Minimum Inhibitory Concentrations of Herbal Essential Oils and Monolaurin for Gram-Positive and Gram-Negative Bacteria

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Abstract

New, safe antimicrobial agents are needed to prevent and overcome severe bacterial, viral, and fungal infections. Based upon our previous experience and that of others, we postulated that herbal essential oils, such as those of origanum, and monolaurin offer such possibilities. We examined *in vitro* the cidal and/or static effects of oil of origanum, several other essential oils, and monolaurin on *Staphylococcus aureus, Bacillus anthracis* Sterne*, Escherichia coli, Klebsiella pneumoniae, Helicobacter pylori*, and *Mycobacterium terrae*. Origanum proved cidal to all tested organisms with the exception of *B. anthracis* Sterne in which it was static. Monolaurin was cidal to *S. aureus* and *M. terrae* but not to *E. coli* and *K. pneumoniae*. Unlike the other two gram-negative organisms, *H. pylori* were extremely sensitive to monolaurin. Similar to origanum, monolaurin was static to *B. anthracis* Sterne. Because of their longstanding safety record, we concluded that origanum and/or monolaurin, alone or combined with antibiotics, might prove useful in the prevention and treatment of severe bacterial infections, especially those that are difficult to treat and/or are antibiotic resistant.

Article Outline

1. Introduction

The search continues for safe and effective antimicrobial agents with which to treat, therapeutically and prophylactically, a wide variety of bacterial infections. This need has been heightened recently by the emergence of many antimicrobial-resistant organisms like staphylococci [1, 2, 3, 4, 5, 6, 7, 8, and 9] and by the potential use of many hard-to-treat, life-threatening microorganisms as weapons of terrorism. Spores of *B. anthracis*, a potentially antibiotic-resistant organism [10, 11 and 12] were recently used as a mass-casualty-producing weapon [13]. Tuberculosis remains a technical and clinical challenge with drug-resistant forms becoming more prevalent throughout the world [14 and 15].

The best therapeutic antimicrobial agents cause virtually no adverse reactions, have a wide spectrum of activity, and are not likely to encounter resistance to their therapeutic effects. A number of natural products, specifically some essential oils and certain fats (monoglycerides), could possess some of these ideal characteristics.

We recently reported the antifungal activity of herbal essential oils both *in vitro* and *in vivo* [16]. Using *Candida albicans* in broth cultures and a macrodilution method, comparative efficacy of origanum oil, carvacrol (an important phenolic constituent of origanum oil), nystatin, and amphotericin B were examined *in vitro*. Origanum oil at 0.250 mg/ml was found to completely inhibit the growth of *C. albicans* in culture. Growth inhibitions of 75% and >50% were observed at 0.125 mg/ml and 0.0625 mg/ml levels respectively. In addition, origanum oil and carvacrol inhibited both the germination and the mycelial growth of *C. albicans* in a dose-dependent manner. Furthermore, the therapeutic efficacy of origanum oil was examined in an experimental murine model. Groups of six mice infected with *C. albicans* (5 x LD_{50}) were fed various amounts of origanum oil in a final volume of 0.1 ml of olive oil (vehicle). The daily administration of 8.6 mg of origanum oil in 0.1 ml of olive oil/kg body weight for 30 days resulted in 80% survivability with no renal burden from *C. albicans* as opposed to that in the group of mice fed olive oil alone. Results similar to these were obtained with carvacrol. However, mice fed origanum oil exhibited smoother fur and were more active than those treated with carvacrol.

In another study [17], we examined the effects of a variety of essential oils and monolaurin on two *Staphylococcus aureus* strains (ATCC 14154 and 14775), which were not used in the study reported here. In that study, origanum oil was the most potent of the essential oils tested and, in cultures of the two *S. aureus* strains, proved bactericidal at 0.250 mg/ml. *In vitro*, monolaurin's effects mirrored those of origanum oil. The combination of monolaurin and origanum oil was bactericidal at the 0.125-mg/ml

concentration of each. In two separate experiments *in vivo*, intraperitoneal injections of *S. aureus* (ATCC 14775) killed all 14 nontreated mice within a one-week period. In treated mice, more than one-third (6 of 14) survived for 30 days when given oral origanum oil daily. Fifty percent of the mice survived for 30 days when receiving daily vancomycin (7/14) and monolaurin (4/8). More than 60% of mice survived when receiving a daily combination of origanum oil and monolaurin (5/8).

