# AGRICULTURAL AND FOOD CHEMISTRY

# Chemical Compositions and Antibacterial Effects of Essential Oils of Turkish Oregano (*Origanum minutiflorum*), Bay Laurel (*Laurus nobilis*), Spanish Lavender (*Lavandula stoechas* L.), and Fennel (*Foeniculum vulgare*) on Common Foodborne Pathogens

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Chemical compositions and inhibitory effects of essential oils of Turkish oregano (*Origanum minutiflorum* O. Schwarz & P. H. Davis), bay laurel (*Laurus nobilis* L.), Spanish lavender (*Lavandula stoechas* subsp. *stoechas* L.), and fennel (*Foeniculum vulgare* Mill.) on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus* were determined. After the essential oils were applied on the foodborne pathogens at doses of 0 (control), 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL, the resultant numbers of cells surviving were counted. Results revealed that all essential oils exhibited a very strong antibacterial activity against the tested bacteria (*P* < 0.05). Gas chromatography–mass spectrophotometry analyses revealed that carvacrol (68.23%), 1,8-cineole (60.72%), fenchone (55.79%), and *trans*-anethole (85.63%) were the predominant constituents in Turkish oregano, bay laurel, Spanish lavender, and fennel essential oils, respectively.

KEYWORDS: Plant essential oil; Escherichia coli O157:H7; Salmonella typhimurium; Staphylococcus aureus; Listeria monocytogenes

# INTRODUCTION

Although humans have used plant extracts for their antifungal, antimicrobial, insecticidal, cytostatic, and therapeutic activities (1, 2), the focus has been on their enhancement of the flavor of foods rather than extension of the shelf life and microbial inactivation. Changes in consumers' preferences toward more natural products than synthetic food additives, and reduction of salt and sugar in foods for dietary reasons, stimulate the use of spices and/or aromatic plants, which are low in sodium and calories (3-5). Aromatic plants have been widely used to extend the shelf life of foods and in folk medicine owing to essential oils that they contain as a product of their secondary metabolisms (6).

Most studies were conducted to determine antifungal and antibacterial activities of plant essential oils against soilborne pathogens, harmful insects, pathogenic fungal strains causing superficial skin infections in human, and food storage fungi (6-15). However, there is a lack of studies performed to determine antibacterial effects of plant essential oils on foodborne pathogenic bacteria. *Escherichia coli* O157:H7, *Staphylococcus aureus, Listeria monocytogenes*, and *Salmonella typhimurium* are common foodborne pathogens causing severe diseases in human. To prevent the growth of these pathogens

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in foods, different attempts have been made recently, with the use of plant extracts and/or essential oils gaining popularity (16-23).

Inactivation effects of thymol, eugenol, menthol, and anethole (major constituents in thyme, oregano, clove, West India bay, aniseed, fennel, and peppermint oil) were tested on *S. typhimurium*, *S. aureus*, and *Vibrio parahaemolyticus*, and they showed different inhibitory impacts on the tested bacteria (24). Thangadurai et al. (15) reported that essential oils extracted from *Decalepis hamiltonii* roots exhibited a strong antimicrobial activity against *Bacillus cereus*, *Bacillus megaterium*, *Candida albicans*, *E. coli*, *Micrococcus luteus*, *M. roseus*, and *S. aureus*.

The essential oils obtained from the aerial parts of Origanum scabrum exhibited an extremely strong activity against S. aureus, Staphylococcus epidermidis, E. coli, Enterobacter colaceae, Pseudomonas aeruginosa, C. albicans, Candida tropicalis, and Torulopsis glabrata. Except for Pseudomonas aeruginosa and Klebsiella pneumoniae, the essential oil of Origanum microphyllum showed weaker antimicrobial activities against the above tested microorganisms (13).

Turkish oregano (*Origanum minutiflorum* O. Schwarz & P. H. Davis), bay laurel (*Laurus nobilis* L.), Spanish lavender (*Lavandula stoechas* subsp. *stoechas* L.), and fennel (*Foeniculum vulgare* Mill.) are natural floristic elements of Turkey and widely used as traditional medicines and flavor enhancers in foods. However, there is a lack of information about their

antimicrobial activities and essential oil constituents in the literature. In this study, we aimed at determining the chemical compositions and inhibitory effects on *E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium*, and *S. aureus* of essential oils of Turkish oregano, bay laurel, Spanish lavender, and fennel.

