

Antibacterial Activity of Some Essential Oil Components against Five Foodborne Pathogens[†]

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Antibacterial activity of 11 essential oil constituents against *Escherichia coli*, *E. coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Vibrio vulnificus* was tested at 5, 10, 15, and 20% in 1% Tween 20 using a paper disk method. Eight constituents were then tested in liquid medium to determine minimum inhibitory and minimum bactericidal concentrations (MIC and MBC, respectively). *V. vulnificus* was most susceptible using disk assay. Carvacrol showed strong bactericidal activity against all tester strains, while limonene, nerolidol, and β -ionone were mostly inactive. Carvacrol was highly bactericidal against *S. typhimurium* and *V. vulnificus* in liquid medium (MBC 250 μ g/mL). Citral and perillaldehyde had MBCs of 100 and 250 μ g/mL against *V. vulnificus*. Terpeneol and linalool were least potent against tester strains, with MBCs of 1000 μ g/mL. Citral, geraniol, and perillaldehyde at 500 μ g/mL completely killed *E. coli*, *E. coli* O157:H7, and *S. typhimurium*, while citronellal at 250 μ g/mL killed *V. vulnificus*. Therefore, these compounds could serve as potential antibacterial agents to inhibit pathogen growth in food.

Keywords: Antibacterial activity; essential oils; foodborne pathogens

INTRODUCTION

For years, foodborne illness resulting from consumption of food contaminated with pathogenic bacteria and/or their toxins has been of vital concern to public health. Among foodborne outbreaks in the United States during 1983–1987 in which the etiology was determined, bacterial pathogens caused the largest number of outbreaks (66%) and cases (92%) (Bean et al., 1990). Thus, controlling pathogens could reduce foodborne outbreaks and assure consumers a safe, wholesome, and nutritious food supply.

Antimicrobial agents, including food preservatives and organic acids, have been used to inhibit foodborne bacteria and extend the shelf life of processed food. Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against food pathogens (Deans and Ritchie, 1987; Janssen et al., 1985). Phenolic compounds and their subclasses, such as coumarins, flavonoids, and essential oils, have antimicrobial function (Jurd et al., 1971; Wyman and VanEtten, 1978; Kubo et al., 1993). The antimicrobial activity of spices and essential oils is well recognized (Shelef, 1983; Farag et al., 1989). Essential oils of thyme, cinnamon, bay, and clove are known to possess antimicrobial activity (Deans and Ritchie, 1987). Essential oils and their constituents have been used extensively as flavor ingredients in a wide variety of food, beverage, and confectionery products and also in numerous applications, including toothpaste. Many of these compounds are classified as Generally Recognized As Safe (GRAS).

Since foodborne pathogens were seldom included in previous antimicrobial studies of essential oil constitu-

ents, five pathogenic strains, *Escherichia coli*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Vibrio vulnificus*, were used in this study to evaluate the antibacterial activity of 11 commercially available essential oil compounds using a paper disk assay. These compounds were chosen because they have previously been shown to possess antibacterial and antifungal activities against many plant and food microorganisms (Kurita et al., 1979, 1981). *E. coli* O157:H7 is an important foodborne pathogen found in animal feces. This microorganism can cause hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) and is of great concern to the meat industry. Therefore, those compounds showing potent antibacterial activities were then tested in liquid culture medium to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

MATERIALS AND METHODS

Test Compounds. Carvacrol, citral, citronellal, limonene, and perillaldehyde were purchased from Aldrich Chemical Co. (Milwaukee, WI), and eugenol, geraniol, β -ionone, linalool, nerolidol, and α -terpineol were obtained from Sigma Chemical Co. (St. Louis, MO). All test compounds were checked for purity by thin-layer chromatography and used without further purification. Test solutions containing the desired concentrations of essential oil compounds were freshly prepared before each use by dissolving in 10 mL of sterile distilled water containing 0.1 g of Tween 20 (Fisher Scientific, Orlando, FL).

Test Microorganisms. *E. coli* (ATCC 33985), *E. coli* O157:H7 (ATCC 43895), *S. typhimurium* (ATCC 6539), *V. vulnificus* (ATCC 27562), and *L. monocytogenes* Scott A were used. Except for *V. vulnificus*, all stock cultures were grown on tryptic soy agar (TSA, Difco Laboratories, Detroit, MI) plates. *V. vulnificus* was grown on TSA containing 1% NaCl. Test strain was transferred to fresh tryptic soy broth (TSB, Difco) before use.

