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The investigation of antimicrobial activity of thyme and oregano essential oils

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Abstract: The aim of this study is to investigate in-vitro antimicrobial effects of the essential oils from oregano (*Origanum acutidens* and *Origanum rotundifolium*) and thyme (*Thymus sipyleus* subsp. *sipyleus* var. *rosulans*). The chemical composition and antimicrobial attributes of the essential oils obtained from the aerial parts of the plants, of which there were 3 Lamiaceae species, have been studied. A total of 43 microorganisms, including 26 bacteria, 14 fungi, and 3 yeasts species, have been studied by using disc-diffusion (DD) and minimal inhibition concentration (MIC) methods. Mean inhibition zones and MIC values of bacterial strains varied from 8 and 72 mm to 7.8 and 500 μg mL^{-1}, respectively. The maximal inhibition zones and MIC values of the yeast and fungi species sensitive to the essential oils were 8-74 mm and 7.8-500 μg mL^{-1}, respectively. The susceptibility of the tested microorganisms varied depending on the essential oil composition. In general, the essential oils showed higher DD values than tested antibiotics. The essential oils of oregano and thyme may be considered a potential source of a natural antimicrobial for the food industry after testing the toxic and irritating effects on humans.

Key words: Antimicrobial activity, essential oil, *Origanum acutidens*, *Origanum rotundifolium, Thymus sipyleus* subsp. *sipyleus* var. *rosulans*

Thyme ve oregano uçucu yağlarının antimikrobiyal etkilerinin araştırılması

Özet: Bu araştırmada, oregano (*Origanum acutidens*, *Origanum rotundifolium*) ve thyme (*Thymus sipyleus* subsp. *sipyleus* var. *rosulans*) uçucu yağlarının in-vitro şartlarda antimikrobiyal etkileri araştırılmıştır. Üç Lamiaceae türünün toprak üstü kısımlarından elde edilen uçucu yağların kimyasal kompozisyonu ve antimikrobiyal etkileri çalışılmıştır. Toplam 43 mikroorganizma - 26 bakteri, 14 küf ve 3 maya- türüne karşı etkileri, disk difüzyon (DD) ve "en düşük engelleme konsantrasyonu" yöntemleri ile belirlenmiş; bakteri türlerinin ortalama engelleme zonları ve en düşük engelleme konsantrasyonu değerleri sırasıyla 8 ve 72 mm ile 7.8 ve 500 μg mL^{-1} arasında değişirken maya ve küflerin en yüksek inhibisyon zonu ve en düşük engelleme konsantrasyonu değerlerinin 8–74 mm ile 7.8–500 μg mL^{-1} arasında değiştiği görülmüştür. Test edilen mikroorganizmaların uçucu yağ kompozisyonuna duyarlılığının farklı olduğu ve genellikle uçucu yağların test edilen antibiyotiklerden daha büyük DD değerine sahip olduğu görülmüştür. Thyme ve oregano uçucu yağları -insan için toksik ve tahriş edici etkileri test edildikten sonra- gıda endüstrisi için doğal antimikrobiyal kaynak olarak önerilebilir.

Anahtar sözcükler: Antimikrobiyal aktivite, uçucu yağ, *Origanum acutidens*, *Origanum rotundifolium*, *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*

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Introduction

Aromatic plants have been used since ancient times for their preservative and medicinal attributes, as well as to impart flavor to food. Recently, there has been considerable interest in essential oils and extracts of medicinal and edible plants, herbs, and spices for the development of alternative food additives, in order to prevent the growth of food-borne pathogens or to delay the onset of food spoilage (Marino et al. 2001; Baydar et al. 2004; Sokmen et al. 2004; Rota et al. 2008; Oke et al. 2009).

Several *Thymus* species are known locally as "kekik" in Turkey and their dried herbal parts are used in herbal tea, condiments, and folk medicine. *Thymus* species have a very rich flora in Turkey. The essential oils of some *Thymus* species are characterized by the presence of a high concentration of the isomeric phenolic monoterpenes thymol and/or carvacrol (Baser 1995; Özgüven and Tansı 1998). *Origanum acutidens* is an endemic, herbaceous, and perennial plant growing mainly in calcareous and noncalcareous rocks, slopes, and screes in the Central Anatolia region of Turkey (Davis 1982).

