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Antimicrobial effects of essential oils in combination with chlorhexidine digluconate

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The aim of the present study was to compare antimicrobial effects of essential oils alone and in combination with chlorhexidine digluconate against planktonic and biofilm cultures of *Streptococcus mutans* and *Lactobacillus plantarum*. The essential oils included cinnamon, tea-tree (*Melaleuca alternifola*), manuka (*Leptospermum scoparium*), *Leptospermum morrisonii*, arnica, eucalyptus, grapefruit, the essential oil mouthrinse Cool Mint Listerine® and two of its components, menthol and thymol. Cinnamon exhibited the greatest antimicrobial potency (1.25–2.5 mg/ml). Manuka, *L. morrisonii*, tea-tree oils, and thymol also showed antimicrobial potency but to a lesser extent. The combination effect of the essential oil–chlorhexidine was greater against biofilm cultures of both *S. mutans* and *L. plantarum* than against planktonic cultures. The amount of chlorhexidine required to achieve an equivalent growth inhibition against the biofilm cultures was reduced 4–10-fold in combination with cinnamon, manuka, *L. morrisonii*, thymol, and Listerine®. We conclude that there may be a role for essential oils in the development of novel anticaries treatments.

Key words: biofilm; chlorohexidine; essential oils; *Lactobacilli*; *Streptococcus mutans*

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Antimicrobial resistance in bacteria raises serious concern for the continued efficacy of antimicrobial agents in medicine, agriculture, and industry (19). With the increase in the prevalence of microbial resistance to conventional antiseptics and antibiotics, attention is now turning to the use of natural antimicrobial compounds (5). The escalating demand for new antimicrobials has prompted several investigations into the antimicrobial effects of phytochemicals extracted from a range of botanic origins, most of which have been used traditionally for many years. Such sources include Goldenseal (*Hydrastis canadensis*) (13), Bakuchiol (*Psoralea corylifolia*) (15), and garlic (*Allium sativum*) (26). Essential oils, the fragrant component of plants, are secondary metabolites that contain substantial concentrations of isoprene compounds (5). The antimicrobial properties of essential oils have been known for many years and have been used against a wide variety of bacteria and fungi (14), including oral pathogens (11, 29, 33). Essential oils have been formulated into several over-the-counter oral hygiene products and the efficacy of the essential oil-containing mouthrinse, Listerine® has been reported since the 1890s (18).

Chlorhexidine is one of the most widely used biocides in antiseptic products, in both hand washing and oral products and as a disinfectant and preservative (17). Chlorhexidine is regarded as the 'gold standard' antiplaque treatment and is particularly effective against gingivitis and widely used as an adjunct treatment for periodontitis (28). However, there are side-effects to chlorhexidine treatment such as an objectionable taste, tooth discoloration, and desquamation and soreness of the oral mucosa (28). Its activity is pH dependent and is greatly reduced in the presence of organic matter (17). Recent reports have suggested that chlorhexidine was ineffective against dental caries in clinical trials (7), and it has been implicated as the potential cause of the selection and persistence of bacteria with low level antibiotic resistance (17, 27).

Preliminary research in this laboratory indicated that the oil extracted from *Leptospermum scoparium* (manuka), a native New Zealand gymnosperm (Myrtaceae), may have the ability to potentiate the antimicrobial effects of essential oils, including thymol. The aim of this study was first to assess the antimicrobial effects of a range of essential oils and a mouthrinse containing essential oils (Cool Mint Listerine®) against two important

cariogenic bacteria, *Lactobacillus plantarum* and *Streptococcus mutans*, in particular against their biofilm growth. Secondly, we sought to evaluate the effect of combining these essential oils and mouthrinse with chlorhexidine with a view to reducing the concentration of chlorhexidine necessary to achieve a particular level of growth inhibition against these bacteria.

Material and methods

Bacterial strains and growth conditions

L. plantarum SA-1 and *S. mutans* ATCC 25175 were grown in TSBYK medium containing (per liter): 15 g tryptic soy broth (Beckton, Dickinson and Co., Sparks, MD); 18.5 g brain heart infusion (Difco; Detroit, MI); 10 g yeast extract (Difco) and 5 mg hemin (Sigma Chemical Co., St. Louis, MO) autoclaved and supplemented with Vitamin K (4 μg/ml final concentration) (34). All cultures of *L. plantarum* were incubated aerobically. *S. mutans* in a 10% CO_2 in air atmosphere at 35°C. *L. plantarum* was kindly supplied by Dr. S. Socranksy and Dr. A. Haffajee from the Forsyth Institute, Boston, MA.