Antibacterial Assay Using a Paper Disk Method. Eleven essential oil compounds were tested on five pathogenic bacteria using a zone of inhibition assay on TSA (*E. coli*, *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* Scott A) or TSA containing 1% NaCl (*V. vulnificus*). Bacterial number

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Table 1. Zone of Inhibition of Eight Essential Oil Constituents against Five Bacterial Tester Strains

chemical	concn (%)	<i>E. coli</i>	<i>E. coli</i> O157:H7	<i>S. typhimurium</i>	<i>L. monocytogenes</i> ^c	<i>V. vulnificus</i>
citral	5	0.89 ± 0.06 ^{A a}	0.88 ± 0.09 ^A	0.86 ± 0.02 ^A	0.84 ± 0.06 ^A	1.45 ± 0.13 ^A
	10	0.90 ± 0.07 ^A	1.01 ± 0.10 ^B	0.93 ± 0.08 ^A	1.13 ± 0.11 ^B	1.79 ± 0.19 ^B
	15	0.95 ± 0.11 ^A	0.98 ± 0.11 ^{AB}	1.07 ± 0.09 ^B	1.24 ± 0.16 ^B	2.48 ± 0.43 ^C
	20	0.96 ± 0.04 ^A	0.98 ± 0.15 ^{AB}	1.12 ± 0.14 ^B	2.33 ± 0.24 ^C	3.02 ± 0.26 ^D
carvacrol ^b	5	1.26 ± 0.13 ^A	1.20 ± 0.05 ^A	1.37 ± 0.05 ^A	0.98 ± 0.06 ^A	1.86 ± 0.14 ^A
	10	2.19 ± 0.18 ^B	1.93 ± 0.09 ^B	1.89 ± 0.08 ^B	1.53 ± 0.25 ^B	2.87 ± 0.28 ^B
geraniol	5	0.94 ± 0.04 ^A	0.91 ± 0.06 ^A	0.92 ± 0.06 ^A	0.96 ± 0.06 ^A	1.32 ± 0.16 ^A
	10	1.06 ± 0.07 ^B	1.03 ± 0.08 ^B	1.05 ± 0.11 ^B	1.20 ± 0.19 ^B	1.46 ± 0.21 ^{AB}
	15	1.16 ± 0.13 ^C	1.13 ± 0.14 ^B	1.11 ± 0.13 ^B	1.29 ± 0.13 ^B	1.55 ± 0.14 ^B
	20	1.56 ± 0.09 ^D	1.39 ± 0.12 ^C	1.33 ± 0.13 ^C	1.27 ± 0.11 ^B	1.75 ± 0.25 ^C
terpineol	5	1.04 ± 0.07 ^A	0.99 ± 0.08 ^A	0.98 ± 0.04 ^A	0.91 ± 0.05 ^A	1.04 ± 0.05 ^A
	10	1.23 ± 0.12 ^B	1.04 ± 0.04 ^A	1.31 ± 0.10 ^B	0.90 ± 0.04 ^A	1.41 ± 0.16 ^B
	15	1.52 ± 0.16 ^C	1.34 ± 0.11 ^B	1.79 ± 0.26 ^C	0.91 ± 0.03 ^A	1.73 ± 0.17 ^C
	20	1.48 ± 0.12 ^C	1.44 ± 0.06 ^C	1.81 ± 0.26 ^C	1.01 ± 0.10 ^B	1.88 ± 0.14 ^D
perillaldehyde	5	0.85 ± 0.03 ^A	0.86 ± 0.07 ^A	0.93 ± 0.05 ^A	0.82 ± 0.04 ^A	0.99 ± 0.08 ^A
	10	0.95 ± 0.04 ^B	0.95 ± 0.09 ^{AB}	1.10 ± 0.09 ^B	0.83 ± 0.04 ^A	1.07 ± 0.09 ^A
	15	0.96 ± 0.05 ^{BC}	1.01 ± 0.10 ^{BC}	1.26 ± 0.16 ^C	0.83 ± 0.03 ^A	1.23 ± 0.13 ^B
	20	1.01 ± 0.08 ^C	1.08 ± 0.13 ^C	1.32 ± 0.24 ^C	0.88 ± 0.06 ^B	1.42 ± 0.11 ^C
eugenol	5	0.98 ± 0.04 ^A	0.96 ± 0.04 ^A	0.96 ± 0.04 ^A	0.91 ± 0.06 ^A	1.14 ± 0.09 ^A
	10	1.01 ± 0.02 ^{AB}	1.05 ± 0.03 ^B	1.11 ± 0.13 ^B	0.90 ± 0.05 ^A	1.23 ± 0.09 ^A
	15	1.09 ± 0.10 ^B	1.11 ± 0.03 ^B	1.28 ± 0.18 ^C	0.92 ± 0.03 ^A	1.50 ± 0.09 ^B
	20	1.26 ± 0.14 ^C	1.27 ± 0.11 ^C	1.50 ± 0.17 ^D	1.03 ± 0.10 ^B	1.79 ± 0.17 ^C
linalool	5	0.96 ± 0.08 ^A	0.94 ± 0.07 ^A	0.95 ± 0.07 ^A	0.87 ± 0.09 ^A	1.13 ± 0.08 ^A
	10	1.06 ± 0.08 ^B	1.05 ± 0.04 ^B	1.00 ± 0.10 ^A	0.89 ± 0.05 ^A	1.24 ± 0.15 ^A
	15	1.08 ± 0.07 ^B	1.13 ± 0.08 ^{BC}	1.03 ± 0.12 ^A	0.88 ± 0.04 ^A	1.49 ± 0.27 ^B
	20	1.12 ± 0.10 ^B	1.07 ± 0.06 ^C	1.17 ± 0.13 ^B	0.86 ± 0.06 ^A	1.59 ± 0.18 ^B
citronellal	5	0.83 ± 0.05 ^A	0.85 ± 0.04 ^A	0.83 ± 0.04 ^A	0.84 ± 0.03 ^A	1.11 ± 0.11 ^A
	10	0.86 ± 0.04 ^A	0.83 ± 0.03 ^A	0.84 ± 0.03 ^A	0.86 ± 0.05 ^A	1.16 ± 0.14 ^{AB}
	15	0.84 ± 0.04 ^A	0.84 ± 0.03 ^A	0.84 ± 0.02 ^A	0.88 ± 0.04 ^{AB}	1.28 ± 0.08 ^{BC}
	20	0.86 ± 0.05 ^A	0.86 ± 0.02 ^A	0.84 ± 0.02 ^A	0.92 ± 0.08 ^B	1.38 ± 0.11 ^C

