microorganisms under identical conditions showed similar results (unpublished data).

Antioxidative Capacity. In contrast to antimicrobial activity results, both the polar phase of the methanol extract and the essential oil exhibited strong antioxidant activity. The concentration of the polar phase providing 50% inhibition in DPPH assay was almost identical with that of positive control (curcumin). The same phase was also observed to possess stronger activity than that of ascorbic acid in terms of superoxide radical scavenging (104.7 ± 3.8 μg/mL). In DPPH and radical scavenging assays, the essential oil exhibited stronger activity than positive controls (BHT, curcumin, and ascorbic acid) and the extract (**Tables 3** and **4**). Also, the essential oil had 50% inhibition concentration (9.5 μg/mL), which was better than that of curcumin and similar to that of BHT in the lipid peroxidation assay. The main components of the essential oil, carvacrol, thymol, borneol, and *p*-cymene, were also tested for their antioxidant activity under identical conditions. The results showed that carvacrol and thymol were the active components of the essential oil in lipid peroxidation (5.2 μg/mL for carvacrol, 6.5 μg/mL for thymol) (**Table 3**). Borneol and *p*-cymene showed no activity.

These phenols are well-known antioxidants, and our findings are in agreement with the previous reports (*24*−*26*). In the DPPH assay, the crude oil was found to be more effective than its main active components (thymol and carvacrol), indicating the possible synergistic interactions of the essential oil constituents. Also, the involvement of the other components, particularly γ-terpinene, could also be taken into account since its strong antioxidant activity was previously reported (*27*). As can be seen from the **Tables 2**−**4**, essential oil is the most promising for both antimicrobial and antioxidant activity. Therefore, elucidation of the chemical composition was carried out only with the essential oil. The results reported here can be considered as the first information on the antimicrobial and antioxidant properties of *T. pectinatus*, an endemic species of the Turkish flora. This may also contribute to the knowledge about the antimicrobial and antioxidant properties of *Thymus* species reported elsewhere (*5*−*8*).

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