# ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM PLANTS AGAINST SELECTED PATHOGENIC AND SAPROPHYTIC MICROORGANISMS

M. Elgayyar, F. A. Draughon, D. A. Golden, and J. R. Mount

Made in the United States of America
Reprinted from JOURNAL OF FOOD PROTECTION
Vol. 64, No. 7, July 2001
Copyright ©, International Association for Food Protection

# Antimicrobial Activity of Essential Oils from Plants against Selected Pathogenic and Saprophytic Microorganisms

M. ELGAYYAR, F. A. DRAUGHON,\* D. A. GOLDEN, AND J. R. MOUNT

Tennessee Agricultural Experiment Station, Department of Food Science and Technology, 2605 River Drive, Food Safety Center, The University of Tennessee, Knoxville, Tennessee 37919, USA

MS 99-288: Received 22 September 1999/Accepted 17 June 2000

#### **ABSTRACT**

The beneficial health effects of extracts from many types of plants that are used as seasoning agents in foods and beverages have been claimed for centuries. The purpose of this study was to examine the effectiveness of selected herb and spice essential oils for control of growth and survival of microorganisms. Inhibition of growth was tested by the paper disc agar diffusion method. Antibiotic susceptibility discs were used as control. Minimum lethal concentration (MLC) was determined by the tube dilution method. Essential oils from anise, angelica, basil, carrot, celery, cardamom, coriander, dill weed, fennel, oregano, parsley, and rosemary were evaluated. Inhibition ranged from complete with oregano to no inhibition with carrot oil for each of the test strains that included: Listeria monocytogenes, Staphylococcus aureus, Escherichia coli O:157:H7, Yersinia enterocolitica, Pseudomonas aeruginosa, Lactobacillus plantarum, Aspergillus niger, Geotrichum, and Rhodotorula. Oregano essential oil showed the greatest inhibition (zone,  $\geq$ 70 to 80 mm) (MLC,  $\sim$ 8 ppm). Coriander and basil were also highly inhibitory (MLC,  $\sim$ 25 to 50 ppm) to E. coli O:157:H7 and to the other bacteria and fungi tested. Anise oil was not particularly inhibitory to bacteria (inhibition zone,  $\sim$ 25 mm); however, anise oil was highly inhibitory to molds. Because some of the herbal and spice essential oils are highly inhibitory to selected pathogenic and spoilage microorganisms, they may provide alternatives and supplements to conventional antimicrobial additives in foods.

Extracts from many types of plants used as flavoring and seasoning agents in foods and beverages have been used therapeutically for centuries (2, 5). However, there is little quantitative data on the antimicrobial activity of most plant extracts (6, 7). The antimicrobial activity of garlic, onion, mustard, cinnamon, and cloves has been heavily studied since the end of last century, and the active components in these herbs are known (22, 24).

Essential oil extracted from several types of plants either by steam distillation or volatile organic solvent, has been used as a flavoring for years (6, 12, 23). Antimicrobial activity of plant extracts is frequently due to the essential oil fraction, or to sulfur-containing compounds in the aqueous phase (11, 24). These compounds are also responsible for the characteristic aroma and flavor of the spices (12).

Wilkins and Board (25) reported that more than 1,340 plants are known to be potential sources of antimicrobial compounds but that few have been studied scientifically. Ela and coworkers (8) screened 16 essential oils for their antimicrobial activity against Staphylococcus aureus, Escherichia coli, and the fungi Aspergillus niger and Candida albicans, by using the agar diffusion technique and measuring their inhibition zones. They classified the tested oils according to their activity: strongly active (inhibition zone, >8 mm), moderately active (inhibition zone, >6 to <8 mm), and inactive (no inhibition zone, <6 mm). Basil and parsley were inhibitory to S. aureus, E. coli, A. niger, and

C. albicans. Conner and Beuchat (6) investigated the antimicrobial effect of 32 essential oils on 13 food-spoilage and industrial yeasts. They categorized the essential oils as strongly active (inhibition zone, >11 mm), moderately active (inhibition zone, >6 to <11 mm), or inactive (inhibition zone, <6 mm). They found that oregano was the most effective inhibitor against yeasts. Anise, basil, cardamom, celery, dill, fennel, parsley, and rosemary were less effective against yeasts.