The literature abounds with reports concerning the determination of chemical compositions and antimicrobial properties of the essential oils of various *Origanum* and *Thymus* species, as well as their applications in various commercial preparations, mainly as antimicrobial and antioxidant agents (Cosentino et al. 1999; Aligiannis et al. 2001; Baydar et al. 2004). These characteristics depend largely on their chemical compositions and are mainly attributed to their contents in carvacrol and thymol. Previous studies demonstrated that the extract or the essential oil of *Origanum acutidens* (Sokmen et al. 2004) had antagonistic activity against food-borne pathogenic bacteria. However, according to our knowledge on the fungi, yeasts, as well as pathogenic and saprophytic bacteria of the essential oils obtained from *Origanum rotundifolium* and *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*, have never been studied before. Furthermore, it is also known that the antimicrobial effects of essential oils, and the extracts of medicinal plants may be subjected to change based on the variations in the chemical composition of an essential oil that may be observed due to the origin, the locality, the climate conditions, and the harvest time of the collected plant material (Özgüven and Tansı 1998; Marino et al. 2001; Güllüce et al. 2003; Baydar et al. 2004).

The objectives of this study were: (i) to investigate the antimicrobial activity (against fungi, yeasts, and pathogenic and saprophytic bacteria) of the essential oil extracts from oregano (*Origanum acutidens* (Hand.-Mazz.) Letswaart, and *Origanum rotundifolium* Boiss), and thyme (*Thymus sipyleus* Boiss subsp. *sipyleus* var. *rosulans* (Barbas Jalas)), and (ii) to determine the chemical composition of its hydrodistilled essential oils by GC and GC/MS.

Materials and methods

Collection of plant materials

The aerial parts of *T. sipyleus* subsp. *rosulans*, *O. acutidens* (endemic in Turkey) and *O. rotundifolium* were collected in the province of İspir-Erzurum in the northeastern region of Turkey in July 2006 during the flowering stage. The plant samples were identified by Prof.Dr. R. Çakmakçı, and collected plants were deposited in the Biotechnology Research and Application Centre at Atatürk University, Erzurum, Turkey. The essential oil constituents of the plants are summarized in Table 1.

The isolation of the essential oils

The shade-dried plant samples (500 g) were subjected to hydrodistillation by using a Clevenger-type apparatus for 4 h. The oils were extracted with $CHCl_3$, dried over anhydrous Na_2SO_4, and then stored under N_2 atmosphere at 20 °C in a sealed vial until use.

GC analysis conditions

The analysis of the essential oils was performed by using a Thermo Finnigan Trace GC/A1300, (E.I) equipped with a SGE-BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25 mm). Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}. Injection temperature was set at 220 °C. The program was 50-150 °C at a rate of 3 °C min^{-1}, isothermal hold for 10 min, and finally risen to 250 °C by 10 °C min^{-1}. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 mL were injected in the splitless mode. Quantitative data were obtained from FID area percentage data.

Table 1. Main components of the essential oils of plant species.

Plant species	Main components	Composition (%)
Origanum acutidens	Carvacrol	47.46
	p-Cymene	22.22
	Borneol	3.41
	γ-Terpinene	2.91
	β-Caryophyllene	2.70
	Linalool	2.35
	3-Octanone	1.84
Origanum rotundifolium	Carvacrol	54.56
	p-Cymene	12.53
	Borneol	5.86
	Thymol	3.52
	Linalool	1.77
	Terpinene-4-ol	1.54
	Thymohydroquinone	1.14
	β-Caryophyllene	1.09
	Germacrene D	1.08
	Linlyl acetate	1.07
Thymus sipyleus subsp. *sipyleus* var. *rosulans*	Carvacrol	29.99
	Thymol	14.46
	α-Terpinyl acetate	10.42
	p-Cymene	10.15
	Linalool	6.82
	γ-Terpinene	3.37
	β-Caryophyllene	3.30
	α-Terpineol	3.14
	Geraniol	2.98

GC-MS analysis conditions

The analysis of essential oils was performed by using a Thermo Finnigan Trace GC/Trace DSQ/A1300 (E.I Quadrupole) equipped with a SGE-BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The program used was 50-150 °C at a rate of 3 °C min^{-1}, isothermal hold for 10 min, and finally risen to 250 °C by 10 °C min^{-1}. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μL were injected manually in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of the standards Wiley7N library data of the GC-MS system and literature data (Adams 2007). The results were also confirmed by the comparison of the compounds elution order to their relative retention indices of non-polar phases reported in the literature (Adams 2007).