#### MATERIALS AND METHODS

**Bacterial Cultures.** *E. coli* O157:H7 (ATCC 35218), *L. monocy-togenes* (NCTC 2167), *S. typhimurium* (RHSM 1996), and *S. aureus* (ATCC 43300) cultures were obtained from the culture collections of the Department of Health of Refik Saydam Hygiene Center Contagious Diseases Research Department (Ankara, Turkey) in tryptone soy agar (TSA) slants. All of the cultures were transferred into tryptone soy broth (TSB) tubes and incubated at 37 °C overnight. TSA and TSB were purchased from Difco (France).

**Plant Materials.** Leaves and aerial parts of Turkish oregano from the district of Sutculer (Isparta) and leaves and flowers of Spanish lavender from the district of Alahan (Hatay) were collected between April and June of 2002. Fresh bay laurel was collected from the district of Aknehir (Hatay) between June and September of 2001. Fennel seeds were purchased from a local seller in Hatay. Turkish oregano, bay laurel, and Spanish lavender flowers and leaves were separated from their stem parts and air-dried until use.

**Analyses of Essential Oils.** Air-dried parts of the plants and fennel seeds were steam-distilled for 3 h using a Clevenger-type apparatus (Ildam, Ankara) according to the *European Pharmacopoeia* (25). The obtained essential oils were stored at 4 °C in airtight glass vials covered with aluminum foil.

The gas chromatography-mass spectrophotometry (GC-MS) analyses of the obtained essential oils were conducted at the Central Laboratory of Mustafa Kemal University (Hatay) using a Hewlett-Packard GC (model 6890) and a Hewlett-Packard MS (model 5972) equipped with a mass selective detector (MSD). The GC was equipped with a (5%-phenyl)-methyl polysiloxane HP-5MS column (30 m length  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m d<sub>f</sub>) and an HP 18593B automatic injection system. Thirty microliters of bay laurel essential oils was transferred into 1 mL of diethyl ether (Sigma), and 30  $\mu$ L each of essential oils from Turkish oregano, Spanish lavender, and fennel were transferred into 1 mL of hexane (Sigma) and injected to the GC-MS sampling port. The chromatogram was produced by holding the oven temperature at 50 °C for 5 min initially and then increasing the temperature to 90 °C at a rate of 2 °C/min followed by an increase at a rate of 5 °C/min to 210 °C, at which it was held for >40 min. MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 33-330 amu; EM voltage (Atune+200); scan rate, 5 scan/s. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. Identification of components in essential oils was carried out with the Wiley 275 MS data library.

**Determination of Inhibitory Effects of Essential Oils.** One hundred microliters of an overnight-grown culture was added to a test tube containing 900  $\mu$ L of 0.1% peptone water to obtain a total viable bacterial count of ~1 × 10<sup>6</sup>−10<sup>7</sup> colony-forming unit (cfu)/mL in each test tube. The obtained essential oils were added to tubes at doses of 0 (control), 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL. Depending on the doses, serial dilutions were prepared with 0.1% peptone. Appropriate dilutions were plated onto plate count agar (PCA) in triplicate. Plates were incubated at 37 °C for 48 h. Each experiment was repeated three times.

**Data Analysis.** Data were analyzed using Minitab software version 12.1 (Minitab, Inc., State College, PA). One-way analysis of variance (ANOVA) for the microbial inactivation and Tukey's multiple-comparison tests were used to determine significant differences between inactivation effects of different essential oils. Log<sub>10</sub> transformations were performed on microbial data.

### **RESULTS AND DISCUSSION**

**Chemical Compositions of the Essential Oils.** Seventeen constituents in the essential oil of Turkish oregano (*O. minuti-florum*) were determined, with the major components as 68.23%

 
 Table 1. Essential Oil Constituents of Turkish Oregano Determined by GC-MS Analyses

peak	constituent <sup>a</sup>	%	RT <sup>b</sup> (min)	identification method <sup>c</sup>
1	$\alpha$ -thujene	0.50	5.84	GC-MS
2	α-pinene	1.39	6.06	GC-MS
3	camphene	0.67	6.61	GC-MS
4	$\beta$ -pinene	0.31	8.27	GC-MS
5	myrcene	0.47	9.56	GC-MS
6	α-terpinene	1.00	9.97	GC-MS
7	<i>p</i> -cymene	11.84	10.72	GC-MS
8	$\gamma$ -terpinene	8.14	12.69	GC-MS
9	linalool	2.15	15.63	GC-MS
10	terpineol-4	0.18	19.97	GC-MS
11	carvacrol methyl ether	0.73	24.62	GC-MS
12	carvacrol	68.23	29.40	GC-MS
13	$\alpha$ -humulene	0.22	31.16	GC-MS
14	$\beta$ -caryophyllene	3.44	31.39	GC-MS
15	aromadenderene	0.36	31.92	GC-MS
16	ledene	0.28	33.67	GC-MS
17	$\beta$ -bisabolene	0.21	34.10	GC-MS

<sup>a</sup> All constituents were tentatively identified. <sup>b</sup> Retention time on HP-5MS column in minutes. <sup>c</sup> Gas chromatography-mass spectrophotometry (GC-MS).