Previous research has shown that oregano and basil essential oils are antimicrobial, but there is little quantitative data (minimum inhibitory concentration or minimum lethal concentration [MLC]) on the antimicrobial activity of anise, angelica, cardamon, celery, coriander, carrot, dill, fennel, parsley, and rosemary against foodborne pathogens (1, 3, 9, 17–19, 26).

Therefore, this study was undertaken to investigate the effectiveness of selected herbal and spice essential oils on survival and growth of selected pathogenic and spoilage microorganisms.

### MATERIALS AND METHODS

Essential oils. The steam-distilled essential oils of anise, angelica, basil, cardamom, carrot, celery, coriander, dill, fennel, parsley, oregano, and rosemary were purchased from The Essential Oil Company (Portland, Oreg.), which supplies foodgrade oils. Each oil for all replications was from the same source and lot. The oils (100% pure essential oil) were stored in dark bottles and kept refrigerated per manufacturer's recommendation until evaluation.

<sup>\*</sup> Author for correspondence. Tel: 865-974-7425; Fax: 865-974-2750; E-mail: draughon@utk.edu.

Microbial analysis of essential oils. All essential oils used in this study were subjected to microbial analysis for quality control. Decimal dilutions were prepared in 0.1% peptone water and each oil (0.1 ml) was spread onto standard methods agar (SMA) and rose Bengal (Difco, Detroit, Mich.) agar. Aerobic plate counts were enumerated on SMA plates by incubating at 32°C for 48 h and fungi were enumerated on rose Bengal plates by incubating at room temperature ( $\approx 25$ °C) for 7 days. All tests were conducted in triplicate with three replications (n = 9). No colonies were isolated from any of the essential oils.

Culture maintenance and inoculum preparation. E. coli O157:H7 (ATCC 35150, apple cider), Lactobacillus plantarum (ATCC 14917), Listeria monocytogenes Scott A (meat isolate), Pseudomonas aeruginosa (ATCC 9027), Salmonella Typhimurium (ATCC 14028), S. aureus (ATCC 13563), Yersinia enterocolitica (ATCC 228), A. niger (UT stock culture), Geotrichum candidum (ATCC 34614), and Rhodotorula (ATCC 32765) were used. Test strains of bacteria were inoculated into Brucella broth (Difco) and incubated at 35°C for 24 h. Cultures were subjected to three successive 24-h transfers before use. All cultures were kept refrigerated on tryptose soy agar slants during the experiment (up to 2 weeks) and were tested biochemically for viability and purity before each use.

A. niger and G. candidum cultures were maintained on potato dextrose agar at 4°C and revived and cultivated on potato dextrose agar at 25°C. Rhodotorula cultures were maintained on yeast and mold agar and revived on yeast and mold broth at 25°C. Yeast and mold cultures were subjected to two successive transfers in yeast and mold broth and tested for purity and viability using biochemical and morphological characteristics, before each use.

Assay for antimicrobial activity. Antimicrobial activity was tested by the filter paper disc diffusion method (19). SMA and Mueller Hinton agar (Difco) containing 100 ppm of 2,3,5-triphenyltetrazolium chloride (Fisher, Fairlawn, N.J.) were used for antibacterial assay. 2,3,5-Triphenyltetrazolium chloride was added to culture media to differentiate bacterial colonies and to clarify the zone of inhibition (13). SMA without 2,3,5-triphenyltetrazolium chloride added was used for antifungal assays. Each plate was inoculated with bacterial or yeasts culture (0.1 ml) directly from the broth or suspension (24 h, 108 CFU/ml). Fungal spores suspensions were prepared on potato dextrose agar slants by incubating each culture for 5 days at 25°C on potato dextrose agar. Sterile Triton X-100 was added to the slant to prepare a spore suspension, and spore counts were conducted microscopically (13). Fungal spore suspensions were diluted to achieve a final concentration of 108 spores/ml. Each essential oil (24 mg of 100% oil) was placed on each sterile 6-mm-diameter absorbent blank filter paper disc (Difco) that was placed in the center of the inoculated plates. All plates (except mold: 25°C for 7 days) were incubated at 32°C for 4 days, after which the inhibition zones were measured and recorded in millimeters (mm). The scale of measurement was the following (disk diameter included): ≥28 mm zone of inhibition is strongly inhibitory; <28 to 16 mm zone of inhibition is moderately inhibitory; <16 to 10 mm zone of inhibition is mildly inhibitory; and <12 mm is noninhibitory (8, 19). Control plates were prepared by placing sterile water and olive oil on disks for negative controls, and antibiotic disks were used to evaluate cultures for possible antibiotic resistance patterns that might affect sensitivity of assay. The antibiotics used were cephalothin 30 µg, amoxicillin 20 µg and clavulanic acid 10 µg, nalidixic acid 30 µg, tetracycline 30 µg, and neomycin 5 µg from Difco. From BBL (Cockeysville, Md.), penicillin 10 IU, tetracy-