In the present investigation, we assessed *in vitro* the static and/or cidal activity of various essential oils (particularly wild Mediterranean oregano) and the monoglycerides of lauric acid (monolaurin) against gram-positive, gram-negative, and acid-fast microorganisms. We examined *Bacillus anthracis* Sterne, grown from a live veterinary vaccine [10, 11and 12] and *Mycobacterium terrae* [18] as surrogates for virulent *B. anthracis* and *M. tuberculosis*, respectively; in addition, we examined *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Helicobacter pylori*.

2. Methods

2.1. Plant oils and chemicals

Oil of origanum (P73 Oreganol^{TM}), other essential oils, and extra virgin olive oil were provided by North American Herb and Spices, Inc., Waukegan, IL, USA. Monolaurin, the monoglycerides of the 12-carbon fatty acid, lauric acid, was obtained from the Center for Research on Lauric Oils, Bethesda, MD, USA (www.lauric.org), or from Sigma Chemical, St. Louis, MO, USA. Bacteriological media were obtained from Difco Laboratories (Detroit, MI, USA). Antibiotics and all other chemicals used in this

study were obtained from Sigma Chemical Co. and were of analytical grade or the highest commercial grade available. Results on each bacterium were obtained from at least two separate studies.

2.2 Organisms

The organisms studied were: *Staphylococcus aureus* (ATCC 33591), *Bacillus anthracis* Sterne, *Klebsiella pneumoniae*, *Escherichia coli*, *Helicobacter pylori* (ATCC 49503), and *Mycobacterium terrae* (ATCC 15755). *Staphylococcus aureus, H. pylori*, and *M. terrae* were obtained from ATCC, Fairfax, VA, USA, and were grown and maintained in Nutrient Broth (Difco 0003) and on Nutrient Agar (Difco 0001). The strain of *E. coli* was maintained in the laboratory of Dr. Joseph Bellanti at Georgetown University, Washington, DC. Strains of *K. pneumoniae* (AFRRI 7) and *B. anthracis* Sterne, which was derived directly from Anthrax Spore Vaccine, Colorado Serum Company, Denver, CO, were maintained at the Armed Forces Radiobiology Research Institute (AFFRI), Bethesda, MD.

2.3 Susceptibility Testing

A macro-broth-dilution technique was used to determine the susceptibility of the bacteria to oil of origanum, other essential oils as indicated, and monolaurin [16,19 and 20] Susceptibility was expressed as minimum inhibitory concentration (MIC) and/or minimum bactericidal concentration (MBC). The stock solutions of origanum oil, other essential oils, and monolaurin were dissolved in eight parts 50% ethanol and one part Tween 20 solvents. Antibiotics were dissolved in 50% ethanol and used as positive

controls. Solvent controls (addition of carrier without essential oil and/or monolaurin) were also included for reference and additional control.

The Nutrient Broth, which contained logarithmic, serially twofold-diluted amounts of origanum oil, other essential oils, monolaurin, and controls, were inoculated with approximately $5 \cdot 10^5$ cfu of actively dividing bacterial cells or spores. The cultures were incubated for 24 h and 48 h at 30^{O}C on a metabolic rotary shaker (220 rev/min), and the growth was monitored visually and spectrophotometrically (at 540 nm). The MIC was defined as the lowest concentration required to arrest the growth of the bacteria at the end of 24 h of incubation. The MBC was determined by subculturing a 0.01-ml volume of the medium drawn from the culture tubes after 48 h on Nutrient Agar and incubated further for bacterial growth. The growth was scored for relative numbers of the bacterial colonies. The lowest concentration of the antimicrobial agent causing negative growth (fewer than three colonies) was considered the MBC. Since the MIC and MBC were virtually the same, we generally reported only the MBC in the results. The single exception was for *B. anthracis* Sterne. This organism showed only a static response (i.e., MIC) to the test agents.