 
 Table 2. Essential Oil Constituents of Bay Laurel Determined by GC-MS Analyses

peak	constituent <sup>a</sup>	%	RT <sup>b</sup> (min)	identification method <sup>c</sup>
1	$\alpha$ -pinene	6.11	8.12	GC-MS
2	sabinene	12.12	10.30	GC-MS
3	$\beta$ -terpinene	0.06	10.98	GC-MS
4	1,8-cineole	60.72	14.33	GC-MS
5	$\gamma$ -terpinene	1.04	15.59	GC-MS
6	linalool	0.73	19.01	GC-MS
7	terpinen-4-ol	3.29	23.83	GC-MS
8	$\alpha$ -terpineol	2.04	25.18	GC-MS
9	$\alpha$ -terpinene	12.53	35.20	GC-MS
10	eugenol	0.53	36.30	GC-MS
11	$\beta$ -caryophyllene	0.40	38.54	GC-MS
12	methyl eugenol	0.68	38.92	GC-MS

<sup>a</sup> All constituents were tentatively identified. <sup>b</sup> Retention time on HP-5MS column in minutes. <sup>c</sup> Gas chromatography-mass spectrophotometry (GC-MS).

carvacrol, 11.84% *p*-cymene, 8.14%  $\gamma$ -terpinene, and 3.44%  $\beta$ -caryophyllene (**Table 1**). The essential oil constituents of *Thymus revolutus*, an endemic species in Hatay, were determined, with carvacrol as the most dominant constituent (43.1%) (26). The major constituents of the two species ( $\gamma$ -terpinene, *p*-cymene, and  $\beta$ -caryophyllene) were the same; however, their quantities differed.

GC-MS analyses of two *Origanum* species, *O. scabrum* and *O. microphyllum*, revealed that the chemical composition of Turkish oregano was very similar to those of *O. scabrum* and *O. microphyllum* (13) and different from that of *O. syriacum* (14).

Bay laurel essential oils were found to contain 1,8-cineole (60.72%),  $\alpha$ -terpinene (12.53%), sabinene (12.12%), and  $\alpha$ -pinene (6.11%) as major constituents (**Table 2**). A previous study reported that bay leaves contained mostly 1,8-cineole (50%), eugenol, acetyl eugenol, methyl eugenol, and terpineol (27).

A total of 18 constituents were identified in Spanish lavender essential oils, with the major constituents being fenchone (55.79%), camphor (18.18%), 1,8-cineole (8.03%), and myrtenyl acetate (6.25%) (**Table 3**). Studies with *L. angustifolia*, a species closely related to Spanish lavender, indicated that the essential oils contained 20.2% linalool, 18.6% linalyl acetate, 16.0%

 
 Table 3. Essential Oil Constituents of Spanish Lavender Determined by GC-MS Analyses

peak	constituenta	%	RT <sup>b</sup> (min)	identification method <sup>c</sup>
1	$\alpha$ -pinene	1.31	6.73	GC-MS
2	camphene	1.4	7.34	GC-MS
3	1,8-cineole	8.03	11.79	GC-MS
4	fenchone	55.79	15.90	GC-MS
5	linalool	0.29	16.98	GC-MS
6	camphor	18.18	19.14	GC-MS
7	myrtenal	0.25	22.09	GC-MS
8	fenchyl acetate	0.32	23.70	GC-MS
9	carvone	0.33	25.52	GC-MS
10	bornyl acetate	1.32	27.50	GC-MS
11	myrtenyl acetate	6.25	29.45	GC-MS
12	$\delta$ -cadinene	0.9	35.87	GC-MS
13	L-carveol	0.73	36.32	GC-MS
14	$\gamma$ -cadinene	0.80	37.04	GC-MS
15	caryophyllene oxide	0.33	37.18	GC-MS
16	γ-selinene	2.54	37.67	GC-MS
17	aromadenderene	0.41	38.39	GC-MS
18	$\delta$ -cadinene	0.50	38.95	GC-MS

<sup>a</sup> All constituents were tentatively identified. <sup>b</sup> Retention time on HP-5MS column in minutes. <sup>c</sup> Gas chromatography-mass spectrophotometry (GC-MS).