Essential oils

The essential oils (essential oils) were chosen based on documented and/or anecdotal antimicrobial effects. The *Leptospermum morrisonii* oil and the East Cape manuka oil (*L. scoparium*) were provided by Dr. John van Klink (New Zealand Institute for Crop and Food Research, Ltd, Dunedin, New Zealand) and the latter oil was originally sourced from Tairawhiti Pharmaceuticals (Te Araroa, New Zealand) (8). Unless stated, the other essential oils were sourced from Aromatic and Herbal Oils Ltd. (Sydney, Australia). The density of cinnamon, tea-tree, manuka, *L. morrisonii*, arnica, eucalyptus, and grapefruit oils was calculated by weighing 50 μl of oil at room temperature (20°C) and prepared as 40 mg/ml stock solutions in 2% twice-filter sterilized ethanol (0.22 μm Minisart filter, Sartorius Goettingen, Germany) the highest ethanol concentration used in the assay was 0.5%, which did not affect bacterial growth. The essential oil-containing mouthrinse, Cool Mint Listerine® (Listerine®), and two of its essential oil components, thymol and menthol (Sigma Chemical Co.) were also evaluated for antimicrobial activity. Thymol and menthol are present in Listerine® at concentrations of 0.64 and 0.42 mg/ml, respectively, and were prepared as tinctures in 2% twice-filter sterilized ethanol. Chlorhexidine digluconate (chlorhexidine; Sigma Chemical Co.) was prepared as a 40 μg/ml stock solution in twice-filter sterilized water. All experiments were independently repeated a minimum of three times and duplicate samples were performed for each test concentration. The mean data are presented and where appropriate ± SE given. The amount of chlorhexidine in the essential oil–chlorhexidine combinations was analyzed for significance using a two-sample *t*-test at 95% confidence interval with SPSS (v12.0.1 for Windows, SPSS Inc., Chicago, IL).

Antimicrobial determination of essential oils alone or in combination with chlorhexidine against planktonic and biofilm cultures

Inhibition of planktonic growth was determined using a microdilution assay in sterile 96-well microtiter plates (Sarstedt, Sarstedt Australia Pty Ltd, Ingle Farm, Australia) (25). The essential oils cinnamon, manuka, *L. morrisonii,* tea-tree, arnica, eucalyptus, and grapefruit were tested at concentrations ranging from 10 mg/ml to 0.25 mg/ml; Cool Mint Listerine® at concentrations ranging from 50% to 1.5%, and the essential oil components thymol at 0.64–0.01 mg/ml, and menthol at 0.42–0.006 mg/ml. Chlorhexidine was tested at concentrations ranging from 10 μg/ml to 0.25 μg/ml. Each well contained 100 μl twofold serially diluted test agent, and in the combination assays 50 μl of essential oil and 50 μl chlorhexidine, 100 μl double strength TSBYK growth medium and 20 μl of an overnight culture of either *L. plantarum* or *S. mutans*, representing approximately 5×10^7 CFU/ml. The controls comprised inoculated growth medium without test agents, and sample blanks in growth medium only. Planktonic growth was estimated spectrophotometrically (A_{595} nm) after 24 and 48 h using a microtiter plate reader (Benchmark, Bio-Rad, Hercules, CA). The plates were shaken in the plate reader for 30 s prior to each reading. The MIC was defined as the minimum concentration of test agent limiting turbidity to < 0.06 A_{595} nm, which represented complete growth inhibition or at least growth inhibition $> 70\%$ compared to growth of the untreated cultures. To assess the presence of a biofilm formed during planktonic growth and inhibition of its formation, the supernatant was gently aspirated and the remaining bacteria adhered to the base of microtiter plate were viewed after staining with 1% alkaline methylene blue (25).

