

Essential Oils as Components of a Diet-Based Approach to Management of *Helicobacter* Infection

G. E. Bergonzelli,* D. Donnicola, N. Porta, and I. E. Corthésy-Theulaz

Nestle Research Center, Lausanne, Switzerland

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An increased density of *Helicobacter pylori* in the gastric mucosa can be associated with more severe gastritis and an increased incidence of peptic ulcers. Therefore, people with asymptomatic gastritis would certainly benefit from a nutritional approach to help them manage the infection and therefore decrease the risk of development of associated pathologies. We analyzed the activities of 60 essential oils against *H. pylori* P1 and identified 30 oils that affected growth, with in vitro inhibition zones ranging between 0.7 and 6.3 cm in diameter. We further analyzed the effects of 16 oils with different activities on *H. pylori* P1 viability. Fifteen showed strong bactericidal activities, with minimal bactericidal concentrations after 24 h ranging from 0.02 to 0.1 g/liter at pH 7.4. Even though slight variations in activities were observed, the essential oils that displayed the strongest bactericidal potentials against *H. pylori* P1 were also active against other *Helicobacter* strains tested. Among the pure constituents of different essential oils tested, carvacrol, isoeugenol, nerol, citral, and sabinene exhibited the strongest anti-*H. pylori* activities. Although oral treatment of *H. pylori* SS1-infected mice with carrot seed oil did not result in significant decreases in the bacterial loads in the treated animals compared to those in the control animals, in all experiments performed, the infection was cleared in 20 to 30% of carrot seed oil-treated animals. Our results indicate that essential oils are unlikely to be efficient anti-*Helicobacter* agents in vivo. However, their effects may not be irrelevant if one plans to use them as food additives to complement present therapies.

Helicobacter pylori infection is extremely common worldwide: more than two-thirds of the world's population is infected with this organism. *H. pylori* is recognized as the major etiological factor in chronic active type B gastritis, gastric ulcers, and gastric cancer (6, 13, 14, 22). The outcome of the infection depends on complex interactions between the bacterium and the host, such as the virulence of the infecting strain, the genetic constitution and age of the host, environmental factors, and dietary habits.

Present treatments for *H. pylori* infections are based on the combination of a proton pump inhibitor and two antibiotics (triple therapy). Antibiotic resistance and noncompliance due to secondary effects are the major causes of eradication treatment failure. There are several ways to decrease treatment failure: by finding new and more potent drugs to kill the bacteria, by developing a vaccine approach to stimulate the host immune defenses (for a review, see reference 11), or by developing new nutritional approaches to the management of the infection. Treatment is justified only for symptomatic patients (20). Thus, people with asymptomatic gastritis would certainly benefit from a nutritional approach aimed at maintaining a low level of infection, since an increased density of *H. pylori* in the gastric mucosa is associated with more severe gastritis and an increased incidence of peptic ulcer (7, 17).

The antimicrobial effects of many herbs and spices have been well known for centuries, and herbs and spices are used to increase the shelf lives of foods. The antimicrobial properties of these kinds of products are attributed to the essential oil

fraction. Strong in vitro evidence indicates that essential oils can act as antimicrobial agents against a wide range of bacteria (for a review, see reference 3). Although essential oils have a broad spectrum of activity, not all of them are able to kill all bacteria. Several studies have been performed to select natural products with anti-*Helicobacter* activities (for a review, see reference 1). However, only a few articles have described the effects of specific essential oils on *H. pylori* growth and viability (4, 5, 10, 12).

The aim of this work was to identify natural essential oils exhibiting strong inhibitory capacities against *H. pylori*. We have examined the anti-*Helicobacter* properties of 60 different commercial essential oils in vitro. We have identified 15 essential oils with strong anti-*Helicobacter* activities and established that the bactericidal activities are enhanced at acidic pH. In a murine model of *Helicobacter* infection, the infection was cleared from 20% of carrot seed oil-treated mice, while in the remaining animals the bacterial loads in their gastric mucosa were comparable to those in untreated animals.

Our results indicate that essential oils may be envisaged as food additives to complement present therapies.

MATERIALS AND METHODS

Essential oils. Essential oils were obtained from a number of commercial sources (Table 1) and kept at 4°C. The high-pressure liquid chromatography profiles of different essential oils were obtained from the suppliers. The essential oils were tested diluted in 100% ethanol (EtOH) or 100% propylene glycol (PG).

Bacteria. The *H. pylori* strains used in the study were *H. pylori* P1 (2), isolated from a patient with nonulcer dyspepsia (reference strain); *H. pylori* Ly4, isolated from a patient with a family history of gastric cancer; *H. pylori* 1172 (ampicillin and metronidazole resistant); *H. pylori* 2234 (*cag* negative); *H. pylori* 2322.7 (*cag* positive); *H. pylori* ATCC 43504; and two mouse-adapted strains, *H. pylori* P49 (H. Kleantous, T. Tibbits, T. J. Bakios, K. Georgopoulos, G. Myers, T. H. Ermak, J. Fox, and T. Monath, *Gut* 37:A94, 1995 [abstract]) and *H. pylori* SS1

* Corresponding author. Mailing address: Nestle Research Center, P.O. Box 4, CH-1000 Lausanne 26, Switzerland. Phone: 41 21 785 80 44. Fax: 41 21 785 85 44. E-mail: gabriela.bergonzelli@rdls.nestle.com.