Antimicrobial activity

Antibiotics and microbial strains

Tested antibiotics (Tables 2 and 3) were purchased from a local pharmacy in Erzurum province.

Table 2. Antibacterial activities of different essential oils against the bacterial strains tested.

Test Bacteria **Pathogenic and saprophytic**	Tsr		Oa		Or		Antibiotics	
	DD[a]	MIC[b]	DD[a]	MIC[b]	DD[a]	MIC[b]	DD[c]	MIC[d]
Acinetobacter lwoffi BC 2819	44	15.62	39	62.5	26	62.5	18 (OFX10)	7.8
Alcaligenes faecalis BC 0452	44	15.62	52	62.5	28	500	20 (OFX10)	7.8
Bacillus cereus BC 6830	36	31.25	38	125	34	62.5	14(SAM20)	7.8
Bacillus subtilis BC 5211	54	7.8	72	125	34	62.5	36(AMC30)	7.8
Enterobacter cloacae BC 3213	15	125	13	500	8	500	24(KF 30)	7.8
Escherichia coli BC 1402	35	250	43	500	26	62.5	22(OFX10)	15.62
Escherichia coli BC 2326	42	250	38	500	32	62.5	26(AZM15)	7.8
Escherichia coli BC 1818	36	250	36	500	38	62.5	12(AZM15)	31.25
Flavobacterium indologenes BC 1520	48	62.5	53	62.5	29	125	27(AZM15)	7.8
Klebsiella pneumoniae BC 1749	34	250	22	500	26	500	30(OFX10)	62.25
Klebsiella pneumoniae BC 3126	29	250	23	500	16	500	25(OFX10)	31.25
Listeria monocytogenes BC 8353	44	62.5	48	500	35	125	22(SAM20)	7.8
Proteus mirabilis BC 2644	36	500	36	500	26	500	28(OFX10)	125
Proteus vulgaris KÜKEM 1329	48	7.8	47	7.8	34	62.4	18(AMC30)	7.8
Providencia alkalifaciens BC 0236	26	250	34	500	28	250	33(OFX10)	62.5
Pseudomonas aeruginosa BC 4372	54	125	54	500	36	125	28(OFX10)	7.8
Pseudomonas aeruginosa ATCC 9027	50	7.8	50	125	38	62.5	36(TE30)	7.8
Pseudomonas fluorescens BC 7324	19	250	14	500	-[e]	-	31(OFX10)	125
Pseudomonas pseudoalkaligenes BC3445	59	125	31	500	38	500	32(OFX10)	125
Pseudomonas putida BC 1617	18	250	16	500	-	-	16(TE30)	125
Salmonella Typhimurium RSSK 95091	20	250	16	500	-	-	12(TE30)	7.8
Staphylococcus aureus ATCC 29213	44	31.25	44	500	44	62.5	34(TE30)	7.8
Staphylococcus aureus BC 7231	56	7.8	42	128	46	7.8	22(KF30)	7.8
Staphlococcus hominis BC 2288	47	62.5	30	500	32	500	24(KF30)	15.62
Streptococcus pyogenes ATCC 176	50	250	54	500	42	250	25(OFX10)	7.8
Yersinia enterocolitica BC 0184	46	250	49	500	30	500	26(AZM15)	31.25

Tsr: *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*; Oa: *Origanum acutidens*; Or: *Origanum rotundifolium*

[a]DD (Disc Diffusion), inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 10 µL of essential oil [b]Minimal inhibitory concentration as µg mL^{-1}. [c]DD, inhibition zone in diameter (mm) around the standard antibiotic discs; OFX10 (Ofloxacin 10 µg $disc^{-1}$), OFX5 (Ofloxacin 5 µg $disc^{-1}$), SAM20 (10 µg sulbactam + 10 µg ampicillin $disc^{-1}$), AMC30 (20 µg amoxicillin + 10 µg clavulanic acid $disc^{-1}$), KF 30 (30 µg cephalothin $disc^{-1}$), AZM15 (15 µg azithromycin $disc^{-1}$), SAM20 (10 µg sulbactam + 10 µg ampicillin $disc^{-1}$), TE30 (30 µg tetracycline $disc^{-1}$) were used as positive reference standards antibiotic discs (Oxoid). [d]Clarithromycin (µg mL^{-1}) was used as reference antibiotic in micro well dilution assay (Oxoid). [e]Antimicrobial effect was not determined.