TABLE 1. Inhibition of growth of gram-positive bacteria by essential oils

	Inhibition zone diameter (mm) <sup>a</sup>					
Oils	L. monocytogenes	L. plantarum	S. aureus			
Anise	0 DW <sup>b</sup>	25 CU	26 FU			
Angelica	23 BR	0 dv	20 gr			
Basil	15 CU	0 dv	$87^c$ ar			
Cardamom	12 cv	42 BS	39 DT			
Carrot	0 dr	0 fr	0 hr			
Celery	22 BU	0 FW	24 FU			
Coriander	21 BU	11 <b>EW</b>	87 ar			
Dill	0 dv	0 dv	69 br			
Fennel	0 dw	15 DU	38 DT			
Parsley	21 BU	0 fx	33 ES			
Oregano	53 AS	87 ar	87 ar			
Rosemary	0 dv	22 CT	45 CR			

<sup>&</sup>lt;sup>a</sup> The diameter of the filter paper discs (6 mm) is included. No inhibition (<6 mm diameter).

cline 5  $\mu g,$  bacitracin 30  $\mu g,$  and chloramphenicol 30  $\mu g$  were purchased.

MLC setup. The MLC for bacteria was measured by using the twofold serial broth dilution method (9) in brain heart infusion broth for all oils that showed inhibitory activity by the paper disk diffusion method. Initial maximum concentration of each essential oil was 100,000 ppm. Essential oil was diluted to a minimum concentration of 100 ppm. Each tube was inoculated with 0.1 ml of bacterial suspension, prepared as described earlier, to achieve a final concentration of 107 CFU/ml. MLC cultures were incubated at 32°C for 24, 48, and 72 h. Growth of the microorganism from any incubation period at a particular concentration indicated that lethal concentration was not achieved. All tests were conducted in triplicate with three replications (n = 9). Controls consisted of brain heart infusion broth containing 100,000 ppm olive oil and 0.1 ml of bacterial suspension (107 CFU/ml). Olive oil was not inhibitory to microorganisms at this concentration. Survival was determined by plating an aliquot from each tube onto SMA plates (incubated for 48 h at 32°C) and recorded as + for growth or - for no growth. MLC was defined as the lowest concentration of essential oil that killed (no growth or survival at the given concentration within 72 h) of the test organism.

Experimental design and statistical analysis. This experiment was performed as a completely randomized block with three replications (3). Statistical analysis was conducted using SAS version 6.1 (SAS Institute, Cary, N.C.) using PROC MIXED.

## RESULTS AND DISCUSSION

Antimicrobial inhibition zones for essential oils against foodborne pathogens, spoilage bacteria, yeasts, and molds are shown in Tables 1 to 3. Antimicrobial activity of herb and spice essential oils ranged from no inhibition to complete inhibition against the test microorganisms.

Oil of oregano completely inhibited growth of the gram-negative pathogens tested (Salmonella Typhimurium,

<sup>&</sup>lt;sup>b</sup> Least-square means in columns followed by different letters (A through I), are significantly different (P < 0.001). Least-square means in rows followed by different letters (R through Z), are significantly different (P < 0.001).

<sup>&</sup>lt;sup>c</sup> Complete inhibition.