TABLE 1. Effects of essential oils on *H. pylori* growth^a

Supplier	Essential oil	PG			EtOH		
		Inhibition zone (cm)		n	Inhibition zone (cm)		n
		Mean	Range or SD		Mean or value	Range	
Laboratoire Monique Remy	Cinnamon bark (<i>Cinnamomum zeylanicum</i>)	4.5	0.50	2	6.3	0.05	2
Adrian	Lemongrass (<i>Cymbopogon citratus</i>)	3.2	0.70	2	2.3	0.00	2
Purescence	Vervein (<i>Lippia citriodora</i>)	2.9	0.20	2	≤0.6		2
Sunkist Growers, Inc.	White grapefruit (<i>Citrus paradisi</i>) 1	2.9	0.25	2	1.7		1
Purescence	Clove leaf (<i>Eugenia caryophyllus</i>)	2.5	0.50	2	ND		
Purescence	Savory (<i>Satureja montana</i>)	2.5	0.50	3	1.3		1
Purescence	Manuka (<i>Leptospermum scoparium</i>)	2.3	0.30	3	ND		
Food Ingredients Specialities	Pine (<i>Abies maritima</i>)	2.2	0.20	2	1.4		1
Laboratoire Monique Remy	Cypress (<i>Cupressus sempervirens</i>)	1.9	0.35	2	1.1		1
Purescence	Oregano (<i>Origanum vulgare</i>)	1.9	0.40	3	GI		1
Adrian	Red thyme (<i>Thymus zygis</i>)	1.9	0.05	2	ND		
Sunkist Growers, Inc.	Lemon (<i>Citrus limonum</i>)	1.6	0.00	2	1.4		1
Purescence	Thyme (<i>Thymus vulgaris</i>)	1.5	0.50	3	1.2		1
Adrian	Juniper (<i>Juniperus communis</i>)	1.4	0.05	2	1.0	0.1	2
Food Ingredients Specialities	Clove bud (<i>Eugenia caryophyllus</i>)	1.3	0.25	2	ND		
Purescence	Grapefruit (<i>Citrus paradisi</i>)	1.3	0.05	2	GI		1
Pierre Chauvet S.A.	Orange blossom Morocco (<i>Citrus aurantium</i>)	1.2	0.00	2	1.6	0.00	2
Adrian	Caraway (<i>Carum carvi</i>)	1.2	0.00	2	≤0.6		2
Purescence	Eucalyptus (<i>Eucalyptus globulus</i>)	1.0	0.10	3	1.2		1
Purescence	Sage (<i>Salvia sclarea</i>)	1.0	0.20	2	1.0		1
Adrian	Basil (<i>Ocimum basilicum</i>)	0.9	0.05	2	0.8	0.05	2
Purescence	Tea tree (<i>Melaleuca alternifolia</i>)	0.9	0.03	2	ND		
Sunkist Growers, Inc.	Pink grapefruit (<i>Citrus paradisi</i>)	0.9	0.00	2	ND		
Laboratoire Monique Remy	Cistus (<i>Cistus ladanigerus</i>)	0.8	0.00	2	2.0		1
Adrian	Carrot seed (<i>Daucus carota</i>)	0.8	0.05	2	1.6	0.15	2
Sunkist Growers, Inc.	White grapefruit (<i>Citrus paradisi</i>) 2	0.8	0.00	2	ND		
Purescence	Camomile (<i>Matricaria recutita</i>)	0.7	0.00	2	1.5		1
Adrian	Tarragon (<i>Artemisia dracunculus</i>)	0.7	0.00	2	≤0.6		1
Purescence	Sage (<i>Salvia officinale</i>)	0.7	0.00	2	≤0.6		1
Purescence	Sassafras (<i>Sassafras officinale</i>)	0.7	0.00	2	≤0.6		1
Purescence	Anise (<i>Pimpinella anisum</i>)	≤0.6		2	1.2		1
FLUKA 02860	100% EtOH	≤0.6		2	≤0.6		2
FLUKA 82280	100% PG	≤0.6		2	≤0.6		2

^a Five microliters of different essential oils at 100 mg/ml in 100% EtOH or PG or solvents was loaded onto a sterile disk (diameter, 6 mm). The disks were transferred to plates freshly inoculated with *H. pylori* P1. Growth inhibition was measured, and the results are expressed as the mean or the value, according to the number of determinations (*n*). The standard deviations (*n* > 2) or the ranges of variation (*n* = 2) were calculated. GI, partial growth inhibition; ND, not determined; ≤0.6 cm, no inhibition.