 Table 4. Essential Oil Constituents of Fennel Determined by GC-MS

 Analyses

peak	constituent <sup>a</sup>	%	RT <sup>b</sup> (min)	identification method <sup>c</sup>
1	$\alpha$ -pinene	0.27	6.89	GC-MS
2	limonene	3.77	11.88	GC-MS
3	$\alpha$ -thujone	1.23	15.39	GC-MS
4	estragole	5.12	23.06	GC-MS
5	carvone	0.94	25.94	GC-MS
6	p-anisaldehyde	2.61	27.56	GC-MS
7	trans-anethole	83.13	29.08	GC-MS

<sup>a</sup> All constituents were tentatively identified. <sup>b</sup> Retention time on HP-5MS column in minutes. <sup>c</sup> Gas chromatography-mass spectrophotometry (GC-MS).

lavandulyl acetate, and 13.1% 1,8-cineole (6). Except for 1,8cineole, the major constituents of *L. angustifolia* were different from those of Spanish lavender. Karamanoli et al. (28) reported that *L. angustifolia* essential oil had 1,8-cineole (44.9%), camphor (14.3%),  $\beta$ -phellandrene (5.0%), and  $\alpha$ -pinene (4.7%). The amounts of camphor and 1,8-cineole were similar to those of Spanish lavender; however, fenchone (54.2%) was not detected in the related species.

The major constituents in fennel essential oil were 85.63% *trans*-anethole, 5.27% estragole, 3.8% limonene, and 2.68% *p*-anisaldehyde (**Table 4**). Ruberto et al. (29) determined that the major constituents of *F. vulgare* essential oil were 55.3% estragole, 11.3%  $\alpha$ -pinene, and 9.1%  $\alpha$ -phellandrene. Except for estragole, the other major constituents were different from those found in our study.

Inhibitory Effects of Essential Oils. *E. coli* O157:H7inoculated control samples had a 8.10 log cfu/mL of initial count, and this value decreased significantly with an increase in the applied essential oil concentrations of Turkish oregano (P < 0.05). The applied essential oil at doses of 5, 10, 20, 30, 40, 50, and 80 µL/mL led to 5.82, 4.45, 4.04, 3.30, 1.00, 0.00, and 0.00 log cfu/mL for *E. coli* O157:H7, respectively (P <0.05). Inactivation of *E. coli* O157:H7 by bay laurel and Turkish oregano essential oils was similar in that increased doses of both essential oils caused an increase in inactivation of *E. coli* O157:H7. The initial count of *E. coli* O157:H7 of 8.02 log cfu/mL was reduced to 5.10, 4.02, 3.79, 2.90, 0.47, 0.00, and

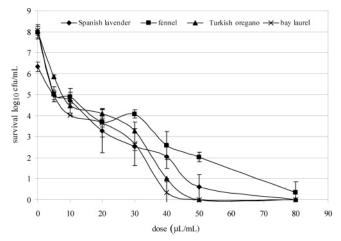


Figure 1. Inactivation of *E. coli* O157:H7 by Turkish oregano, bay laurel, Spanish lavender, and fennel essential oils.

0.00 log cfu/mL with doses of 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL bay laurel essential oils, respectively (P < 0.05). At the same doses, Spanish lavender essential oil reduced the initial count from 6.33 log cfu/mL to 4.99, 4.72, 3.27, 2.52, 2.04, 0.60, and 0.00 log cfu/mL, respectively (P < 0.05). Fennel essential oils applied at 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL doses resulted in survival counts of 5.02, 4.84, 3.76, 4.04, 2.74, 2.08, and 0.46 log cfu/mL, respectively (P < 0.05). The initial level of *E. coli* O157:H7 in the control samples was 7.88 log cfu/mL (**Figure 1**).

When the effects of Turkish oregano, bay laurel, Spanish lavender, and fennel essential oils of the same doses on inactivation of *E. coli* O157:H7 were also compared, bay laurel essential oils were found to be the most effective in inactivation of *E. coli* O157:H7 in all of the doses, followed by Turkish oregano, fennel, and Spanish lavender essential oils (P < 0.05) (**Figure 1**).