Table 3. Antifungal activities of different essential oils against the yeasts and fungus isolates tested.

Test Microorganisms	Tsr		Oa		Or		Antibiotics	
Yeast	DD[a]	MIC[b]	DD[a]	MIC[b]	DD[a]	MIC[b]	DD[c]	MIC[d]
Candida albicans ATCC 1223	60	31.25	68	62.5	46	62.5	15	250
Sacharomyces boulardii BC 6128	50	15.62	74	62.5	38	62.5	9	62.5
Sacharomyces cerevisiae BC 6541	52	15.62	68	62.5	34	62.5	8	62.5
Fungi								
Absidia repens BC 100	54	125	48	250	- [e]	-	13	250
Aspergillus flavus BC 101	66	15.62	59	62.5	41	125	17	250
Aspergillus niger BC 102	55	125	51	125	51	125	21	62.5
Aspergillus niger BC 103	64	62.5	66	62.5	44	62.5	19	62.5
Aspergillus ochraceus BC 104	65	62.5	50	125	61	62.5	14	250
Cladosporium herbarum BC 106	55	125	31	250	26	500	10	250
Geotrichum candidum BC 107	59	31.5	43	62.5	29	250	29	250
Paecilomyces variotii BC 108	35	125	21	250	43	15.62	19	62.5
Penicillium brevicompactum BC 109	54	62.5	59	31.25	19	125	14	62.5
Penicillium jensenii BC 110	61	62.5	58	250	58	125	11	250
Penicillium roqueforti BC 111	62	62.5	63	125	46	250	11	250
Penicillium roqueforti BC 113	62	125	61	250	49	250	8	500
Scopulariopsis chartarum BC 115	59	125	55	250	56	250	8	250
Trichothecium roseum BC 116	68	15.62	68	7.8	62	31.25	32	62.5

[a]DD, inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 10 µL of essential oil [b]Minimal inhibitory concentration as µg mL^{-1}. [c]DD, inhibition zone in diameter (mm) around the standard, Amphotericin B (20 µg $disc^{-1}$ Amphotericin B). [d]Amphotericin B (µg mL^{-1}) was used as reference antibiotic in micro well dilution assay (Sigma). [e]Antimicrobial effect was not determined.

The essential oils were tested individually against a range of 43 microorganisms, including 26 bacteria, 14 fungi, and 3 yeasts species. The microorganisms that were used are listed in Tables 2 and 3. The microorganisms were provided by the Food Microbiology Laboratory, Department of Food Engineering, Faculty of Agriculture, Atatürk University, Erzurum, Turkey. The fungal strains were identified by their morphology (Hasenekoğlu 1991). Identification of the bacteria that were used in the study was confirmed by the Microbial Identification System (Sherlock Microbial Identification System version 4.0, MIDI Inc., Newark, DE, USA), API (BioMerieux, France), BIOLOG (MicroStation ID System, Biolog Inc., Hayward), and classic identification tests from Bergey's manual of determinative bacteriology (Holt et al. 1994).

Disc diffusion assay

Antimicrobial tests were carried out by the disc diffusion method, (Murray et al. 1995) using 100 µL of suspension, containing 10^8 colony forming units (CFU) mL^{-1} of bacteria, 10^6 CFU mL^{-1} of yeast, and 10^4 spores mL^{-1} of fungi spread on Nutrient agar (NA), Sabouraud dextrose agar (SDA), and Potato dextrose agar (PDA) medium, respectively. The discs (6 mm in diameter) impregnated with 10 µL of essential oils were placed on the inoculated agar.