(9). All *H. pylori* strains were grown on 3.6% GC agar (Oxoid) plates supplemented with 1% IsoVitaleX and 10% horse donor serum (Inotech) and maintained in a microaerobic atmosphere (85% N₂, 10% CO₂, 5% O₂) at 37°C for 48 h. Bacteria were harvested in brain heart infusion broth supplemented with 0.25% yeast extract (BHI; BioMerieux). The number of bacteria per milliliter was estimated by measuring the optical density at 600 nm (OD₆₀₀; an OD₆₀₀ of 1 is equal to 10⁸ bacteria/ml).

Enteropathogenic *Escherichia coli* (EPEC) strains B74 and B75 strains were grown on standard Luria-Bertani (LB) agar (Difco Laboratories) plates. The bacteria were harvested in LB agar. The number of bacteria per milliliter was estimated by measuring the OD₆₀₀ (an OD₆₀₀ of 1 is equal to 2 × 10⁸ bacteria/ml).

Growth inhibition assays. The equivalent of 2 × 10⁷ *H. pylori* bacteria was spread on GC serum plates. Five microliters of 100 mg of essential oil per ml in 100% EtOH or 100% PG was loaded onto a sterile disk (diameter, 6 mm). The disks were transferred to the agar plates containing *H. pylori*, and the bacteria were further incubated for 48 to 72 h under microaerobic conditions. After the incubation period, the diameters of the growth inhibition zones were measured and were expressed in centimeters.

Serial dilutions of different essential oils were initially made in PG and were further diluted 1/100 in LB agar. The equivalent of 10⁶ *E. coli* cells was added to 1 ml of LB agar (initial OD₆₀₀, 0.005) containing essential oils at concentrations ranging from 0.02 to 1 g/liter. The mixtures were incubated for 24 h at 37°C with shaking at 220 rpm. Growth was then evaluated by measuring the final OD₆₀₀ and by comparing it to the initial OD₆₀₀ and to the OD₆₀₀ of the control (10⁶ *E. coli* cells per ml of LB agar containing PG alone).

Determination of MBCs. The minimal bactericidal concentrations (MBCs) of 16 essential oils and pure compounds in liquid medium were determined. Serial dilutions were initially made in PG, and the dilutions were further diluted 1/100 in BHI containing 10⁷ *H. pylori* cells. Controls were made by incubating 10⁷ bacteria in the presence of PG diluted 1/100 in BHI. After 1 or 24 h of incubation under microaerobic conditions with mild shaking, the bacteria were pelleted, the supernatants were discarded, and different dilutions of the pellets were plated on GC agar. After incubation for 3 to 5 days, the number of viable bacteria was determined by counting the number of CFU. The MBC is defined as the lowest concentration at which bacteria are killed (no growth in subculture).

Animal studies. Pathogen-free, female C57BL/6 mice (age, 6 to 8 weeks; Iffa-Credo) were allowed free access to food and water during the entire experiment. Mice were inoculated once by gavage with 10⁶ *H. pylori* SS1. Inoculations were performed by gastric intubation with a needle while the mice were under light anesthesia with halothane (Halocarbon Laboratories). Twelve days after inoculation, groups of 10 mice each received either 20 μl of carrot seed essential oil in PG (1/1; 2.5 μg/g of body weight) or 50% PG orally twice a day for 14 days. After the mice were killed, the *H. pylori* infection level was determined by counting the number of CFU present in stomach homogenates. In other sets of experiments, the carrot seed oil was delivered orally as described above and in the drinking water (2 g/liter) for 16 days, including 2 days before *H. pylori* inoculation.

The present study was approved by the State Veterinary Office (authorization no. 1360).

Statistical analysis. The Mann-Whitney U test was used for the analysis of *H. pylori* infection.

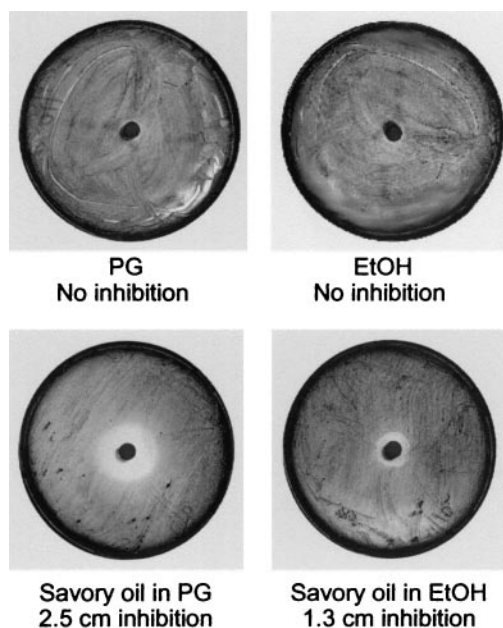


FIG. 1. Disk inhibition assay. Five microliters of 100 mg savory essential oil in 100% EtOH or PG per ml was loaded onto a sterile disk (diameter, 6 mm). The solvents alone were used as controls. Disks were transferred to plates freshly inoculated with *H. pylori* P1. Growth inhibition was measured and is expressed in centimeters.