Turkish oregano essential oils applied at 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL doses reduced the initial L. monocytogenes count from 7.23 log cfu/mL to 4.00, 3.45, 3.54, 2.41, 0.23, 0.12, and 0.00 log cfu/mL, respectively (P < 0.05). Beyond 80  $\mu$ L/mL, no colonies were counted. Bay laurel essential oils of 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL doses reduced the initial level of 6.99 log cfu/mL of L. monocytogenes to 4.14, 4.11, 3.31, 1.57, 0.93, 0.93, and 0.50 log cfu/mL, respectively (P <0.05). At the same doses of Spanish lavender essential oils, survival numbers of L. monocytogenes were decreased to 4.89, 4.78, 3.66, 2.76, 2.23, 2.24, and 1.85 log cfu/mL, respectively, relative to the control samples with the initial count of 6.91 log cfu/mL (P < 0.05). The initial count of 6.28 log cfu/mL for the control sample was reduced to 4.79, 3.66, 3.42, 3.11, 1.70, 0.64, and 0.26 log cfu/mL at doses of 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL fennel essential oils, respectively (P < 0.05) (Figure 2).

When the effects of the applied essential oils on the inactivation of *L. monocytogenes* were compared, the inhibitory effects of the essential oils were found in the following order: Turkish oregano  $\geq$  bay laurel  $\geq$  fennel  $\geq$  Spanish lavender. However, Turkish oregano essential oils had a significantly higher inhibitory effect than those of fennel and Spanish lavender (P < 0.05). Similarly, bay laurel essential oil had a significantly higher inhibitory effect than that of Spanish lavender (P < 0.05) (Figure 2).

The applied Turkish oregano essential oil had a significantly stronger inhibition effect on *S. typhimurium* in that the control samples with the inoculation level of 6.95 log cfu/mL were

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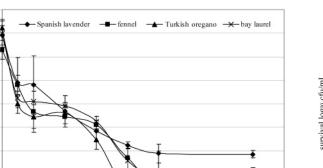
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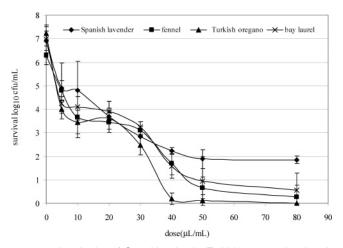
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90

dose(μL/mL) **Figure 2.** Inactivation of *L. monocytogenes* by Turkish oregano, bay laurel, Spanish lavender, and fennel essential oils.

40



**Figure 3.** Inactivation of *S. typhimurium* by Turkish oregano, bay laurel, Spanish lavender, and fennel essential oils.

reduced to 2.90 log cfu/mL after the application of the 5  $\mu$ L/mL Turkish oregano essential oil (P < 0.05). In response to the doses of 10 and 20  $\mu$ L/mL essential oils, the initial number of S. typhimurium was reduced to 2.13 and 0.32 log cfu/mL, respectively (P < 0.05). All S. typhimurium cells were inhibited by the 30, 40, 50, and 80  $\mu$ L/mL Turkish oregano essential oils (P < 0.05). As a function of the 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL bay laurel essential oils, the initial numbers of S. typhimurium cells of 7.44 log cfu/mL were reduced to 4.40, 4.31, 3.86, 3.57, 1.98, 1.81, and 0.94 log cfu/mL, respectively (P < 0.05). The 5  $\mu$ L/mL Spanish lavender essential oil reduced the initial S. typhimurium number in the control samples of 6.57 log cfu/mL to 3.87 log cfu/mL (P < 0.05). The 10, 20, 30, 40, 50, and 80  $\mu$ L/mL doses reduced the survival numbers of S. typhimurium to 3.82, 3.89, 3.20, 2.11, 2.25 and 1.47 log cfu/mL, respectively (P < 0.05). The 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL fennel essential oils caused the initial count of S. typhimurium to decrease from 7.60 to 4.32, 4.12, 4.17, 3.40, 1.39, 0.50, and 0.21 log cfu/mL, respectively (P < 0.05). Compared to the control samples, all of the doses resulted in a significant reduction (P < 0.05) (Figure 3).

When the inactivation effects of Turkish oregano, bay laurel, Spanish lavender, and fennel essential oils on *S. typhimurium* were compared, their inhibitory effects were found in the following order: Turkish oregano > fennel  $\geq$  bay laurel  $\geq$ Spanish lavender. In all of the applied doses, Turkish oregano essential oils appeared to be more effective than bay laurel,

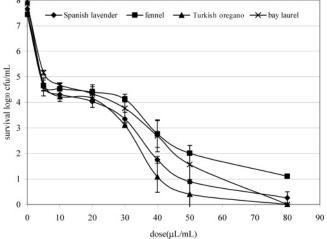


Figure 4. Inactivation of *S. aureus* by Turkish oregano, bay laurel, Spanish lavender, and fennel essential oils.