OFX10 (Ofloxacin 10 μg disc^{-1}), SAM20 (10 μg sulbactam + 10 μg ampicillin disc^{-1}), AMC30 (20 μg amoxicillin + 10 μg clavulanic acid disc^{-1}), KF 30 (30 μg cephalothin disc^{-1}), AZM15 (15 μg azithromycin disc^{-1}), SAM20 (10 μg sulbactam + 10 μg ampicillin disc^{-1}), TE30 (tetracycline), CC2 (2 μg clindamycin disc^{-1}), and NV5 (5 μg novobiocin disc^{-1}) were used as positive reference standards (Oxoid). The inoculated plates were incubated at 30 °C for 24 h for mesophilic bacteria, at 20 °C for 48 h for psychrophiles, at 30 °C for 48 h for the yeast, and at room temperature for 72 h for fungi isolates (Harrigan 1998).

Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was repeated 3 times.

Microwell dilution assay

The MIC values were determined for the bacterial and yeast strains that were sensitive to the essential oil in the disc diffusion assay. The inocula of the strains were prepared from 12 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils dissolved in dimethyl sulfoxide (DMSO) were first diluted to the highest concentration (500 μg mL^{-1}) to be tested, and then serial 2-fold dilutions were made to obtain a concentration range from 500 to 7.8 μg mL^{-1} in 10 mL sterile test tubes containing Nutrient or SAB broths. The MIC values of essential oils against bacterial and yeast strains were determined on the basis of a microwell dilution method (Gulluce et al. 2004) with some modifications. The 96-well plates were prepared by dispensing 95 μL of broth and 5 μL of the inoculum into each well. One hundred microliters from the stock solutions of the essential oils prepared at the 500 μg mL^{-1} concentration was added into the first wells. Then 100 μL from the serial dilutions was transferred into the 6 consecutive wells. The last well containing 195 μL of nutrient broth without compound, and 5 μL of the inoculum on each strip was used as a negative control. The final volume in each well was 200 μL. Clarithromycin at a concentration range of 500-7.8 μg mL^{-1} was prepared in the nutrient broth and used as a standard drug for positive control. The plate was covered with a sterile plate sealer. The contents of each well were mixed and then incubated at appropriate temperatures for 24 h. Microbial growth in each medium was determined by reading the respective absorbance at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, VT, USA) and confirmed by plating 5 μL samples from clear wells on the nutrient agar medium. The oil tested in this study was screened 3 times against each organism.

MIC agar dilution assay

The agar dilution method was used to determine the MIC values of the fungus isolates (Gul et al. 2002). The essential oils of samples were added aseptically to a sterile molten PDA medium, containing Tween 20 (Sigma, 0.5%, v/v), at the appropriate volume to produce the concentration range of 7.8-500 μg mL^{-1}. The resulting PDA solutions were immediately poured into petri plates after vortexing. The plates were spot inoculated with 5 μL (10^4 spore mL^{-1}) of each fungal isolate. Amphotericin B (Sigma A 4888) was used as a reference antifungal drug. The inoculated plates were incubated at room temperature for 72 h. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the essential oils at which the absence of growth was recorded. Each test was repeated at least 3 times.

Results

Chemical composition of the essential oils

The essential oils of hydrodistilled essential oils of the aerial parts of plant species were analyzed by using the GC and GC-MS system. The main component of essential oils ranged according to the species (Table 1). Forty-seven components were identified, comprising 97.1% of the total components in the oil of *Origanum acutidens*. The major components of *O. acutidens* oil were carvacrol (47.5%), *p*-cymene (22.2%), borneol (3.4%), γ-terpinene (2.9%), β-caryophyllene (2.7%), and linalool (2.4%). Oil of *Origanum rotundifolium* contained mainly carvacrol (54.6%), p-cymene (12.5%), borneol (5.9%) and thymol (3.5%) together with linalool (1.8%) and terpinene-4-ol (1.5%). On the other hand, 39 compounds were identified representing 98.7% of the oil of *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*. This oil is characterized by the high monoterpene fraction, and especially by the presence of the phenolic carvacrol (30.0%), thymol (14.5%), and their precursors *p*-cymene

(10.2%), α-terpinyl acetate (10.4%), linalool (6.8%), and γ-terpinene (3.4%).