RESULTS

Disk inhibition assay of *H. pylori* growth. Sixty different essential oils were tested by the disk inhibition assay for their capacities to inhibit *H. pylori* P1 growth in vitro. Figure 1 depicts the results of a typical experiment in which the effect of savory oil was monitored in two different solvents, PG and EtOH, and estimated by measuring the diameter of the zone corresponding to the area of *H. pylori* growth inhibition. The inhibition zone was 2.5 cm for 10% savory oil in PG, whereas it was 1.3 cm for the oil in EtOH, stressing once more the

influence of the solvent on the diffusion of essential oils in agar (3).

Of the 60 essential oils tested, 31 displayed zones of inhibition larger than 0.6 cm (Table 1). Most essential oils displayed inhibitory activities in both solvents, especially those with the highest inhibitory potentials. The exceptions were vervein and cumin, which showed inhibitory activities when they were diluted in PG but not when they were diluted in EtOH, and anise, which was active when it was diluted in EtOH but not when it was diluted in PG.

Occasional differences in the activity of a given essential oil were observed between different lots from the same supplier (Table 1, white grapefruit oils 1 and 2).

Effects of essential oils on *H. pylori* P1 viability. Sixteen essential oils presenting different inhibitory potentials were further tested in liquid medium to determine their effects on *H. pylori* P1 viability. PG was chosen as the solvent for the assay with liquid medium, as most of the essential oils diffused better in PG than in EtOH (Table 1). Different dilutions of the essential oils were incubated with *H. pylori* P1 for 1 or 24 h, and the number of viable bacteria was determined at the end of the incubation period by plating the bacteria and counting the number of CFU. Figure 2 shows the results obtained with four different essential oils, lemongrass, cinnamon bark, vervein, and carrot seed, after 1 h (Fig. 2A) or 24 h (Fig. 2B) of incubation. A marked inhibition of *H. pylori* P1 viability was already observed after 1 h, indicating that the effect is very fast. The most active essential oil was carrot seed oil, with an MBC of 0.5 g/liter. The inhibitory effects of the essential oils were even more pronounced after 24 h of contact, with MBCs ranging between 0.04 g/liter for cinnamon, vervein, and lemongrass oils and 0.02 g/liter for carrot oil.

Table 2 summarizes the MBCs of the 16 essential oils as well as the results obtained by the agar diffusion method. With the exception of eucalyptus oil, all essential oils tested showed strong bactericidal potentials, with MBCs ranging from 0.1 to 0.02 g/liter after 24 h of incubation.

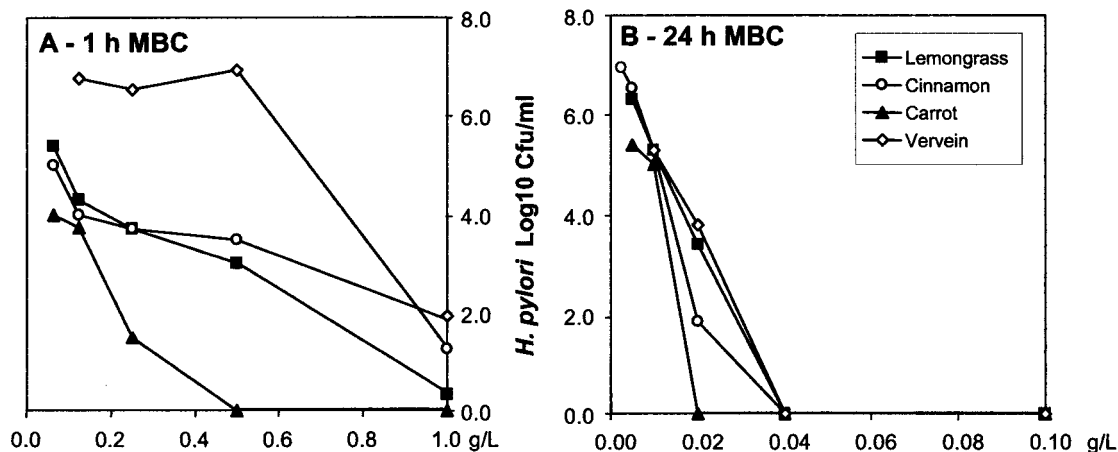


FIG. 2. Determination of MBCs after 1 and 24 h of incubation. Ten microliters of different dilutions of the essential oils were incubated for 1 h (A) and 24 h (B) with 10^7 *H. pylori* P1 cells/ml. Cell viability was assessed by plating and counting the number of CFU. The log CFU per milliliter was plotted as a function of the essential oil concentration (in grams per liter), and MBCs were determined. The results of one representative experiment are shown for each essential oil.