TABLE 2. Inhibition of growth of gram-negative bacteria by essential oils

	Inhibition zone diameter (mm) <sup>a</sup>						
Oils	E. coli O157	Salmonella Typhimurium	Y. entero- colitica	P. aeruginosa			
Anise	19 Ev <sup>b</sup>	22 CU	34 ET	0 ew			
Angelica	11 Gs	12 ES	23 GR	12 cs			
Basil	52 BT	79 BS	$87^c$ ar	15 CU			
Cardamom	12 gv	18 DU	57 CR	19 BU			
Carrot	0 HR	0  fr	0  HR	0 er			
Celery	13 FV	13 FV	38 ET	13 cv			
Coriander	37 CT	22 CU	67 BS	19 BV			
Dill	36 cr	26 ст	47 DS	0 ev			
Fennel	14 FU	18 DU	57 CR	11 DV			
Parsley	13 FW	16 dv	23 FT	16 cv			
Oregano	87 ar	87 ar	87 ar	46 at			
Rosemary	23 DT	23 CT	35 ES	13 cu			

<sup>&</sup>lt;sup>a</sup> The diameter of the filter paper discs (6 mm) is included. No inhibition (<6 mm diameter).</p>

Y. enterocolitica, and E. coli), S. aureus, L. plantarum, and fungi imperfecti (A. niger, Geotrichum, and Rhodotorula). Oregano was significantly less effective in inhibiting P. aeruginosa (P < 0.001), although still strongly inhibitory (zone = 46 mm). L. monocytogenes was also strongly inhibited (zone = 53 mm), but oregano was significantly (P < 0.001) less effective against L. monocytogenes compared to other microorganisms. These data confirm the findings that oregano inhibits Aspergillus spp. (20, 21). Biondi et al. (4) also reported that oregano (unspecified amount) completely inhibited S. aureus and E. coli and that it was antifungal against A. niger. Conner and Beuchat (6) reported that oregano essential oil was strongly inhibitory for 13 yeasts at the 10% level. Earlier studies have demonstrated that oregano progressively delayed growth and acid production of lactic acid bacteria (16, 26). Oil of oregano exhibited the highest antimicrobial activity of any essential oil in our study against all test microorganisms (inhibition zone = 45 to 87 mm). The powerful antimicrobial activity of oregano against gram-negative pathogens, S. aureus, and L. monocytogenes suggests that oregano oil may be useful in some food formulations as an antimicrobial. Because the aroma of oil of oregano is quite strong, its usefulness may be limited to foods where the oregano flavor would be desirable (i.e., meat marinades, salad dressings, etc.).

Oil of coriander completely inhibited growth of the S. aureus and fungi imperfecti (A. niger, Geotrichum, and Rhodotorula) (Tables 1 and 3). Coriander was less effective in inhibiting Y. enterocolitica and E. coli (P < 0.001), although still strongly inhibitory. Coriander was moderately inhibitory to E. monocytogenes, Salmonella Typhimurium, and E. aeruginosa, but E. plantarum was resistant to the antimicrobial activity of coriander. Conner and Beuchat (E)

TABLE 3. Inhibition of growth of fungi by essential oils

	Inhibition zone diameter $(mm)^a$					
Oils	A. niger	G. candidum	Rhodotorula			
Anise	56 CT <sup>b</sup>	42 cs	0 DW			
Angelica	0 DV	12 HS	2 cr			
Basil	$87^c$ ar	77 BS	87 ar			
Cardamom	18 DU	20 gu	0  pw			
Carrot	0 fr	0 ir	0 dr			
Celery	16 dv	41 cs	45 BR			
Coriander	87 ar	87 ar	87 ar			
Dill	12 EU	38 dr	44 BS			
Fennel	60 BR	43 cs	0 DW			
Parsley	17 DV	29 ET	44 BR			
Oregano	87 ar	87 ar	87 ar			
Rosemary	0 DV	24 FT	0 dv			

<sup>&</sup>lt;sup>a</sup> The diameter of the filter paper discs (6 mm) is included. No inhibition (<6 mm diameter).

stated that oil of coriander was only slightly inhibitory toward most yeasts selected for evaluation. Our results showed that coriander completely inhibited *Rhodotorula* (zone > 87 mm), which was not evaluated in previous studies. In addition, Meena and Sethi (19) found that coriander has no inhibitory effect on *A. niger* and a mild inhibitory effect on *Lactobacillus acidophilus* at both room temperature and at 37°C. Our data showed that coriander (24 mg) completely inhibited *A. niger* (zone > 87 mm), and that it has little or no inhibitory effect on *L. plantarum*. The antimicrobial effect of coriander appeared to be highly variable depending on cultural conditions and species. This variation may limit commercial applications.