Antimicrobial activity

The antimicrobial activities of the essential oils of *T. sipyleus* subsp. *sipyleus* var. *rosulans*, *O. acutidens*, and *O. rotundifolium* assayed against the microorganisms in the present study were qualitatively and quantitatively assessed by evaluating the presence of inhibition, zone diameter, and MIC values. The results are given in Tables 2 and 3. The essential oils of 3 plant species showed different antimicrobial activity against 26 pathogenic and/or saprophytic, 14 fungi, and 3 yeast species tested. The essential oils of *O. rotundifolium* showed remarkable antimicrobial activity (Tables 2 and 3), exhibiting an inhibitory effect against 23 of the 26 bacteria, 13 of the 14 fungi, and the yeast species tested, whereas all tested microorganisms were inhibited by essential oils of *T. sipyleus* subsp. *sipyleus* var. *rosulans* and *O. acutidens*.

The maximal inhibition zones and MIC values of the essential oils of *T. sipyleus* subsp. *sipyleus* var. *rosulans*, *O. acutidens*, and *O. rotundifolium* for bacterial strains were in the range of 15-59 mm and 7.8-500 μg mL^{-1}, 13-72 mm and 7.8-500 μg mL^{-1}, and 8-46 mm and 7.8-500 μg mL^{-1}, respectively (Table 2). *T. sipyleus* subsp. *sipyleus* var. *rosulans* showed the highest inhibitory effects on *Pseudomonas pseudoalkaligenes* (59 mm) and *Staphylococcus aureus* (56 mm), followed by *Bacillus subtilis, P. aeruginosa*, *Streptococcus pyogenes*, and *P. vulgaris* nearly to the same extent, and a lower inhibition effect on *Enterobacter cloacae*. The inhibitory effects of *O. acutidens* exhibited the highest activity on *B. subtilis* (72 mm), and the same inhibition zone (54 mm) on both *S. pyogenes* and *P. aeruginosa*. *O. rotundifolium* revealed the highest activity against *S. aureus* (44-46 mm), *S. pyogenes* (42 mm), and the same inhibition zone (38 mm) on 3 species: *E. coli*, *P. aeruginosa*, and *P. pseudoalkaligenes*.

In general, *T. sipyleus* subsp. *sipyleus* var. *rosulans* essential oils showed the greatest minimum inhibitory concentration for yeast and fungi (MIC, equal to 15.62 to 125 μg mL^{-1}). *O. acutidens* also showed minimum inhibition concentration (MIC, equal to 7.8 to 250 μg mL^{-1}, and 62.5-500 μg mL^{-1} for Amphotericin B). The maximal inhibition zones and MIC values for fungal strains, which were sensitive to the oils of *T. sipyleus* subsp. *sipyleus* var. *rosulans*, *O. acutidens*, and *O. rotundifolium*, were in the range of 35-68, 21-68, and 19-62 mm, and 15.62-125, 7.8-250, and 15.62-500 μg mL^{-1}, respectively (Table 3).

Discussion

The oils that were studied show a similar composition when compared to oils from a previous study (Baser et al. 1997; Sokmen et al. 2004; Figuérédo et al. 2006; Dikbas et al. 2009) (Table 1). The essential oil of *O. acutidens* was previously investigated (Kordali et al. 2008), and it was shown that the major constituents of the 2 chemotypes were the same; however, their quantities were different. This finding indicates that the composition of *O. acutidens*' essential oil that was studied was influenced by the presence of several factors, such as local, climatic, seasonal, and experimental conditions (Nevas et al. 2004; Çakmakçı et al. 2009). Also, essential oil content may be affected by the influence of water stress, the origin, the chemotype, chemical polymorphism, and the stage of the collected plant material. In the Coruh Valley, a wide range of variations in oil characteristics and distribution were observed, indicating that the İspir region is an important centre of diversity and a potentially source of *O. acutidens*. The phytochemical contents among the respective essential oils of *O. acutidens*' chemotypes varied greatly. In the previous study, it was indicated that essential oils isolated from *O. acutidens*, growing in different regions of the İspir region, exhibited varying chemical compositions (Çakmakçı et al. 2009). The results suggested that most *O. acutidens* populations show a wide genetic variation in the region due to their wild plant characteristic; thus, valuable genotypes, in respect to yield and essential oil, are possible. Essential oils are produced in a variety of microclimatic areas of the İspir region, demonstrating the selection of a specific chemotype with the highest oil yield and an acceptable chemical composition. The essential oil of *Thymus sipyleus* subsp. *rosulans* showed a similar composition when compared to oils studied by Tepe et al. (2005).