^a Zone of inhibition is expressed as the diameter in centimeters. Values represent means ± standard deviations of inhibition zones from four experiments, each using duplicate plates. Within each column and for each compound, means having the same superscripts (A, B, and C) are not significantly different ($P > 0.05$) from each other. ^b Carvacrol was dissolved in 2% Tween 20. This compound at 15 and 20% did not dissolve in 2% Tween 20 and was therefore not tested. ^c The control (1 or 2% Tween 20) showed a zone of inhibition of about 0.7–0.8 cm for *L. monocytogenes* Scott A. For the other tester strain, the control showed no zone of inhibition.

of a 16 h culture in TSB was estimated by comparing the absorbance at 540 nm, using a Beckman DU-40 spectrophotometer (Fullerton, CA), after dilution with TSB and calibration to a standard curve. The concentration was then adjusted to 4×10^8 colony forming units (CFU)/mL using Butterfield's phosphate buffer (pH 7.2), and a 100 μ L aliquot was evenly spread on agar plates using a glass rod spreader. The plates were left at room temperature for 20 min to allow the agar surface to dry. Sterilized filter paper disks (Whatman No. 1 filter paper, 0.6 cm in diameter) were arranged on the plate, and a 10 μ L aliquot of test solutions was added to the paper disks. Five disks were arranged on a plate: two each for 5 and 20% concentrations of the test chemical and one for the control (or two each for 10 and 15% concentrations of the test chemical and one for control). Only two disks were placed on the plate for testing with *V. vulnificus* (one for control and the other for test chemical at any concentration). After the plates were incubated at 35 °C for 24 h, the diameters of the distinctly clear zones were measured using a metric ruler with the aid of a Darkfield Quebec colony counter (American Optical Co., Buffalo, NY). Four disks were used for each test compound at each concentration. The zone of inhibition experiment was repeated four times, and the data were statistically analyzed using Duncan's multiple range test (Duncan, 1955).