3. Results

Not all of the six bacteria were examined in the same fashion. Therefore, results for each are described separately (Table 1).

3.1 Gram-Positive Organisms

Staphylococcus aureus: We found that *S. aureus* ATCC 33591 was resistant to streptomycin and penicillin at the concentrations of 0.020 mg/ml and 0.062 mg/ml respectively; but the organism was killed by vancomycin at 0.032 mg/ml. The MBC for oil of origanum was 0.500 mg/ml and for monolaurin was 0.0625 mg/ml. At these concentrations, no growth was seen when the organism was subcultured in brain-heart infusion (BHI) for 24 h. For a combination of oil of origanum and monolaurin, the MBC was 0.0625 mg/ml of each.

Bacillus anthracis Sterne: This organism grew in a concentration of penicillin at 0.062 mg/ml, but it did not grow at concentrations of 0.020 mg/ml of streptomycin or 0.032 mg/ml of vancomycin. No growth was seen in broth at 1.000 mg/ml for bay leaf, 2.000 mg/ml for cumin, 0.500 mg/ml for cassia, and 0.250 mg/ml for oil of origanum. However, all cultures showed growth when subcultured in BHI. Accordingly, the above values represent an MIC. The MIC for monolaurin was 0.0625 mg/ml and the MIC for the combination of oil of origanum and monolaurin was not greater than that for monolaurin alone, that is, the combined concentration of 0.0625 mg/ml of each.

3.2. Gram-Negative Organisms

Escherichia coli: This organism showed no inhibition to pumpkin seed or sage oils at any concentration tested whereas the MBC for myrtle and lavender oils alone was 4.000 mg/ml, for bay leaf was 1.000 mg/ml, and for oil of origanum and cassia was 0.500 mg/ml. *E. coli* grew in the presence of monolaurin at all concentrations tested.

Klebsiella pneumoniae: A concentration of 0.020 mg/ml of streptomycin killed *K. pneumoniae*. In the case of oil of origanum, the MBC was 0.500 mg/ml whereas the addition of monolaurin did not kill *K pneumonia*.

Helicobacter pylori: At a concentration of 0.063 mg/ml of amoxicillin, no *H. pylori* grew on the culture plates. When several herbal essential oils were examined, oil of origanum at a concentration of 0.250 mg/ml prevented growth. The following oils were effective at the concentrations indicated: cassia, cumin, lavender and all spice at 2.000 mg/ml; myrtle and bay leaf at 4.000 mg/ml; and pumpkin seed oil and sage at 8.000 mg/ml. Olive oil did not prevent growth. Monolaurin was cidal at a concentration of 0.0625 mg/ml whereas a combination of 0.0312 mg/ml each of the oils of origanum and monolaurin was cidal.

3.3 Acid-Fast Bacillus

Mycobacterium terrae: The MBC for oil of origanum and monolaurin were, respectively, 0.500 mg/ml and 0.250 mg/ml. When oil of origanum and monolaurin were combined, the MBC was 0.125 mg/ml. Cassia was cidal at 0.500 mg/ml and combining cassia with oil of origanum did not change the MBC of either agent alone; that is, the MBC was 0.500 mg/ml.

Overall, we found that the antimicrobial activity of monolaurin was greater against the two gram-positive bacteria *S. aureus* and *B. anthracis,* against the acid-fast bacteria *M. terra,e* and against the gram-negative bacteria *H. pylori* than it was against

the gram-negative bacteria *K. pneumoniae* and *E. coli*. Oil of origanum essentially achieved the same level of antimicrobial activity against all of the bacteria tested, which was approximately 4 to 8 times less activity than that of monolaurin against the gram-positive bacteria and *H. pylori* but more than monolaurin against the gram-negative bacteria. The combination of the two substances did not consistently augment the effect of either one alone.