To measure inhibition of biofilm growth, preformed biofilms of each of the test bacteria were established in 96-well microtiter plates by inoculating 20 μl of overnight cultures in each of the wells, except the agent control wells, containing fresh 200 μl TSBYK growth medium and incubated for 24 h under the appropriate atmospheric conditions. The plate was shaken, as above, to remove loosely attached cells and the supernatant removed by gentle aspiration (25). The essential oils cinnamon, manuka, *L. morrisonii,* tea-tree, arnica, eucalyptus, and grapefruit were tested at concentrations of 20–0.25 mg/ml; Cool Mint Listerine® at concentrations of 1.5–50%, the essential oil components thymol at 0.04 −1.28 mg/ml, and menthol at 0.03–0.84 mg/ml. Chlorhexidine was tested at concentrations ranging from 20 μg/ml to 0.6 μg/ml. The test agents (100 μl final volume) that were serially diluted twofold prior to addition to the wells, and fresh double strength TSBYK (100 μl) were added. Measurements of biofilm growth were taken 24 h and 48 h after aspirating the supernatant and preparing biofilm cell suspensions by adding 200 μl of 1 M NaOH, mixing by repeated plunging motion of the pipette (25). The biofilm suspension was then vigorously shaken in the microtiter plate reader for 90 s prior to reading (A_{595} nm). The biofilm MIC was defined as the minimum concentration of test agent that inhibited further growth of the initial biofilm.

To assess whether the action of cinnamon, manuka oils, and chlorhexidine, separately and in combination, was bacteriostatic or bactericidal to biofilms, 20 μl of biofilm culture resuspended in 200 μl sterile water was taken from concentrations equal to or higher than MIC and streaked on TSBYK agar plates supplemented with 5% defibrinated sheep blood (Invitrogen New Zealand Ltd, Auckland, New Zealand) and incubated under the appropriate conditions for 48 h. Growth at concentrations higher than or equal to the MIC indicated bacteriostatic action and no growth indicated bactericidal action.

Within the limits of a twofold dilution scheme a Fractional Inhibition Concentration index (FIC) was estimated for both the planktonic and biofilm cultures for each combination to determine the effect of the combinations (35). Synergy was defined as an FIC index of 0.5. Nonantagonistic interactions were defined as an FIC index of > 0.5 but < 4. Antagonistic interactions were defined as an FIC index of > 4 (35). The FIC index was calculated by the

following: (MIC essential $oil_{combination}$/MIC essential oil_{alone}) + (MIC $chlorhexidine_{combination}$/MIC $chlorhexidine_{alone}$) where MIC represents either the planktonic or the biofilm MIC.

Results

Growth inhibitory effects of the essential oils, Listerine®, and chlorhexidine

The degree of growth inhibition exhibited by the essential oils, Listerine®, and chlorhexidine, as measured by a twofold microdilution assay, depended on species and growth mode (Tables 1 and 2). The planktonic cultures of both *S. mutans* and *L. plantarum* were more susceptible to all the agents tested individually than the biofilm cultures by at least twofold (Tables 1 and 2).

Cinnamon oil demonstrated greatest antimicrobial potency against both planktonic and biofilm growth. The other essential oils – manuka, *L. morrisonii*, tea-tree, and thymol – showed antimicrobial activity but to a lesser extent against biofilm growth (Tables 1 and 2). Biofilm formation during planktonic growth of *S. mutans* and *L. plantarum*, evaluated with MB staining, was prevented in the presence of these oils at the MICs. The other essential oils did not demonstrate antimicrobial effects at test concentrations. At half-strength concentrations, Listerine® was ineffective in inhibiting the biofilm growth of either *S. mutans* or *L. plantarum* more than 50%, although growth inhibition against the planktonic cultures was ~70% against *L. plantarum* and ~80% against *S. mutans*.

With *S. mutans*, the amount of chlorhexidine required to achieve the same or a greater level of growth inhibition as chlorhexidine used alone was reduced in combination with the essential oils cinnamon and *L. morrisonii* ($P < 0.05$) and Listerine®, particularly against the biofilm cultures. A nonantagonistic antimicrobial effect was observed with the chlorhexidine and cinnamon oil combination against both the planktonic (FIC 0.75) and biofilm (FIC 0.62 ± 0.05) cultures (Tables 1 and 2). At the biofilm MIC, chlorhexidine was bacteriostatic only against *S. mutans* biofilm cultures. Cinnamon was bactericidal alone and in combination with chlorhexidine, with the same activity (Tables 1 and 2). An apparent nonantagonistic antimicrobial effect was observed with chlorhexidine and manuka oil against both the biofilm (FIC 0.62 ± 0.3) and planktonic (FIC 1.25) cultures of *S. mutans*. However, manuka alone and in combination with chlorhexidine, even though bactericidal alone and in combination with chlorhexidine, yielded ~45% growth inhibition against the biofilm cultures (Table 2). The activity of *L. morrisonii* was greater than manuka oil and a nonantagonistic effect was achieved in combination with chlorhexidine against the biofilm culture only (FIC 0.68; Table 2). A nonantagonistic antimicrobial effect was observed with chlorhexidine and Listerine® against the biofilm cultures of *S. mutans* (FIC 0.58 ± 0.07) and against the planktonic cultures (FIC 2.25).