TABLE 2. Effects of essential oils on *H. pylori* viability by MBC determination in liquid medium and comparison with the growth inhibition assay in solid medium^a

Essential oil	MBC (g/liter) in liquid medium at:		Diffusion (cm) in solid medium (PG)
	1 h	24 h	
Carrot seed	0.50	0.02	0.8
Cinnamon bark	1.00	0.04	4.5
Clove	>1.00	0.10	2.5
Caraway	1.00	0.10	1.2
Eucalyptus	>1.00	>0.10	1.0
Pink grapefruit	0.75	0.10	0.9
White grapefruit	1.00	0.10	0.8
Lemongrass	1.00	0.04	3.2
Manuka	0.50	0.04	2.3
Oregano (vulgaris)	1.00	0.04	1.9
Sage	1.00	0.10	0.7
Savory	1.00	0.04	2.5
Tarragon	1.00	0.10	0.7
Thyme (red)	>1.00	0.10	1.9
Thyme (vulgaris)	1.00	0.04	1.5
Vervein	>1.00	0.04	2.9

^a Ten microliters of different dilutions of essential oils was incubated for 1 or 24 h with 10⁷ *H. pylori* P1 cells/ml. Cell viability was assessed by plating and counting the number of CFU. The results are expressed as mean values (n = 2).

Sensitivities of *H. pylori* strains to essential oils. To determine whether other *H. pylori* strains were sensitive to the selected essential oils, seven oils that inhibited *H. pylori* P1 viability were further tested with different *H. pylori* strains from patients presenting with different pathologies or strains with antibiotic resistance. The essential oils that displayed the strongest bactericidal potential against *H. pylori* P1, carrot seed, cinnamon bark, manuka, and savory oils, were also active against the other strains tested (Table 3); the exception was manuka oil, which showed partial inhibition of *H. pylori* ATCC 43504 at the highest concentration tested (i.e., a 4-log reduction in the number of live bacteria compared to that for the control at a concentration of 0.10 g/liter). White grapefruit and clove oils were less active against *H. pylori* P1 as well as against the other strains tested. Eucalyptus oil completely inhibited *H. pylori* 2234 and Ly4 (MBCs, 0.10 g/liter) but did not affect the viability of *H. pylori* P1, ATCC 43504, 1172, 2322.7, P49, or SS1 at a similar concentration.

To determine the selectivities of these seven essential oils, we also tested their inhibitory capacities against two EPEC strains. Neither bactericidal activity against the two EPEC

strains nor inhibition of the growth of the two EPEC strains was observed after 24 h in liquid medium at concentrations as high as 1 g/liter (data not shown).

Effects of pure compounds on *H. pylori* P1 viability. The principal components of the essential oils were then tested for their activities on *H. pylori* P1 viability to trace the ingredients responsible for the observed inhibitory activity. Table 4 shows the MBCs of the compounds at 24 h as well as their relative amounts in seven essential oils. In the case of clove, white grapefruit, and lemongrass oils, the anti-*Helicobacter* activities of the oils are likely due to their principal compounds (more than 70% of the total composition); the MBC of the oil is indeed equal to the MBC of its principal compound (Tables 2 and 4). We were not able to obtain and test the activity of carotol, the principal compound of carrot seed oil, but we may infer that it is the component conferring activity to the oil because the other most abundant compounds of the carrot oil, alpha-pinene (13.30%) and geranyl acetate (10.39%), were less active than the oil itself (Tables 2 and 4). In addition, alpha-pinene is present in eucalyptus oil (18.50%), an oil that was not active against *H. pylori* P1. The savory oil has the same MBC as its principal compound, carvacrol. However, carvacrol constitutes less than 50% of the oil, indicating that other compounds may interact with carvacrol to confer the high level of activity to the oil. Cinnamon oil was used as a positive control, as it has already been demonstrated that the oil and its extracts are active against *H. pylori* (18, 19).

Effect of acidic pH on anti-*Helicobacter* properties of essential oils. To determine the influence of the stomach environment on the anti-*Helicobacter* activities of carrot seed, lemongrass, and white grapefruit oils, the liquid medium assay was performed in parallel under neutral and acidic conditions. Figure 3 depicts the results of a typical experiment in which the effect of carrot seed oil on *H. pylori* P1 viability was monitored. A decrease in the pH from 7.4 to 4.0 resulted in a marked reduction in the minimal concentration required to completely inhibit *H. pylori* P1 cell viability (MBCs, 0.75 g/liter at pH 7.4 and 0.13 g/liter at pH 4.0). The assays performed under acidic conditions were also repeated in the presence of urea to more closely mimic gastric conditions. The addition of urea increased the MBC of carrot seed oil to 0.50 g/liter. The same observations were obtained with lemongrass and white grapefruit oils (Table 5).