4.0 Discussion

Although this study was performed with only one strain of each species, the results indicate the potential inhibition of bacteria by herbal essential oils. The benefits of essential oils to preserve various foods have been known since the days of the explorers sailing to India and the Spice Islands [19]. Subsequently, many essential oils have been found to be effective against many pathogenic organisms [19, 20, 21, 22, 23, 24, 25, 26 and 27]. In recent findings [22], the inhibitory effects of origanum oil, cinnamon, and clove on *Clostridium botulinum* were judged "very active." In addition to its effects on *K. pneumoniae* and *S. aureus*, origanum oil can be fungicidal toward *Candida albicans* [16 and 21]. Also, antiviral actions of origanum and clove oils against RNA and DNA viruses have been reported [28]. As a potential mechanism of action, the outer protective membrane of the viruses, when viewed by electron microscopy, disintegrated after exposure to the origanum oil [28].

Importantly, most essential oils of spices are classified by the U.S. Food and Drug Administration as "generally recognized as safe," indicating that consumers can eat them

without fear. Accordingly, the benefit/risk ratio for essential oils would be high. Other potential advantages of essential oils over antibiotics are that bacteria do not appear to develop resistance to the relatively inexpensive essential oils [21]. However, future rigorous testing is necessary to rule out conclusively the potential development of resistance against essential oils.

Origanum oil is among the essential oils that possess antimicrobial activity. The major components that confer the antibiotic properties are two phenols, carvacrol and thymol [26 and 27]. Phenols are antiseptic substances used commonly in mouthwashes and throat lozenges.

Monolaurin is another natural substance composed of monoglycerides and fatty acids with potential antimicrobial properties [29]. Kabara [30] champions the use of certain lipids as antimicrobials. He measured the optimum antimicrobial activity for fatty acids and their corresponding monoglycerides and reported that the optimum chain length for therapy is 12 carbons (C 12) [31]. Lauric acid (C12) has greater antiviral activity than caprylic acid (C8), capric acid (C10), or myristic acid (C14). In contrast to monolaurin, the dilaurin derivative was inactive. It is now generally accepted that monoglycerides are active whereas diglycerides and triglycerides are inactive against microorganisms [30 and 31].

In one study of several fatty acid esters of polyhydric alcohols, a broth-dilution method was used to determine the MICs effectiveness against gram-negative and gram-

positive organisms [32]. Of the tested compounds, gram-positive organisms were affected to the greatest extent by monolaurin [32]. In general, gram-negative organisms were not affected. Monolaurin is effective in blocking or delaying production of exotoxins by pathogenic gram-positive bacteria [33] and inhibits the synthesis of most staphylococcal and other exoproteins at the level of transcription [34]. Monolaurin also inhibits signal transduction pathways and, thereby, the expression of virulence factors including protein A, alpha-hemolysin, B-lactamase, and toxic shock syndrome toxin 1 in *S. aureus* and the induction of vancomycin resistance in *Enterococcus faecalis* [35 and 36].

When the bactericidal effects of several fatty acids and monoglycerides on *Chlamydia trachomatis* bacteria were studied *in vitro*, the cidal effects appeared to be related to the disruption of the membrane of the elementary body [37]. Corroborating that finding are those of viral studies that suggest the inactivation effects are due to membrane disintegration caused by fatty acids [38, 39, and 40], a finding similar to that in a report of the action of origanum oil on viruses [28].

Concerning gram-negative organisms, the failure of monolaurin against *E. coli* and *K. pneumoniae* was expected, because monolaurin is known to kill primarily gram-positive organisms [29]. However, this study corroborates previous findings [41] showing that monolaurin is exquisitely effective against *H. pylori*, a gram-negative organism that is difficult to culture. Because approximately two-thirds of the world's human population is colonized or infected with this organism, a safe and effective herb-

derived and/or natural fat therapy would provide an alternative to the current antimicrobial therapy [42].