Oil of anise (Tables 1 to 3) was inhibitory for all organisms in our study except for L. monocytogenes, Pseudomonas, and Rhodotorula. Greatest inhibition of growth by anise was found against Y. enterocolitica, A. niger, and, G. candidum. Anise was also moderately inhibitory against L. plantarum, S. aureus, E. coli, and Salmonella Typhimurium. Conner and Beuchat (6) investigated the antimicrobial effect of essential oils of anise and stated that these oils had only mild effects on all tested yeasts, which is consistent with our findings with Rhodotorula.

Oil of angelica did not completely inhibit any of the bacteria used in our study and was only moderately inhibitory against L. monocytogenes, S. aureus, Y. enterocolitica, and Rhodotorula (zone = 23.3, 20.3, 23, and 22 mm, respectively). Oil of angelica was weakly inhibitory against E. coli, Salmonella Typhimurium, P. aeruginosa, G. candidum (zone = 10.3, 11.7, 12, and 12 mm, respectively) and had no inhibitory effect against L. plantarum and A. niger. This is the first scientific study to report antimicrobial activity of angelica. Angelica was one of the few oils that had antimicrobial activity against L. monocytogenes in our study.

<sup>&</sup>lt;sup>b</sup> Least-square means in columns followed by different letters (A through 1), are significantly different (P < 0.001). Least-square means in rows followed by different letters (R through Z), are significantly different (P < 0.001).

<sup>&</sup>lt;sup>c</sup> Complete inhibition.

<sup>&</sup>lt;sup>b</sup> Least-square means in columns followed by different letters (A through I), are significantly different (P < 0.001). Least-square means in rows followed by different letters (R through Z), are significantly different (P < 0.001).

<sup>&</sup>lt;sup>c</sup> Complete inhibition.

1022 ELGAYYAR ET AL. J. Food Prot., Vol. 64, No. 7

TABLE 4. Inhibition zones (mm) of growth of tested microorganisms by antibiotics

Antibiotic	Inhibition zone diameter (mm) <sup>a</sup>								
	L. monocy- togenes	L. plantarum	S. aureus	E. coli O157	Salmonella Typhimurium	Y. entero- colitica	P. auruginosa	Aspergillus and Geotrichum	Rhodotorula
Tetracycline	11	0	28	19	22	23	11	0	0
Neomycin	0	0	0	11	0	0	0	0	0
Chloramphenicol	10	0	30	26	26	30	20	0	0
Cephalothin	0	0	25	17	24	14	12	0	0
Ampicillin	0	0	0	0	0	0	0	0	0
Bacitracin	0	0	11	0	0	0	0	0	0
Nalidixic acid	10	0	0	30	29	34	18	0	0
Penicillin	0	0	0	0	0	0	0	0	0

<sup>&</sup>lt;sup>a</sup> The diameter of the antibiotic discs (6 mm) is included. No inhibition (<6 mm diameter).

Oil of basil (Tables 1 to 3) completely inhibited growth (zone = 87 mm) of S. aureus, Y. enterocolitica, and the fungi imperfecti (A. niger and Rhodotorula). Basil was also strongly inhibitory to E. coli, Salmonella Typhimurium, and G. candidum (P < 0.001). Basil was only mildly inhibitory against L. monocytogenes and P. aeruginosa. Several investigators have studied antimicrobial effectiveness of basil with varying results. Lachowicz et al. (17) found that essential oils of five different varieties of basil possessed mild antimicrobial activity against three gram-positive bacteria (L. plantarum, L. monocytogenes, S. aureus) and several gram-negative bacteria (E. coli, P. aeruginosa, Salmonella Typhimurium, Y. enterocolitica), yeasts (Rhodotorula), and molds. Fyfe et al. (9) reported that oil of basil at 0.2% was a potent inhibitor of L. monocytogenes and Salmonella Enteritidis. Meena and Sethi (19) also found that basil essential oil has a moderate antimicrobial effect on A. niger and L. acidophilus. Our data expand and support the findings of Ela et al. (8), who showed that basil essential oil had antibacterial and antifungal activity against S. aureus, E. coli, and A. niger.