Determination of MIC and MBC. The MIC of the test compounds was determined using the broth dilution method. To each duplicate 50 mL Erlenmeyer flask containing 19.6 mL of sterile TSB was added a 200 μ L aliquot of bacterial suspension at 10^6 CFU/mL and a test compound preparation to give a final concentration of 100, 250, 500, or 1000 μ g/mL. Test chemicals showing inhibitory effect at 100 μ g/mL were retested at 25, 50, and 100 μ g/mL. Since most of the test chemicals were poorly soluble at above 1000 μ g/mL in TSB, they were not tested at concentrations above that level.

Flasks were then incubated with shaking at 140 oscillations/min in a 35 °C water bath. At 0, 6, 12, 18, 24, and 36 h, a 1.0 mL aliquot was drawn from each flask and turbidity at 540 nm measured. Duplicate flasks were run for each compound at each concentration. The lowest concentration at which no growth occurred in either flask was taken as the MIC. After the MIC was determined, the flask showing no increase in turbidity at each time interval (0, 6, 12, 18, 24, and 36 h) was streaked on appropriate agar plates to check bacterial growth/survival. The MBC was the lowest concentration at which the test compound killed the bacteria. This experiment was conducted twice with duplicate samples for each compound at each test concentration.

RESULTS AND DISCUSSION

Antibacterial Activity Using Paper Disk Assay.

The 11 test compounds showed various degrees of inhibition against the five bacterial strains using the paper disk assay. Except for nerolidol, limonene, and β -ionone, the assay results related to the other eight compounds are shown in Table 1. Carvacrol and geraniol showed a dose-related increase in zone of inhibition against all five tester strains, while citral showed a similar effect only against *E. coli* O157:H7, *S. typhimurium*, *L. monocytogenes*, and *V. vulnificus*. For each of these compounds with the tester bacteria, the zone of inhibition was significantly different ($P < 0.05$) from each other between the lower and higher concentrations (Table 1).

Terpineol, perillaldehyde, and eugenol showed dose-related increases in zone of inhibition against the five