The antimicrobial potential of herbal essential oils and monolaurin could be of great importance for two reasons. First, many antimicrobial agents produced by the pharmaceutical industry have been associated with serious side effects that limit their long-term use. Second, the potential for creating microorganisms that are resistant to antimicrobial agents is a major concern. Hence, the accepted practice is to encourage the use of antimicrobial agents only when necessary to treat infections, thus precluding their prophylactic use under many circumstances. On the other hand, natural products, many of which can be used for long periods, might be less likely to produce side effects, and resistance to natural herbal essential oils has not been shown. Accordingly, medical countermeasures against bioterrorism would benefit especially from the development of a safe and efficacious natural alternative with a broad range of uses.

Results of this study suggested that oil of origanum and monolaurin may be useful either alone or combined with antimicrobial agents to treat bacterial infections. The proven safety of these natural substances support their long-term use and their possible use for prophylaxis.

References

1. U.S. Congress, Office of Technology Assessment. Impacts of antibiotic resistant bacteria, OTA-H-629. U.S. Government Printing Office, Washington DC; 1995.

2. Ayliffe GA. The progressive intercontinental spreads of methicillin-resistant Staphylococcus aureus. Clin Infect Dis 1997; 24, (Suppl 1): S74-S79.

3. Cunha BA. Strategies to control antibiotic resistance. Sem Resp Infect 2002; 17: 250-258.

4. Edmond MB, Wenzel RP, Pasculle AW. Vancomycin-resistant Staphylococcus aureus: perspectives on measures needed for control. Ann Intern Med 1996; 124: 329-334.

5. Waldvogel, W. New resistance in Staphylococcus aureus. N Eng J Med 1999; 340: 556-557.

6. Burnie J, Matthews R, Jiman-Fatami A, Gottardello P, Hodgetts S, D'arcy S. Analysis of 42 cases of septicemia caused by an epidemic strain of methicillin-resistant Staphylococcus aureus: evidence of resistance to vancomycin. Clin Infect Dis 2000; 31: 684-689.

7. Hiramatsu K, Hanake H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. J. Antimicrob Chemother 1997; 40: 135-136.

8. Sieradzki K, Roberts RB, Haber SW, Tomasz A. The development of vancomycin resistance in a patient with methicillin-resistant Staphylococcus infection. N Eng J Med 1999; 340: 517-523.

9. Denis O, Nonhoff C, Byl B Knoop C, Bobin-Dubreux S, Struelens MJ. Emergence of vancomycin-intermediate Staphylococcus aureus in a Belgian hospital: microbiological and clinical features. J Antimicrob Chemother 2002; 50: 383-391.

10. Brook I, Elliott T B, Pryor HI, et al. In vitro resistance of Bacillus anthracis Sterne to doxocycline, macrolides and quinolones. Intern J Antimicrob Agents 2001; 18: 559-562.

11. Brook I, Elliott TB, Harding RA, Bouhaouala SS, et al. Susceptibility of irradiated mice to Bacillus anthracis Sterne by the intratracheal route of infection. J Med. Microbiol. 2001; 50: 702-711.

12. Choe CH, Bouhaouala SS, Brook I, Elliott TB, Knudson GB. In vitro development of resistance to ofloxacin and doxycycline in Bacillus anthracis Sterne. Antimicrob Agents Chemother 2000; 44: 1766.

13. Pile JC, Malone JD, Eitzen EM, Friedlander AM. Anthrax as a potential biological warfare agent. Arch Intern Med 1998; 158: 429-434.

14. Horsburgh Jr CR, Felman S, Ridzon R. Practice guidelines for the treatment of tuberculosis. Clin Infect Dis 2000; 31: 633-639.

15. Remis RS, Jamieson F, Chedore P, Haddad A, Vernich L. Increasing drug resistance of Mycobacterium tuberculosis isolates in Ontario, Canada, 1987-1998. Clin Infect Dis 1998; 31: 427-432.

16. Manohar V, Ingram C, Gray J, et al. Antifungal activities of Origanum oil against Candida albicans. Molec Cell Biochem 2001; 228: 111-117.