Spanish lavender, and fennel essential oils in the inactivation of *S. typhimurium* (P < 0.05). No significant difference was found among the inhibitory effects of bay laurel, Spanish lavender, and fennel essential oils on *S. typhimurium* (P > 0.05) (**Figure 3**).

As for inactivation of S. aureus under the same doses of Turkish oregano, bay laurel, Spanish lavender, and fennel essential oils, the 5 and 10  $\mu$ L/mL Turkish oregano essential oils decreased the initial count of 7.92 log cfu/mL in the control samples to 4.76 and 4.22 log cfu/mL, respectively (P < 0.05). After doses of 20, 30, 40, and 50 µL/mL, the numbers of survivors were detected as 4.10, 3.11, 1.08, and 0.30 log cfu/mL, respectively. No colony was counted after the application of the 80 µL/mL Turkish oregano essential oil. Applied bay laurel essential oils at 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL doses were found to reduce the initial level of 7.95 log cfu/mL for S. aureus to 5.17, 4.62, 4.36, 4.11, 3.23, 2.01, and 0.50 log cfu/mL, respectively (P < 0.05). Applied Spanish lavender essential oils at the same doses reduced the initial number of 7.66 log cfu/mL for S. aureus to 4.73, 4.31, 4.07, 3.28, 1.71, 0.75, and 0.17 log cfu/mL, respectively (P < 0.05). The number of surviving S. aureus cells in response to doses of 5, 10, 20, 30, 40, 50, and 80  $\mu$ L/mL fennel essential oils decreased from the inoculation level of 7.44 log cfu/mL in the control samples to 4.66, 4.52, 4.41, 4.12, 2.88, 2.04, and 1.11 log cfu/mL, respectively (Figure 4).

For inactivation of *S. aureus*, the degree of inhibitory effects of the essential oils of the plants was Turkish oregano > Spanish lavender  $\geq$  bay laurel > fennel. All of the doses of Turkish oregano essential oils had significantly more inhibitory effects than those of bay laurel, Spanish lavender, and fennel essential oils on *S. aureus* (P < 0.05) (**Figure 4**).

The results support previous studies that determined antimicrobial activities of plant extracts or essential oils. Essential oils obtained from the aerial parts of *T. revolutus* (a species closely related to *O. minutiflorum*) were used at different doses against *E. coli, S. aureus*, and *L. monocytogenes* to determine minimum inhibitory concentrations (MICs). Compared to *L. monocytegenes* and *S. aureus, E. coli* O157:H7 required a higher dose of *T. revolutus* essential oil to be inactivated (26). Aligiannis et al. (13) reported MICs of *O. scabrum* and *O. microphyllum* essential oils for *E. coli, S. aureus*, and *S. epidermidis*. The orders of inactivation degree of the two *Origanum* species and *T. revolutus* for *E. coli, S. epidermidis*, and *S. aureus* found by Aligiannis et al. (13), Karaman et al. (26), and our study were

Essential oils obtained from bay laurel were applied on *S. typhimurium, S. aureus*, and *E. coli* at doses of 1, 10, and 15% (v/v). The results revealed that the effective dose of bay laurel essential oils for the three bacteria was 15% (*30*). A study conducted with ground bay laurel and its alcohol extract showed that ground bay laurel had the least inactivation effect after thyme and mint as spices. Ground bay laurel was reported to be more effective in the inactivation of *S. aureus* than in that of *S. typhimurium*. The alcohol extract was effective at the 2000 ppm level for both *S. typhimurium* and *S. aureus* (*31*). Our findings resulted in the following order of inhibitory effects of bay laurel essential oil on the pathogens: *E. coli* O157:H7 > *S. aureus* > *S. typhimurium* > *L. monocytogenes*.

Spanish lavender essential oils appeared to be ineffective against *Pseudomonas syringae* and had an inhibition zone of <8 mm against *Erwinia herbicola* (28). According to Adam et al. (6), *Lavandula angustofolia* showed moderate to low antifungal activities against *Malassezia furfur*, *Trichophyton rubrum*, and *Trichosporon beigelii*. Because previous studies were concentrated on inactivation effects of lavender essential oils on fungi, comparison could not be made for the bacteria in this study. The degree of antibacterial effects of Spanish lavender essential oils was determined as *S. typhimurium* > *S. aureus* > *L. monocytogenes* > *E. coli* O157:H7 in the present study.