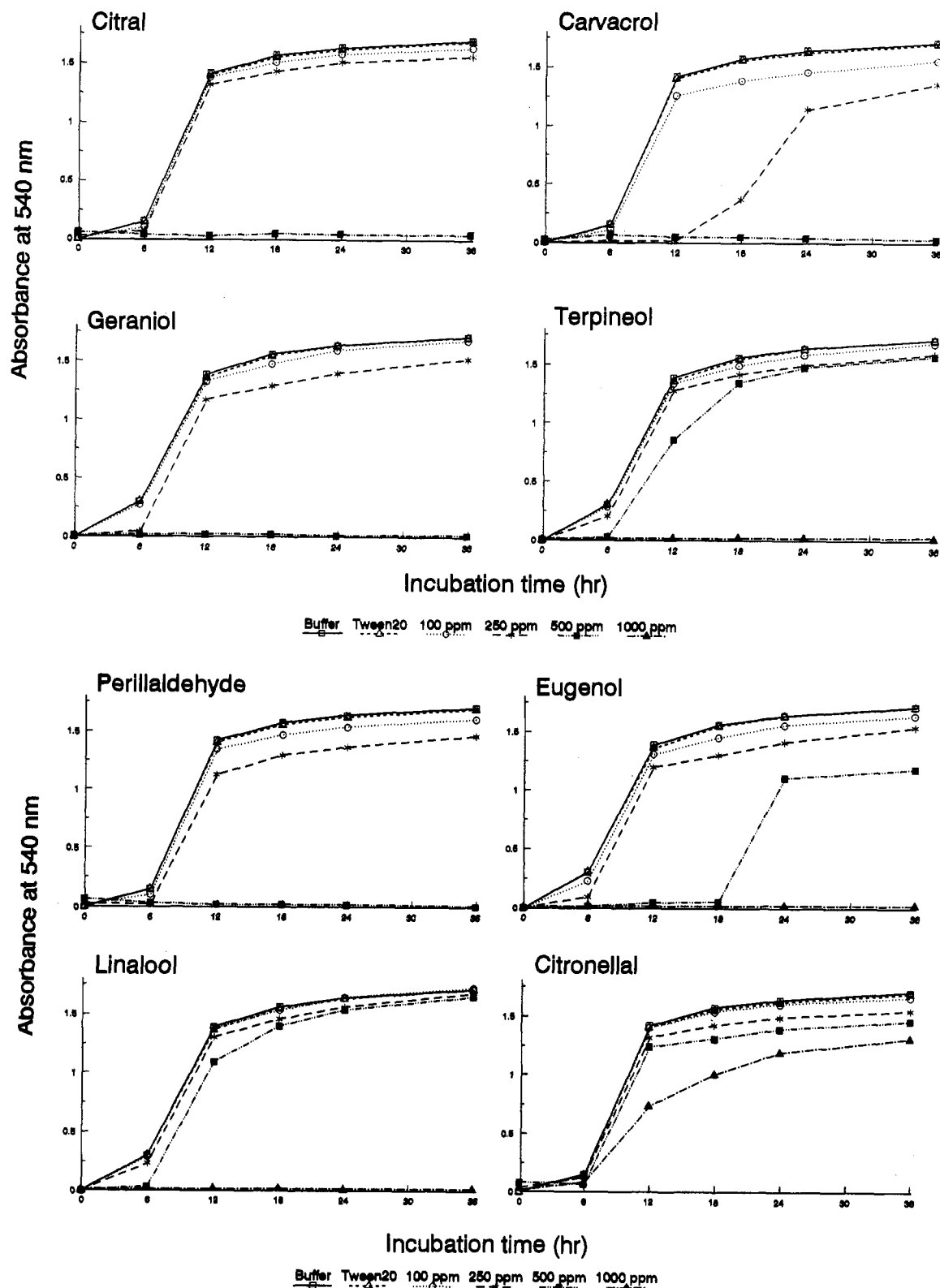


Figure 1. Time-related growth of *E. coli* O157:H7 in tryptic soy broth in the presence of eight essential oil constituents.

strains, while linalool showed a similar effect against *E. coli*, *E. coli* O157:H7, *S. typhimurium*, and *V. vulnificus* and citronellal against *L. monocytogenes* and *V. vulnificus* (Table 1). Again, the zones of inhibition for these five compounds between the lower and higher concentrations were significantly different from each other ($P < 0.05$). Linalool also inhibited the growth of *L. monocytogenes* on agar plates, and citronellal inhibited *E. coli*, *E. coli* O157:H7, and *S. typhimurium*. However, the inhibition was not dose-related, and the

difference in zone size between the test concentrations was not significantly different (Table 1).

Nerolidol, limonene, and β -ionone did not show any inhibition against *E. coli*, *E. coli* O157:H7, and *S. typhimurium* at the doses tested (data not shown). These three compounds showed a zone of inhibition (0.85 cm) against *L. monocytogenes*; however, the inhibition was not dose-related. Limonene and β -ionone showed a weak but dose-related increase in antibacterial activity against *V. vulnificus* (0.74–0.86 cm for limonene