17. Manohar V, Ingram C, Gray J, Talpur N, Echard BW, Preuss HG. Antibacterial effects of the edible oil of oregano against Staphlococcus aureus. J Amer Coll. Nutr 2001; 20: 66 (abstract).

18. Griffiths PA, Babb JR, Fraise AP. Mycobacterium terrae: a potential surrogate for Mycobacterium tuberculosis in a standard disinfectant test. J Hosp Infect 1998; 38: 183-192.

19. Kim J, Marshall MR, Wei, C-I. Antibacterial activity of some essential oil components against five foodborne pathogens. J Agric Food Chem 1995; 43: 2839-2845.

20. Carson CF, Cookson BD, Farrelly HD, Riley TV. Susceptibility of methicillin resistant Staphylococcus aureus to the essential oil of Melaleuca alternifolia. J Antimicrob Chemother 1995; 35: 421-424.

21. Schmidt MA, Sehnert KW, Smith LH. Beyond Antibiotics. 50 (Or So) Ways to Boost Immunity and Avoid Antibiotics, North Atlantic Books, Berkeley, CA, 1994.

22. Ismaiel A, Pierson MD. Inhibition of growth and germination of C botulinum 33A, 40B, and 1623E by essential oil of spices. J Food Sci 1990; 55: 1676-1680.

23. Mansour M, Bouttefroy AD, Linder M, Milliere JB. Inhibition of Bacillus licheniformis spore growth in milk by nisin, monolaurin and pH combinations. J Appl Microbiol 1999; 86: 311-324.

24. Kivanc M, Akgul A, Dogan A. Inhibitory and stimulatory effects of cumin, oregano and their essential oils on growth and acid production of Lactobacillus plantarum and Leuconostoc mesenteroides. Intern. J Food Microbiol 1991; 13: 81-86.

25. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999; 86: 985-990.

26. Tucker AO, Maciarello MJ. Oregano: botany, chemistry, and cultivation. In: Charalambus G, ed), Spices, Herbs, and Edible Fungi, Elsevier Science, St Louis, MO. 1994; 439-456.

27. Sivropoulus A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M. Antimicrobial and cytoxic activities of origanum essential oils. J Agric Food Chem 1996; 44: 1202-1205.

28. Siddiqui YM, Ettayebi M, Haddad A, Al-Ahdal MN. Effect of essential oils on enveloped viruses: antiviral activity of oregano and clove oils on herpes simplex virus type 1 and Newcastle disease virus. Medi Sci Res 1996; 24: 185-186.

29. Enig MG. Lauric oils as antimicrobial agents: theory of effect, scientific rationale, and dietary application as adjunct nutritional support for HIV-infected individuals. In: Watson RR, ed. Nutrients and Foods in AI6/14/04 CRC Press, Boca Raton, FL, 1998; 81-97.

30. Kabara JJ. Lipids as host-resistance factors of human milk. Nutr Rev 1980; 38: 65-73.

31. Kabara JJ, Vrable R. Antimicrobial lipids: natural and synthetic acids and monoglycerides. Lipids 1977; 12: 753-759.

32. Conley AJ, Kabara JJ. Antimicrobial action of esters of polyhydric alcohols. Antimicrob Agents Chemother 1973; 4: 501-506.

33. Schlievert PM, Deringer JR, Kim MH, Projan SJ, Novick RP. Effect of glycerol monolaurate on bacterial growth and toxin production. Antimicrob Agents Chemother 1992; 36: 626-631.

34. Projan SJ, Brown-Skrobot S, Schlievert PM. Glycerol monolaurate inhibits the production of B-lactamase, toxic shock syndrome toxin-1, and other staphylococcal exoproteins by interfering with signal transduction. J Bacteriol 1994; 176: 4204-4209.

35. Ruzin A, Novick RP. Equivalence of lauric acid and glycerol monolaurate as inhibitors of signal transduction in Staphylococcus aureus. J Bacteriol 2000; 182: 2668-2671.