The inhibitory effect of fennel essential oil on *L. monocytogenes* resulted in a reduction of  $1.7-3 \log$  at 4, 8, and 24 h. Fennel oil also had an inhibitory effect on *Salmonella enteritidis* in that the culture growth was reduced by 4 log for 48 h (*32*). The antibacterial activity of *F. vulgare* essential oil revealed that *E. coli, S. aureus*, and *Salmonella pullorum* had inhibition zones of 7.25, 16.5, and 8.05 mm, respectively. The order for the degree of inhibitory effect was found as *S. aureus* > *S. pullorum* > *E. coli* (29). The inhibitory effect of fennel essential oils on the tested bacteria was *S. typhimurium*  $\geq$  *E. coli* O157:H7 > *L. monocytogenes*  $\geq$  *S. aureus*.

The results showed that inhibitory effects of the plant essential oils on the tested bacteria varied. The different inhibitory effects may be attributed to the differences in the biological properties of the main compounds in the essential oils. Turkish oregano essential oils showed a higher antibacterial activity against the tested bacteria (except for *E. coli* O157:H7). Its strong inhibitory effect appeared to be due to the higher content of carvacrol, defined as a strong antimicrobial agent. However, Spanish lavender essential oils usually showed a weak antibacterial activity, and its inhibitory effect was less than those of the other essential oils as supported by the literature (6, 28) reporting the main component of Spanish lavender (fenchone) to be a weak antibacterial agent.

This study reported the antibacterial activity of the selected plant essential oils against the selected foodborne pathogen bacteria in the selected media, but not in food samples. Therefore, further studies need to be carried out to determine the potential of plant essential oils as antimicrobial agents in foods in order for extension of shelf life as well as prevention of food deterioration under acceptable doses to be ensured.

#### ACKNOWLEDGMENT

We thank Dr. F. Durlu-Ozkaya for providing bacterial cultures, Seher Misirlioglu for helping with GC-MS analyses, and Drs. E. M. Soylu, S. Soylu, Y. K. Avsar, and F. Evrendilek for valuable comments on an earlier version of the manuscript.

## LITERATURE CITED

- Franzios, G.; Mirotson, M.; Hatziapostolou, E.; Kral, J.; Scouras, Z. G.; Mavragani Tsipidou, P. Insecticidal and genotoxic activities of mint essential oils. *J. Agric. Food Chem.* **1997**, *45*, 2690–2694.
- (2) Pruthi, J. S. Spices and Condiments: Chemistry, Microbiology, Technology. In Advances in Food Research; Academic Press: New York, 1980; Suppl. 4.
- (3) Beuchat, L. R. Sensitivity of *Vibrio parahaemolyticus* to spices and organic acids. *J. Food Sci.* **1976**, *41*, 899–902.
- (4) Huntanen, C. N. Inhibition of *Clostridium botulinum* by spice extracts and aliphatic alcohols. *J. Food Prot.* **1980**, *43*, 195– 197.
- (5) Shelef, L. A. Antimicrobial effects of spices. J. Food Saf. 1983, 6, 29–44.
- (6) Adam, K.; Sivripoulou, A.; Kokkini, S.; Lanaras, T.; Arsenakis, M. Antifungal activities of *Origanum vulgare* subsp. *hitrum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. J. Agric. Food Chem. **1998**, 46, 1739–1745.
- (7) Thompson, D. P. Fungitoxic activity of essential oil components on food storage fungi. *Mycologia* **1989**, *81* (1), 151–153.
- (8) Dwivedi, S. K.; Dubey, N. K. Potential use of the essential oil of *Trachyspermum ammi* against seed-borne fungi of guar (*Cyamopsis tetragonoloba* L. (Taub)). *Mycopathology* **1993**, *121*, 101–104.
- (9) Kishore, N.; Mishra, A. K.; Chansouria, J. P. N. Fungitoxicity of essential oils against dermatophytes. *Mycoses* 1993, 36, 211– 215.
- (10) Mishra, A. K.; Dubey, N. K. Evaluation of some essential oil for their toxicity against fungi causing deterioration of stored food commodities. *Appl. Environ. Microbiol.* **1994**, *60*, 1101– 1105.
- (11) Rana, B. K.; Singh, U. P.; Taneja, V. J. Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos. J. Ethnopharm.* **1997**, *57*, 29–34 1997.
- (12) Vieira, R. F.; Simon, J. E. Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. *Econ. Bot.* **2000**, *54*, 207–216.
- (13) Aligiannis, N.; Kalpoutzakis, E.; Mitaku, S.; Chinou, I. B. Composition and antimicrobial activity of the essential oils of two *Origanum* species. J. Agric. Food Chem. 2001, 49, 4168– 4170.
- (14) Abou-Jawdah, Y.; Sobh, H.; Salameh, A. Antimycotic activities of selected plant flora, growing wild in Lebanon, against phytopathogenic fungi. J. Agric. Food Chem. 2002, 50, 3208– 3213.
- (15) Thangadurai, D.; Anita, S.; Pullaiah, T.; Reddy, P. N.; Ramanchandraiah, O. S. Essential oil constituents and in vitro antimicrobial activity of *Decalepis hamiltonii* roots against foodborne pathogens. J. Agric. Food Chem. **2002**, 50, 3147–3149.
- (16) Olson, D. G. Irradiation of food. Food Technol. 1998, 52 (1), 56–62.
- (17) Reina, L. D.; Jin, Z. T.; Zhang, Q. H.; Yousef, A. E. Inactivation of *Listeria monocytogenes* in milk by pulsed electric fields. *J. Food Prot.* **1998**, *61* (9), 1203–1206.
- (18) Kim, J.; Yousef, A. E.; Dave, S. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J. Food Prot. **1999**, 62 (9), 1071–1087.
- (19) Evrendilek, G. A.; Zhang, Q. H.; Richter, R. E. Inactivation of *E. coli* O157:H7 and *E. coli* 8739 in apple juice by pulsed electric fields. *J. Food Prot.* **1999**, *62* (7), 793–796.
- (20) Evrendilek, G. A.; Jin, Z. T.; Ruhlman, K. T.; Qiu, X.; Zhang, Q. H.; Richter, E. R. Microbial safety and shelf life of apple juice and cider processed by bench and pilot plant scale PEF systems. *Innovative Food Sci. Emerg. Technol.* **2000**, *1*, 77– 86.
- (21) Evrendilek, G. A.; Zhang, Q. H.; Richter, R. E. Application of pulsed electric fields to skim milk inoculated with *Staphylococcus aureus*. *Biosyst. Eng.* **2004**, 87 (2), 137–144.