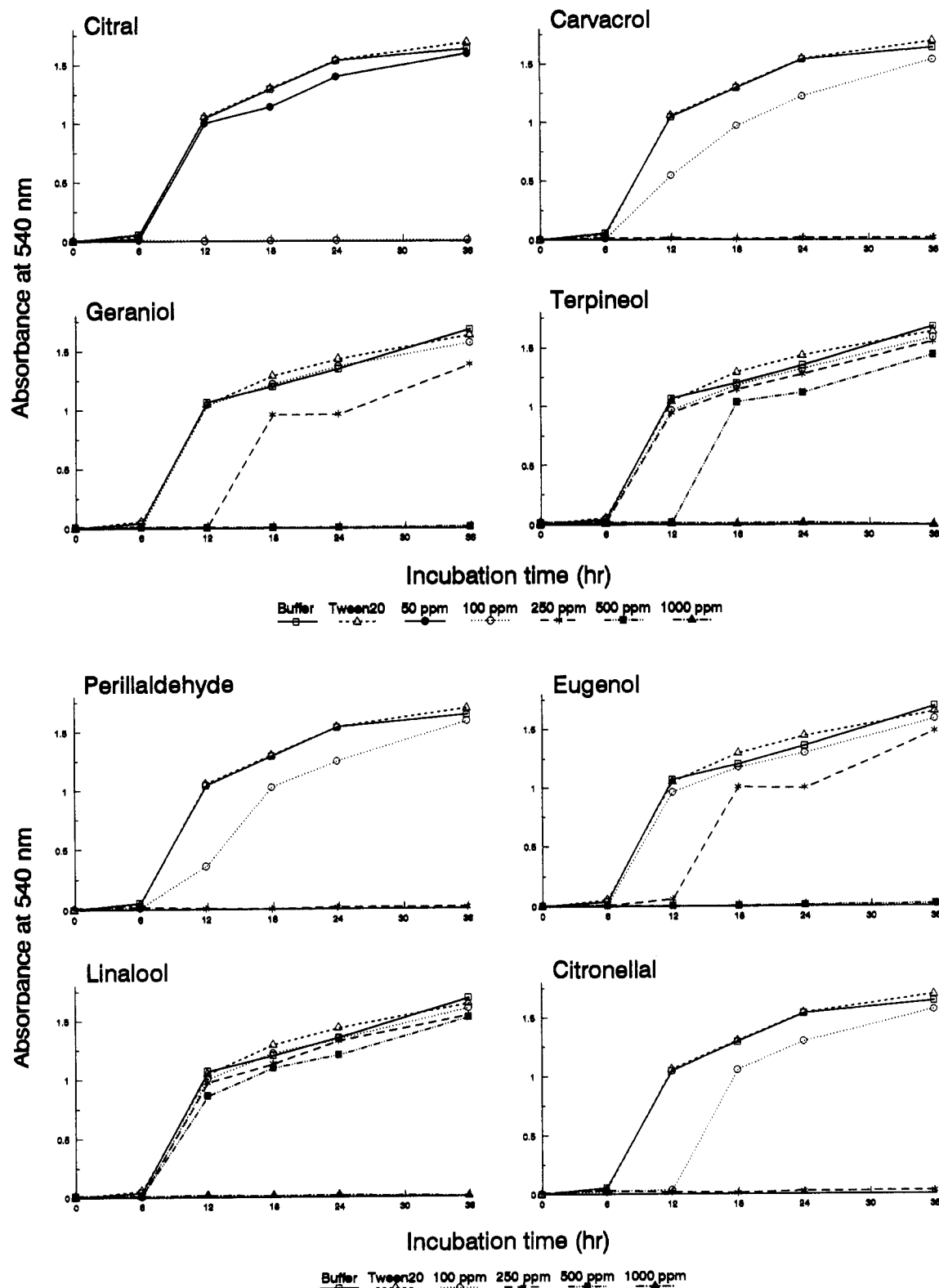


Figure 2. Time-related growth of *V. vulnificus* in tryptic soy broth in the presence of eight essential oil constituents.

and 0.85–0.93 cm for β -ionone). The difference in the size of the zones between the lower and higher concentrations was significantly different ($P < 0.05$). Nerolidol was not inhibitory against *V. vulnificus* (data not shown).

Overall, carvacrol was the most potent inhibitor against the five tester bacteria using the disk method. This compound at 5% had the greatest zone of inhibition against *E. coli* (1.26 cm), *E. coli* O157:H7 (1.20 cm), *S. typhimurium* (1.37 cm), *L. monocytogenes* (0.98 cm), and

V. vulnificus (1.86 cm), while *V. vulnificus* was the most susceptible microorganism for most of the test compounds except nerolidol. For other bacterial strains, the resistance was dependent on the compounds tested. Each bacterial strain responded differently to the various essential oil constituents. Thus, the essential oil constituents may exhibit different modes of action against the bacterial strains. These include (1) interference with the phospholipid bilayer of the cell membrane, causing increased permeability and loss of cellular

Table 2. MIC and MBC of Eight Essential Oil Constituents^a against Five Bacterial Tester Strains

chemical	MIC and/or MBC ($\mu\text{g/mL}$)				
	<i>E. coli</i>	<i>E. coli</i> O157:H7	<i>L. monocytogenes</i>	<i>S. typhimurium</i>	<i>V. vulnificus</i>
citral	500	500	500	500	100
carvacrol	500	500	500	250	250
geraniol	500	500	1000	500	500
terpineol	1000	1000	>1000	1000	1000
perillaldehyde	500	500	1000	500	250
eugenol	1000	1000 ^b	>1000	500	500
linalool	1000	1000	1000 ^b	1000	1000
citronellal	>1000	>1000	1000	1000	250

^a Essential oil constituents were dissolved in 1% Tween 20 and then added in liquid media with test bacteria. ^b The MBCs for eugenol against *E. coli* O157:H7 and for linalool against *L. monocytogenes* are greater than 1000 $\mu\text{g/mL}$.

constituents (Knobloch et al., 1986); (2) impairment of a variety of enzyme systems, including those involved in the production of cellular energy and synthesis of structural components (Conner and Beuchat, 1984); and (3) destruction or inactivation of genetic material.