The oil with the strongest antifungal activity was obtained from *T. sipyleus* subsp. *sipyleus* var. *rosulans*, inhibiting 12 of the fungi tested. All 3 plant species were found to have activities against all of the yeast species tested.

This is the first study to provide data about the essential oils of *T. sipyleus* subsp. *sipyleus* var. *rosulans*, *O. acutidens*, and *O. rotundifolium* evaluated against a wide range of microorganisms possessing potential antibacterial, antifungal, and anti-candidal activities. This result may indicate that their essential oils can be used as natural preservatives in food against the well-known causal agents of food-borne diseases and food spoilage, such as *E. coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Staphylococcus* spp., *Acinetobacter lwoffii*, *Alcaligenes faecalis*, *Bacillus* spp., *E. cloacae*, *Flavobacterium indologenes*, *Klebsiella pneumoniae*, *Proteus* spp., *Pseudomonas* spp., *S. pyogenes*, and *Yersinia enterocolitica*.

The results, presented in Tables 2 and 3, indicated that all oils possess remarkable antibacterial and antifungal activities against the different strains tested. The antimicrobial tests showed that essential oils of the 3 plant species had different antimicrobial activity against test microorganisms. Generally, it can be suggested that the essential oils of the 3 species possess strong antibacterial and antifungal activities on a wide variety of organisms, which have importance to food spoilage and/or poisoning, as well as to those of interest to the medical field such as *Salmonella*, *Staphylococcus*, *E. coli*, *Klebsiella*, and *Listeria*. Thyme and oregano essential oils can inhibit some pathogenic bacterial strains, such as *E. coli*, *Salmonella* Enteritidis, *Salmonella* Choleraesuis, and *Salmonella* Typhimurium (Peñalver et al. 2005), with direct correlation to carvacrol and thymol as phenolic components. The presence of a phenolic hydroxyl group, in carvacrol particularly, is credited with its activity against pathogens such as *B. cereus* (Ultee et al. 2002). Our results showed the wide variation in the antimicrobial attributes of the oils with inhibition diameters ranging from 8 to 72 mm. Considered as an economical source, this study could provide useful information on the utilization of *Thymus* and *Origanum* oils as natural antimicrobial preservatives in food and pharmaceutical systems. These essential oils may find industrial applications as natural preservatives and conservation agents in the food and/or cosmetic industries, and as active ingredients in medical preparations.

Oregano and thyme have been studied widely for their antimicrobial activity, due to the higher content of phenolic compounds. The results showed that the activity of the oils could be attributed, to a considerable degree, mostly to the existence of carvacrol and thymol. Essential oils with high concentrations of thymol and carvacrol, e.g. oregano and thyme, usually inhibit pathogenic bacteria (Nevas et al. 2004; Bozin et al. 2006; Rota et al. 2008). The antimicrobial nature of the essential oils that have been studied is apparently related to their high phenolic contents, particularly carvacrol and thymol, and this finding is in agreement with a previous report (Cosentino et al. 1999; Boyraz and Özcan 2006). The inhibitory activity of thyme and oregano is mainly due to a phenolic constituent (carvacrol 30.0%, 47.5%, and 54.6%). The same correlation was also confirmed for oils that are only rich in carvacrol (Santoyo et al. 2006). Also, the inhibition of the growth of several pathogens by carvacrol was reported in various articles (Sivropoulou et al. 1996; Ultee et al. 2002). Essential oils that are rich in phenolic compounds, such as carvacrol, are widely reported to possess high levels of antimicrobial activity (Aligiannis et al. 2001; Baydar et al. 2004), which has been confirmed and extended by the present studies.

The current results also show that gram-positive bacteria were more affected than gram-negative ones (Table 2). On the other hand, yeast and fungi were more sensitive than bacteria, and all essential oil samples were more effective than positive control (antibiotic discs). The essential oils of oregano (*Origanum acutidens* and *Origanum rotundifolium*) and thyme (*Thymus sipyleus* subsp. *sipyleus* var. *rosulans*) may be suggested as a new potential sources of a natural antimicrobial for the food industry after testing the toxic and irritating effects on humans. Therefore, further studies are necessary to estimate the potential for utilizing oregano and thyme essential oils as additives for extending the safety and shelf-life of food products.

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