- (22) Farkas, D.; Hoover, D. High-pressure processing. J. Food Sci. **2000**, 65 (8), 47–64.
- (23) Yuste, J.; Fung, D. Y. C. Inactivation of *Salmonella typhimurium* and *Escherichia coli* O157:H7 in apple juice by a combination of nisin and cinnamon. J. Food Prot. 2004, 67 (2), 371–377.D
- (24) Karapinar, M.; Esen Aktuğ, Ş. Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethole. *Int. J. Food Microbiol.* **1987**, *4*, 161–166.
- (25) European Pharmacopoeia; Maissonneuve: Sainte-Ruffine, France, 1975; Vol. 3, p 68.
- (26) Karaman, S.; Digrak, M.; Ravid, U.; Ilcım, A. Antibacterial and antifungal activity of the essential oils of *Thymus revolutus* Celak from Turkey. *J. Ethnopharm.* 2001, *76*, 183–186.
- (27) Bayrak, A.; Akgül, A.; Kıvanç, M. Chemical composition antimicrobial effects of Turkish laurel leaf oil. J. Essent. Oil Res. 1989, 1, 277–280.
- (28) Karamanoli, K.; Vokou, D.; Menkissoglu, U.; Constantinidou, H.-I. Bacterial colonization of phyllosphere of Mediterranean aromatic plants. J. Chem. Ecol. 2000, 26 (9), 2035–2048.
- (29) Ruberto, G.; Tizina Baratta, M.; Deans, S. G.; Damien Dorman, H. J. Antioxidant and antimicrobial activity of *Feoniculum*

vulgare and Crithum maritimum essential oils. Planta Med. 2000, 66, 687–693.

- (30) Ozcan, M.; Erkmen, O. Antimicrobial activity of the essential oils of Turkish plant species. *Eur. Food Res. Technol.* 2001, 212, 658–660.
- (31) Esen Aktuğ, Ş.; Karapınar, M. Sensitivity of some common foodpoisoning bacteria to thyme, mint and bay leaves. *Int. J. Food Microbiol.* **1986**, *3*, 349–354.
- (32) Fyfe, L.; Armstrong, F.; Stewart, J. Inhibition of *Listeria monocytogenes*, *Salmonella enteritidis* by combinations of plant oils and derivatives of benzoic acid: the development of synergistic antimicrobial combinations. *Int. J. Antimicrob. Agents* 1998, 9, 195–199.

Received for review June 15, 2004. Revised manuscript received October 8, 2004. Accepted October 10, 2004. This project was funded by the Research Grant of Mustafa Kemal University.

JF049033E