Antibacterial Activity Using Liquid Culture Assay. Eight essential oil constituents (citral, carvacrol, geraniol, terpineol, perillaldehyde, eugenol, linalool, and citronellal) showing the most potent antimicrobial activity using the paper disk assay were selected for determination of MICs and MBCs. Only the results with *E. coli* O157:H7 and *V. vulnificus* are shown in Figures 1 and 2. The growth patterns of the other microorganisms, although slightly different in TSB, showed trends similar to those of *E. coli* O157:H7 and *V. vulnificus* in the presence of these eight test compounds (data not shown). The growth of *E. coli* O157:H7 was completely inhibited by citral, carvacrol, geraniol, and perillaldehyde at 500 $\mu\text{g/mL}$, by terpineol, eugenol, and linalool at 1000 $\mu\text{g/mL}$, and by citronellal at greater than 1000 $\mu\text{g/mL}$ (Figure 1). Carvacrol at 250 $\mu\text{g/mL}$ and eugenol at 500 $\mu\text{g/mL}$ delayed the lag phase of the growth curve of *E. coli* O157:H7. These compounds at the test concentrations might have killed some of the initial bacterial numbers and then affected the cellular structures or biochemical reactions of the growing bacterial cells. Once the bacteria overcame the inhibitory effect, they multiplied rapidly.

The growth of *V. vulnificus* was completely inhibited by citral at 100 $\mu\text{g/mL}$, by carvacrol, citronellal, and perillaldehyde at 250 $\mu\text{g/mL}$, by eugenol and geraniol at 500 $\mu\text{g/mL}$, and by linalool and terpineol at 1000 $\mu\text{g/mL}$ (Figure 2). Citronellal at 100 $\mu\text{g/mL}$, eugenol and geraniol at 250 $\mu\text{g/mL}$, and terpineol at 500 $\mu\text{g/mL}$ delayed the lag phase of the growth curve for *V. vulnificus*.

Table 2 is the summary of the MIC and MBC values of these eight essential oil constituents with the five bacterial strains. A comparison of the bacteriostatic and bactericidal results of the test compounds showed that, in most cases, the MIC values were the same as the MBCs. The compounds at these levels showed inhibitory effect against bacterial growth by killing the bacteria. Eugenol and linalool are the only two exceptions. Eugenol showed an inhibitory effect against *E. coli* O157:H7 and linalool against *L. monocytogenes* at 1000 $\mu\text{g/mL}$ but failed to kill the bacteria at this concentration. Therefore, the MBC values for these two compounds against these two tester strains were greater than 1000 $\mu\text{g/mL}$.

It seemed that carvacrol and citral had the most potent inhibitory/bactericidal activity against the five bacterial strains, followed by geraniol, perillaldehyde, and eugenol. Carvacrol at 250 $\mu\text{g/mL}$ was highly bactericidal against *S. typhimurium* and *V. vulnificus*.

Citral at 100 $\mu\text{g/mL}$ and perillaldehyde at 250 $\mu\text{g/mL}$ completely killed *V. vulnificus*, while citral, geraniol, and perillaldehyde at 500 $\mu\text{g/mL}$ showed bactericidal activity against *E. coli*, *E. coli* O157:H7, and *S. typhimurium*. Linalool, terpineol, and citronellal had the least bacteriostatic/bactericidal activity against the five tested strains. A MBC value of 1000 $\mu\text{g/mL}$ was determined for these five strains; however, citronellal at 250 $\mu\text{g/mL}$ did kill *V. vulnificus*.

The MIC values obtained for citral (500 $\mu\text{g/mL}$ for *E. coli*, *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*) were similar to that reported by Onawunmi (1989), who demonstrated a value of 0.05% (v/v) against *E. coli*.

The liquid culture method is useful for determining the MIC and MBC of an antimicrobial compound. However, one of the major problems with the use of turbidimetric analysis to determine MIC and MBC values is the minimal range of detection. A bacterial culture with a concentration of 10^6 – 10^7 CFU/mL is always needed for the spectrophotometer to show a meaningful reading. Thus, a bacterial culture with a concentration below 10^5 CFU/mL, and actively growing, is undetected and shows no increase in absorbance using the spectrophotometer. Therefore, periodic sampling of test cultures to determine bacterial survival is needed.