36. Ruzin A, Novick RP. Glycerol monolaurate inhibits induction of vancomycin resistance in Enterococcus faecalis. J Bacteriol 1998; 180: 182-185.

37. Bergsson G, Arnfinnsson J, Karlsson SM, Steingrimsson O, Thormar H. In vitro inactivation of Chlamydia trachomatis by fatty acids and monoglycerides. Antimicrob Agents Chemother 1998; 42: 2290-2294.

38. Thormar H, Isaacs CE, Brown HR, Barshatzky MR, Pessolano T. Inactivation of enveloped viruses and killing of cells by fatty acids and monoglycerides. Antimicrob Agents Chemother 1987; 31: 27-31.

39. Isaacs CE, Kashyap S, Heird WC, Thormar H. Anitiviral and antibacterial lipids in human milk and infant formula feed. Arch Dis Childhood 1991; 65: 272-273.

40. Isaacs CE, Thormar H. The role of milk-derived antimicrobial lipids as antiviral and antibacterial agents. Adv Exper Med Biol 1991; 310: 159-165.

41. Petschow BW, Batema RP, Ford LL. Susceptibility of Helicobacter pylori to bactericidal properties of medium-chain monoglycerides and free fatty acids. Antimicrob Agents Chemother 1996; 40: 302-306.

42. CDC: Helicobacter pylori and peptic ulcer disease. http//www.cdc.gov/ulcer/md.htm (entered May 12, 2003).

Table 1. MIC of control antimicrobial agents compared with MIC of herbal essential oils effective against microorganisms.

Microorganism (strain)	**Test drug**	**MIC[a] or MBC[b] (mg/ml)**	**Subculture (growth/ no growth)[c]**
Staphylococcus aureus	Streptomycin	0.020	G
(ATCC 33591)	Penicillin	0.063	G
	Vancomycin	0.032	NG
	Origanum	0.500	NG
	Monolaurin	0.063	NG
	Origanum + monolaurin	0.063	NG
Bacillus anthracis Sterne	Streptomycin	0.020	NG
	Penicillin	0.063	G
	Vancomycin	0.032	NG
	Origanum	0.250	G
	Monolaurin	0.063	G
	Origanum + monolaurin	0.063	G
	Pumpkin seed oil	>8.000	G
	Bay leaf	1.000	G
	Cumin	2.000	G
	Cassia	0.500	G
Escherichia coli Easter	Amoxicillin	0.063	NG
	Origanum	0.500	NG
	Monolaurin	>8.000	NG
	Cassia	0.500	NG
	Bay leaf	1.000	NG
	Myrtle	4.000	NG
	Lavender	4.000	NG
	Sage	>8.000	NG
	Pumpkin seed oil	>8.000	NG
Klebsiella pneumoniae	Streptomycin	0.020	NG
(AFRRI 7)			
	Origanum	0.500	NG
	Monolaurin	>8.000	NG

Helicobacter pylori	Amoxicillin	0.063	NG
(ATCC 49503)			
	Origanum	0.500	NG
	Monolaurin	0.063	NG
	Origanum + monolaurin	0.031	NG
	Cassia	2.000	NG
	Bay leaf	4.000	NG
	Myrtle	4.000	NG
	Lavender	2.000	NG
	Sage	>8.000	NG
	Pumpkin seed oil	>8.000	NG
	All spice	2.000	NG
	Cumin	2.000	NG
	Olive oil	>8.000	NG
Mycobacterium terrae	Streptomycin	0.020	NG
(ATCC 15755)			
	Origanum	0.500	NG
	Monolaurin	0.250	NG
	Origanum + monolaurin	0.125	NG
	Cassia	0.500	NG
	Cassia + origanum	0.500	NG
	Eucalyptus	4.000	NG

[a]MIC = lowest concentration at which microorganisms do not grow in the presence of a test drug

[b]MBC = lowest concentration at which microorganisms do not grow in subcultured test suspension

[c]G = growth of bacteria; NG = no growth of bacteria after 48 h of incubation