In vivo effect of carrot seed oil on *H. pylori* infection. As in vitro studies are not necessarily predictive of clinical activity,

TABLE 3. Sensitivities of *H. pylori* strains to essential oils^a

Essential oil	MBC (g/liter) at 24 h							
	P1	ATCC 43504	1172	2234	2322.7	Ly4	P49	SS1
Carrot seed	0.02	0.04	0.02	0.04	0.02	0.02	0.02	0.04
Cinnamon bark	0.04	0.10	0.10	0.04	0.04	0.04	0.02	0.04
Manuka	0.04	>0.10	0.02	0.04	0.02	0.02	0.01	0.04
Savory	0.04	0.10	0.10	0.04	0.10	0.04	0.04	0.04
White grapefruit	0.10	>0.10	0.10	0.04	>0.10	0.04	0.10	>0.10
Clove	0.10	0.10	>0.10	0.10	>0.10	0.10	>0.10	>0.10
Eucalyptus	>0.10	>0.10	>0.10	0.10	>0.10	0.10	>0.10	>0.10

^a Ten microliters of different dilutions of essential oils was incubated for 24 h with 10⁷ bacteria of each strain per ml. Cell viability was assessed by plating and counting the number of CFU. The results are expressed as two identical values (n = 2).

TABLE 4. MBCs of pure compounds present in essential oils^a

Pure compound name	MBC (g/liter) at 24 h	Content (%) in essential oil						
		Carrot seed	Cinnamon bark	Clove	Eucalyptus	White grapefruit	Lemongrass	Savory
Alpha-copaen	ND			0.15				
Alpha-cubeben	ND			0.15	0.01			
Alpha-pinene	0.10	13.30	0.79		18.50			0.08
Alpha-selinene	ND	2.39						
Beta-caryophyllene	ND	1.91		9.86	0.01	0.35	1.75	1.85
Beta-pinene	0.10	4.40	0.33		0.40			0.58
Beta-selinene	ND	0.14						
Camphor	ND		0.04					0.58
Carotol	ND	34.80						
Carvacrol	0.04		0.10					36.50
Cinnamaldehyde	ND		41.80					
Citral	0.04						72.90	
Citronellal	0.10						0.75	
Decanal	ND					0.30		
Eucalyptol	>>0.10		1.80		62.50			1.00
Eugenol	0.10		23.28	76.60				
Gamma-terpinene	ND		0.03		0.40			1.04
Geraniol	0.10	2.34					6.80	
Geranyl acetate	>>0.10	10.39			1.30		1.40	0.12
Isoeugenol	0.04			0.19				
Limonene	0.10	1.73	0.64		4.00	92.31	3.00	0.28
Linalool	0.10	1.35	5.37		0.01		2.00	0.91
Myrcene	ND	1.61	0.12		0.30	1.86		0.30
Nerol	0.04	1.69						
Nootkatone	ND					0.55		
Octanal	ND					0.15		
para-Cymene	ND	0.98	0.40		0.50			12.00
Sabinene	0.04	3.70	0.06					
Thymol	0.10				0.01			6.30

^a Ten microliters of different dilutions of the principal pure compounds was incubated for 24 h with 10^7 *H. pylori* P1 cells/ml. Cell viability was assessed by plating and counting the number of CFU. The results are expressed as mean values ($n = 2$). ND, not determined.

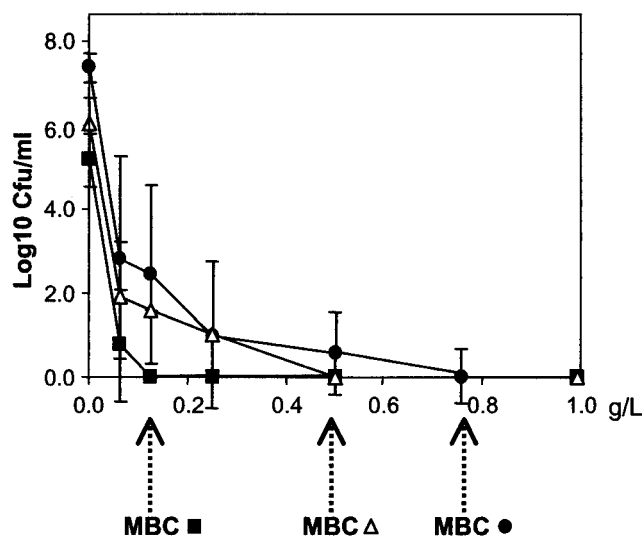


FIG. 3. Influence of pH on the effect of carrot seed essential oil on *H. pylori* urease activity and viability. Ten microliters of different dilutions of the essential oil were incubated with 10^7 *H. pylori* P1 cells/ml at pH 7.4 (●) and at pH 4.0 in the absence (△) and the presence (■) of 10 mM urea. After 1 h of contact, cell viability was assessed by plating and counting the number of CFU. The MBC of the oil was determined at each pH. The results are expressed as means \pm standard deviations ($n = 3$).