Carrot essential oil (Tables 1 to 3) was not inhibitory to any of the tested microorganisms. Babic et al. (3) reported that purified ethanolic extracts of peeled and shredded carrot have an antimicrobial effect against a range of foodborne microorganisms such as *L. monocytogenes*, *S. aureus*, and *E. coli*. It should be noted that the water extract of carrot gives highly polar compounds, whereas the essential oil extract using ethanol or petroleum ether gives a variety of both polar and nonpolar compounds.

Oil of celery was strongly inhibitory against Y. enterocolitica, G. candidum, and Rhodotorula, moderately inhibitory against L. monocytogenes and S. aureus, and only weakly inhibitory to E. coli O157H7, Salmonella Typhimurium, P. aeruginosa, and A. niger. Conner and Beuchat (6) reported weak inhibitory activity of essential oil of celery on yeasts.

Oil of dill was strongly antimicrobial against S. aureus, E. coli, Y. enterocolitica, G. candidum, and Rhodotorula and moderately inhibitory against Salmonella Typhimurium. Dill was weakly inhibitory against A. niger. Oil of dill had no inhibitory effect against L. monocytogenes, L.

plantarum, or P. aeruginosa. Traditional use of dill in many fermented foods may reflect the preservative effects of dill against spoilage yeasts such as Rhodotorula.

Both oil of fennel and oil of parsley were strongly inhibitory against *S. aureus, Y. enterocolitica*, and fungi, moderately inhibitory against *Salmonella* Typhimurium, and only weakly or not inhibitory to *L. plantarum, E. coli*, *P. aeruginosa, L. monocytogenes*, and *Rhodotorula*. Oil of fennel was reported by Conner and Beuchat (6) to be mildly effective against selected yeasts. Others have reported that fennel may be inhibitory in combination with known antimicrobials (9, 14).

Oil of rosemary was not inhibitory to *L. monocytogenes*, *A. niger*, or *Rhodotorula* and was only weakly inhibitory to *Pseudomonas*. Zones of inhibition for the other spoilage and pathogenic organisms exposed to oil of rosemary ranged from 23 to 45 mm, which made rosemary moderately to strongly inhibitory to a wide range of organisms. This was the only oil that was moderately inhibitory to one of the fungi imperfecti but not inhibitory to the others. Conner and Beuchat (6) reported that oil of rosemary was mildly inhibitory to some of the yeasts that they tested. In general, oil of rosemary was only weakly or not inhibitory to fungi imperfecti or yeasts but showed a fairly broad range of activity against gram-positive and gram-negative bacteria.

Antibiotic sensitivity of the bacterial pathogens and saprophytic organisms used in this study is documented in Table 4. None of the cultures used showed atypical or unusual antibiotic resistance patterns. *Salmonella* and *Yersinia* were resistant to neomycin, ampicillin, bacitracin, and penicillin, as were most of the other gram-negative bacteria (Table 4). The fungi were not inhibited by any of the antibiotic disks (negative controls).

The MLC values for the individual oils are shown in Table 5. Among the gram-positive bacteria tested, *S. aureus* was the most sensitive bacterium. The MLC for *S. aureus* ranged from as low as 400 ppm for both oregano and coriander to 100,000 ppm with angelica essential oil. *L. monocytogenes* was, in general, the most resistant organism to the various essential oils with MLC ranging from 6,250 ppm for oregano to greater than 100,000 ppm for several