Relationship between Paper Disk Assay and Liquid Culture Assay. The size of the zone of inhibition in the paper disk assay does not accurately reflect the relative antimicrobial effectiveness of the test compounds in liquid culture. Generally, antimicrobial agents with a low bacteriostatic activity against a tested strain will have a high MIC and produce only a small zone of inhibition or no zone at all on agar plates. However, a highly active compound will have a low MIC value and produce a large zone of inhibition. However, in this study, some compounds producing small zones of inhibition had a potent bactericidal activity in liquid culture (i.e., citral against *E. coli* and *E. coli* O157:H7, Tables 1 and 2). On the other hand, some compounds producing large zones of inhibition in agar plates had high MIC values (i.e., terpineol against *E. coli*, *E. coli* O157:H7, and *S. typhimurium*, Tables 1 and 2). Therefore, the bacteriostatic results obtained from paper disk and liquid culture assays were not correlated with zone of inhibition. Furthermore, some compounds showed a potent inhibitory effect against a specific bacterial species at a relatively low concentration but failed to inhibit others at the same test concentration. For example, citronellal completely killed *V. vulnificus* at 250 $\mu\text{g/mL}$, while a concentration of 1000 $\mu\text{g/mL}$ was needed to kill *L. monocytogenes* and *S. typhimurium*. Eugenol at 500 $\mu\text{g/mL}$ completely killed *V. vulnificus* and *S. typhimurium*, while a dose of 1000 $\mu\text{g/mL}$ was needed to kill the other tested strains.

The paper disk assay is a practical approach for screening large numbers of potential antibacterial compounds. However, using the size of inhibition zone to indicate relative antimicrobial activity of the essential oil constituents was not adequate. The zone of inhibition may be affected by the solubility and rate of diffusion of the test compounds in agar medium. Moreover, evaporation of these essential oil constituents can affect the doses applied to paper disks and thus the results. Tween 20 was used in this study to increase the solubility of the hydrophobic compounds and aid in their penetration into bacterial cell wall and membrane.

Essential oil constituents are, in general, more effective inhibitors of fungi than of bacteria (Koedam, 1977; Morozumi, 1978; Zaika, 1988). It is also believed that essential oils are more active against Gram-positive than Gram-negative bacteria (Maruzzella and Sicurella, 1960; Farag et al., 1989; Lemos et al., 1990). However, in this study, *V. vulnificus* (Gram-negative) was the most sensitive to test compounds, while *L. monocytogenes* (Gram-positive) was the most resistant. Thus, there was no discernible trend of inhibition reflected in the type of bacterial strains studied; both Gram-negative and -positive organisms were affected.

CONCLUSION

The results of this study further confirmed the possibility of using plant essential oil constituents as potent antibacterial agents to control foodborne pathogens such as *E. coli*, *E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium*, and *V. vulnificus*. Essential oils capable of preventing the growth of foodborne microorganisms have been well documented (Morris et al., 1979; Shelef et al., 1980; Huhtanen, 1980; Saleem and Al-Delaimy, 1982; Gnan and Sheriha, 1986; Deans and Ritchie, 1987; Karapinar and Aktug, 1987). Subba et al. (1967) showed that orange and lemon oils were inhibitory to a wide range of food spoilage microorganisms. Essential oils of cinnamon, clove, garlic, onion, oregano, and thyme were shown to be inhibitory to selected food spoilage organisms and industrial yeasts (Conner and Beuchat, 1984). Maruzzella and Sicurella (1960) examined the antibacterial activity of 133 essential oils against *Staphylococcus*, *Escherichia*, *Bacillus*, *Streptococcus*, *Salmonella*, and *Mycobacterium* species using zone of inhibition assay. Bullerman et al. (1977) reported that cinnamic aldehyde, the principal component of cinnamon oil, at 150 ppm and eugenol, the principal component of clove oil, at 125 ppm inhibited the growth and toxin production by *Aspergillus parasiticus* in candies and baked goods. The antimicrobial effect of condiments added to fish sausage was believed to be attributed to essential oils (Subba et al., 1967). Dabbah et al. (1970) used orange oil to extend the shelf life of milk. Liquid seasonings (sauce, dressing) containing emulsified essential oils and their components also serve as antimicrobials against food microorganisms. Essential oil components with potent antibacterial activities could also be incorporated in toothpaste, cosmetics, skin ointment, disinfectants, and shampoo.

Although only a limited number of essential oil constituents were evaluated in this study, the results suggest that many of them, especially carvacrol, citral, geraniol, and perillaldehyde, could have the potential to be used in food systems to inhibit the growth of foodborne pathogens. Since these natural compounds occurring in edible and medicinal plants, herbs, and

spices are of GRAS status, the safety concern of using them to control foodborne pathogens is minimal.

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