we analyzed the capacity of carrot seed oil to diminish *H. pylori* infection in mice. Carrot seed essential oil was administered orally for 14 days to mice that had been inoculated with *H. pylori* SS1. After the mice were killed, the infection level was determined by counting the number of CFU in stomach homogenates. Figure 4 shows the results of two separate experiments. Administration of carrot seed oil to mice did not result in significant decreases in the bacterial loads in the group of treated animals compared to those in the control group ($P = 0.056$). However, no bacteria were recovered from 4 of 20 animals (20%). Interestingly, a comparable level of eradication (25%) was observed in two other independent experiments in

TABLE 5. Influence of pH on MBCs of four different essential oils after 1 h of incubation^a

Essential oil	Mean MBC (g/liter) at 1 h		
	pH 7.4 without urea	pH 4.0 without urea	pH 4.0 with urea
Carrot seed	0.75	0.13	0.50
Lemongrass	1.00	0.13	0.50
White grapefruit	1.00	0.25	0.50

^a *H. pylori* P1 cells were incubated with different dilutions of the essential oil at pH 7.4 and pH 4.0 in the absence and the presence of 10 mM urea. Cell viability was assessed after 1 h by plating and counting the number of CFU. The results are expressed as mean values ($n = 2$ or 3).

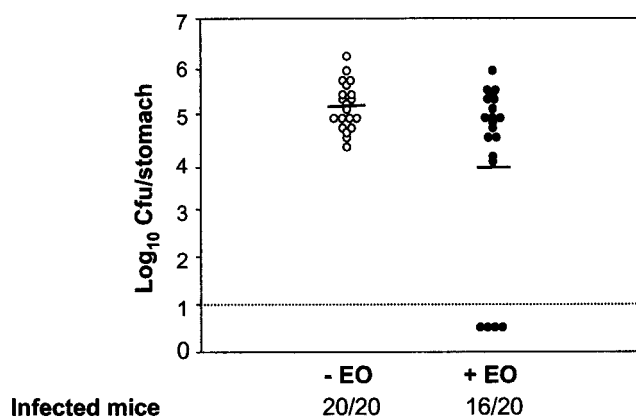


FIG. 4. Treatment of *H. pylori* infection with carrot seed essential oil. Carrot seed essential oil was administered for 14 days to mice inoculated with *H. pylori* SS1. After the mice were killed, the infection level was determined by counting the number of CFU in stomach homogenates. The results of two independent experiments with 10 mice per group are shown. Differences in infection levels between the control groups (not treated with essential oil [- EO]; open circles) and the treated groups (treated with essential oil [+ EO]; closed circles) were evaluated by the Mann-Whitney U test ($P = 0.056$). The dotted line indicates the limit of detection.

which carrot seed oil was added to the drinking water (data not shown).

DISCUSSION

We screened 60 different commercial essential oils for their anti-*Helicobacter* properties by using two different methods, the disk inhibition assay, based on the diffusion of the oil in agar that measured growth inhibition, and a liquid assay that measured bactericidal activity. We have identified 30 essential oils able to inhibit *H. pylori* growth in vitro. We have confirmed that the efficacies of the essential oils in solid medium depend on the carrier used to increase the solubility of the oil components in the medium (3).

Sixteen essential oils presenting different growth-inhibitory potentials were further evaluated for their effects on *H. pylori* P1 viability by the liquid assay. With the exception of eucalyptus oil, all essential oils tested showed strong bactericidal activities. The activities of essential oils that inhibited *H. pylori* P1 viability were further tested for their activities against different *H. pylori* strains. Even though slight variations in activities were observed, the essential oils that displayed the strongest bactericidal potential against *H. pylori* P1 were also active against the other strains. Interestingly, several oils completely inhibited *H. pylori* 1172, a strain resistant to ampicillin and metronidazole, two drugs that are part of present anti-*H. pylori* therapies. Furthermore, none of these essential oils was active against two EPEC strains, suggesting that the inhibitory effect is specifically directed toward *Helicobacter* strains.

Several differences between the levels of growth inhibition and the bactericidal capacities of the essential oils were observed. For example, eucalyptus oil showed a relatively strong growth-inhibitory potential both in PG and in EtOH (Table 1). However, it exhibited no bactericidal activity (Table 2). In addition, carrot seed oil exhibited a much smaller inhibition

diameter than cinnamon bark oil; in contrast, the carrot seed oil MBC (0.02 g/liter) was two times lower than the cinnamon bark oil MBC (0.04 g/liter), demonstrating that carrot seed oil is more active than cinnamon bark oil in liquid culture. These results indicate that it is not possible to predict the results in liquid culture from the results obtained by the disk inhibition assay even when the same solvent is used.

We also observed differences in the activities of essential oils from the same plant but from different suppliers (Table 1, grapefruit oils from Sunkist Growers and Pureissance). These findings are not surprising, since the compositions of essential oils from the same species vary with geographic location and climate. Occasional differences in the activities of different lots of essential oils from the same supplier were also detected, despite similar high-pressure liquid chromatography profiles. Identification and tracing of the active component(s) should allow better standardization of the antimicrobial activity. This is of particular concern when essential oils are incorporated into foods or beverages, as a lack of standardization can compromise the quality of the final product.