TABLE 5. MLC of essential oils for tested bacteria

Oils				MLC (ppm)			
	L. monocytogenes	L. plantarum	S. aureus	E. coli O157	Salmonella Typhimurium	Y. enterocolitca	P. aeruginosa
Anise	>100,000	50,000	50,000	50,000	25,000	50,000	>100,000
Angelica	50,000	>100,000	>100,000	>100,000	>100,000	>100,000	>100,000
Basi1	>100,000	>100,000	1500	6000	6000	<800	50,000
Cardamom	>100,000	25,000	12,500	50,000	50,000	3000	12,500
Carrot	>100,000	>100,000	>100,000	>100,000	>100,000	>100,000	>100,000
Celery	50,000	>100,000	25,000	>100,000	>100,000	50,000	>100,000
Coriander	50,000	>100,000	400	50,000	50,000	3000	12,500
Dill	>100,000	>100,000	3000	12,500	50,000	12,500	>100,000
Fennel	>100,000	>100,000	25,000	50,000	50,000	12,500	25,000
Parsley	50,000	>100,000	50,000	>100,000	>100,000	50,000	>100,000

of the essential oils. The gram-negative pathogenic and spoilage bacteria had MLCs ranging from 400 ppm to >100,000 ppm depending on the essential oil. Essential oils of angelica, carrot, celery, and parsley were not significantly (P > 0.05) inhibitory to most gram-negative bacteria tested in our study. Jay (15) stated that generally grampositive bacteria are more sensitive than gram-negative bacteria to the spice essential oils, with the lactic acid bacteria being the more resistant among gram-positive bacteria. We would disagree with this statement based on our results because S. aureus was very sensitive to essential oils but L. monocytogenes was highly resistant. It is difficult to make such generalizations because each oil (or plant family) is unique and gram-positive bacteria vary widely in structure and functionality.

Essential oil of oregano was the only oil in this study containing primarily thymol and carvacrol. Another interesting finding was that the only two oils containing linalool (coriander and basil) were both highly inhibitory to fungi, but not necessarily bacteria. Studies are needed to identify the active antimicrobial components in each oil to facilitate their use in food formulations. One of the negative aspects of using spices and herbs in food processing is that some types have been found to be heavily contaminated by mold flora. These mold flora were reported to be dominated by Aspergillus spp. that may produce aflatoxin (10). However, preliminary data collected on all oils at the beginning of our study showed that the essential oil, which is derived by extraction or steam distillation, was relatively free of microorganisms (<10 CFU/g), probably due to the harsh environmental conditions during preparation of the oil and to the antimicrobial activity of the pure oil after preparation.

#### CONCLUSION

Because several of the essential oils have potent antimicrobial effects against some of the foodborne pathogens in the ppm range (>400 ppm), further studies are needed on incorporation of these oils into appropriate food formulations to evaluate flavor, chemical changes, and antimicrobial effect in a food system. Some essential oils were highly inhibitory to pathogens at low levels comparable to organic acids used in food processing. It has to be emphasized that these oils will probably not be useful as preser-

vatives in foods that will receive heat treatment unit operations after addition of essential oil because it is very likely that the antimicrobial compounds would be evaporated. Studies incorporating these essential oils into meat marinades and other food products are currently in progress in our laboratory.