In an attempt to identify the active ingredient(s), we tested the effects of several compounds of different essential oils on *H. pylori* viability. The anti-*H. pylori* activities of oils such as carrot seed, clove, white grapefruit, and lemongrass oils may be attributed to their principal ingredients. However, the possibility of synergistic or even antagonistic effects among different compounds cannot be excluded, as such effects were clearly evidenced for the savory essential oil. Most of the ingredients of essential oils belong to the terpenoid family. Terpenoids are weakly to moderately soluble in water, but they readily dissolve in the phospholipid bilayer of biological membranes. It is generally assumed that the antimicrobial activities of terpenoids are due to their ability to disrupt the lipid structure, thus causing a loss of membrane integrity, dissipation of the proton motive force, and impairment of intracellular pH homeostasis (15, 16). This has been particularly well described for the effect of carvacrol on *Bacillus cereus* (21). Specific functional groups of terpenoids can additionally be effective. Among these, phenolic and nonphenolic alcohols revealed the strongest inhibitory activities against several bacteria and fungi, followed by aldehydes, ketones, and finally, pure hydrocarbons (8). In our study, the compounds tested contained different specific groups; but apart from eucalyptol, an oxide, and geranyl acetate, all the others showed inhibitory activities. The compounds that were the most active against *H. pylori* (MBC, 0.04 g/liter) were carvacrol and isoeugenol, two phenolic alcohols; nerol, a nonphenolic alcohol; citral, an aldehyde; and sabinene, a hydrocarbon. Furthermore, eugenol and isoeugenol, two isomers in which the positions of the double bond in the aliphatic chain differ, did not show the same activities, suggesting that passage through the *H. pylori* membrane is conditioned by the conformation of the molecule.

Several studies have been performed to select natural products with anti-*Helicobacter* activities. However, only a few have shown a reduction in the number of bacteria and/or inflammation in vivo (for a review, see reference 1). Recently, it has been established that essential oils obtained from the aerial parts of *Nepeta* subsp. *camphorata* (5), *Mentha piperita* L. and *Mentha spicata* L. (4), and *Allium sativum* L. (12) are able to inhibit the growth and viability of *H. pylori* in vitro. However,

when a 4-mg garlic oil capsule was administered to *H. pylori*-positive dyspeptic patients, no evidence of eradication, suppression, or symptom improvement was found (10), indicating that positive test results obtained in vitro do not allow one to predict the results that will be obtained in vivo.

To determine whether our essential oils could be active in vivo, we first reevaluated the in vitro bactericidal activities of three of them at acidic pH and in the presence of urea to closely mimic gastric conditions. Lowering of the pH resulted in a marked reduction of the MBCs, even in the presence of urea, indicating that the anti-*Helicobacter* potential may be enhanced in the human stomach environment.

The essential oil interventions resulted in the eradication of *H. pylori* from 20 to 30% of the mice and did not significantly decrease the bacterial loads in the oil-treated mice, which did not clear the infection. Thus, it appears that mice either respond to the treatment by eradicating the infection or do not respond, since their infection levels were barely affected. At present, we do not understand why only a subgroup of mice responded to the essential oil interventions, but similar observations have already been reported for drugs. We could not evaluate whether the essential oil had an effect on the *H. pylori*-associated gastritis, since the stomachs of the infected mice did not show any signs of gastric inflammation during the experimental period.

If one compares the efficacies of essential oils to those of the therapies used at present, one might conclude that essential oils are unlikely to be efficient anti-*Helicobacter* agents in vivo. However, their effects may not be irrelevant if one plans to use them as food additives. Indeed, helping a fifth of the *Helicobacter* carriers manage their infections would be a significant achievement. Given the fact that the gastric milieu is more acidic in humans than in mice and that acidic pH enhances the inhibitory potentials of the oils, essential oils might turn out to be more effective in humans than in mice.

The MBC represents an acute effect aimed at killing 2×10^7 bacteria at one time; but when essential oils are present in food, and therefore ingested more than once, the concentrations needed to act on the gastric *Helicobacter* population are likely to be much lower than those necessary to demonstrate an acute effect. On the other hand, one needs to take into account the dilution effect of a bolus in the stomach and the feeding regimen, which may delay the diffusion of the essential oil from the gastric lumen to the mucosa. The normal levels of essential oils used in foods have already been established for the majority of the essential oils selected. However, more precise safety data are required to accurately evaluate the amount of essential oils that could be added in food when prolonged ingestion is envisaged.

Even though further studies are required to prove the efficiency and safety of essential oils in humans, we believe that essential oils may be envisaged as components of a diet-based approach to the clearance of gastric *H. pylori* infection in part of the asymptomatic population or as an adjuvant supplement to increase the efficacies of the present drug therapies.

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