#### REFERENCES

- Arora, D. K., K. G. Mukerji, and E. H. Marth. 1991. Handbook of applied mycology, chapter 16. In M. B. Liewen (ed.), Antifungal food additives. Marcel Dekker, Inc., New York.
- Ayres, J. C., J. O. Mundt, and W. E. Sandine. 1980. Microbiology of foods, chapters 11 and 18. In Spices and condiments, fish and shellfish. W. H. Freeman and Company, San Francisco, Calif.
- Babic, I., C. Nguyen-the, M. J. Amiot, and S. Aubert. 1994. Antimicrobial activity of shredded carrot extracts on food-borne bacteria and yeasts. J. Appl. Bacteriol. 76:135-141.
- Biondi, D., P. Cianci, C. Geraci, and G. Ruberto. 1993. Antimicrobial activity and chemical composition of essential oils from sicilian aromatic plants. Flavour Fragrance J. 8:331-337.
- Charalambous, G. 1994. Spices, herbs and edible fungi, chapters 3, 12, 19, and 20. Elsevier Science B.V., Amsterdam.
- Conner, D. E., and L. R. Beuchat. 1984. Effect of essential oils from plants on growth of spoilage yeasts. J. Food Sci. 49:429–434.
- Davidson, P. M., and A. L. Branen. 1993. Antimicrobials in foods, chapter 13. In D. E. Conner (ed.), Naturally occurring compounds. Marcel Dekker, Inc., New York.
- Ela, M. A., N. S. El-Shaer, and N. B. Ghanem. 1996. Antimicrobial evaluation and chromatographic analysis of some essential and fixed oils. Pharmazie 51:993–995.
- Fyfe, L., F. Armstrong, and J. Stewart. 1998. Inhibition of *Listeria monocytogenes* and *Salmonella enteriditis* by combinations of plant oils and derivatives of benzoic acid the development of synergistic antimicrobial combinations. Int. J. Antimicrob. Agents 9:195–199.
- Garrido, D., M. Jodral, and R. Pozo. 1992. Mold flora and aflatoxinproducing strains of Aspergillus flavus in spices and herbs. J. Food Prot. 55:451-452.
- Gould, G. W. 1995. New method of food preservation, 1st ed. Blackie Academic and Professional Pub., London.
- Gould, G. W. 1996. Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. J. Food Prot. 59(Suppl.):82–86.
- Harrigan, W. F., and M. E. McCance. 1976. Laboratory methods in food and dairy microbiology. Academic Press, New York.
- Hodgson, I., J. Stewart, and L. Fyfe. 1998. Inhibition of bacteria and yeasts by oil of fennel and paraben: development of synergistic antimicrobial combinations. J. Essent. Oil Res. 10:293-297.
- 15 Jay, J. M. 1996. Modern food microbiology, 5th ed. Van Nostrand Reinhold, New York.
- 16. Kivanc, M., A. Akgul, and A. Dogan. 1991. Inhibitory and stimu-

- latory effect of cumin, oregano and their essential oils on growth and acid production of *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. Int. J. Food Microbiol. 13:81-86.
- Lachowicz, K. J., G. P. Jones, D. R. Briggs, F. E. Bienvenu, J. Wan, A. Wilcock, and M. J. Coventry. 1998. The synergistic preservative effects of essential oil of sweet basil (*Ocimum basilicum* L.) against acid-tolerant food microflora. Lett. Appl. Microbiol. 26:209-214.
- Manderfeld, M. M., H. W. Schafer, P. M. Davidson, and E. A. Zottola. 1997. Isolation and identification of antimicrobial furocoumarins from parsley. J. Food. Prot. 60:72-77.
- Meena, M. R., and V. Sethi. 1994. Antimicrobial activity of essential oils from spices. J Food Sci. Technol. 31:68-70.
- Paster, N., M. Menasherov, U. Ravid, and B. Juven. 1995. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J. Food Prot. 58:81-85.
- 21. Salmeron, J., R. Jordano, and R. Pozo. 1990. Antimycotic and an-

- tiaflatoxinogenic activity of oregano (*Origanum vulgare L.*) and thyme (*Thymus vulgaris*, L.). J. Food Prot. 53:697-700.
- Saxena, A. P., and K. M. Vyas. 1986. Antimicrobial activity of seeds of ethnomedicinal plants. J. Econ. Taxon. Bot. 8:291–299.
- Thomann, R. J., J. Ehrich, and U. Bauermann. 1993. Distillation and use of essential oils from dill, celery, lovage, and parsley made in Germany. Acta Hortic. 333:101-111.
- Uda, Y., H. Matsuoka, H. Shima, H. Kumagami, and Y. Maeda. 1993. Antimicrobial activity of water-soluble products derived from radish mustard oil and identification of an active component in there. Nippon Shokuhin Kogyo Gakkaishi 40:801-806.
- Wilkins, K. M., and R. G. Board. 1989. Natural antimicrobial systems, chapter 11. In G. W. Gould (ed.), Mechanisms of action of food preservation procedures. Elsevier Applied Science, London.
- Zaika, L. L., J. C. Kissinger, and A. E. Wasserman. 1983. Inhibition of lactic acid bacteria by herbs. J. Food Sci. 48:1455–1459.