With *L. plantarum*, the amount of chlorhexidine required to achieve the same or a greater level of growth inhibition as chlorhexidine used alone was reduced by combination with the essential oils cinnamon and *L. morrisonii* and thymol, and by Listerine® ($P < 0.05$), particularly against the biofilm cultures (Tables 1 and 2). A nonantagonistic antimicrobial effect was observed with chlorhexidine and cinnamon oil against the planktonic (FIC 0.75) and biofilm (FIC 0.63 ± 0.05) cultures (Tables 1 and 2). Both cinnamon and chlorhexidine, alone and in combination, were bactericidal against the biofilm cultures of *L. plantarum* (Table 2). An apparent synergistic antimicrobial and bactericidal effect was observed between chlorhexidine and manuka oil (FIC 0.29 ± 0.04) against the biofilm cultures of *L. plantarum*. This interaction was nonantagonistic against the planktonic cultures (FIC 1.25). Again, manuka alone and in combination with chlorhexidine, though bactericidal, yielded only ~40% growth inhibition against the biofilm cultures (Table 2). The combination of *L. morrisonii* oil and chlorhexidine (FIC 0.5), and Listerine® and chlorhexidine (FIC 0.5), and thymol and chlorhexidine (FIC 0.53 ± 0.03) yielded nonantagonistic antimicrobial effects against the biofilm cultures of *L. plantarum* (Table 2).

Table 1. The inhibitory concentrations of essential oils (EO), Cool Mint Listerine® alone and in combination with chlorhexidine digluconate (CHX) against planktonic cultures of *S. mutans* and *L. plantarum*

	S. mutans			*L. plantarum*		
		MIC combination			MIC combination	
Test agent (unit)	MIC alone	EO	CHX	MIC alone	EO	CHX
Chlorhexidine (μg/ml)	2.5	–	–	2.5	–	–
Cinnamon oil (mg/ml)	1.25	0.62	0.62*	1.25	0.62	0.62*
Manuka oil (mg/ml)	0.62	0.62	0.62*	1.25	1.25	1.25
L. morrisonii oil (mg/ml)	0.62	1.25	1.25	1.25	1.25	1.25
Tea-tree oil (mg/ml)	10	2.5	2.5	10	2.5	2.5
Arnica oil (mg/ml)	> 10	2.5	12.5	> 10	2.5	2.5
Eucalyptus oil (mg/ml)	> 10	2.5	12.5	> 10	2.5	12.5
Grapefruit oil (mg/ml)	> 10	2.5	2.5	> 10	2.5	12.5
Listerine® (%)	50	12.5	5	12.5	6.25	2.5
Thymol (mg/ml)	0.64	0.16	2.5	0.64	0.16	2.5
Menthol (mg/ml)	> 0.42	2.5	2.5	> 0.42	0.1	2.5

The values represent data obtained from three independent assays after 48 h incubation and where appropriate the mean ± SE is given.
* Indicates a reduction in the amount of chlorhexidine required to achieve the same or greater level of growth inhibition compared to chlorhexidine used alone ($P < 0.05$).

Discussion

Under the conditions described, this study has shown that the essential oils, cinnamon and *L. morrisonii*, in particular, and to lesser extent manuka, tea-tree oils, and thymol exhibited growth inhibitory effects against two important cariogenic bacteria, *S. mutans* and *L. plantarum*. They inhibited biofilm formation during planktonic growth and inhibited growth of preformed biofilms.

Combining chlorhexidine with essential oils, in particular with cinnamon, *L. morrisonii*, and Listerine®, and manuka oil at a lower level of inhibition, resulted in a substantial reduction in the amount of chlorhexidine required to achieve the same level of growth inhibition compared to chlorhexidine used alone. The increased antimicrobial activity of the essential oil–chlorhexidine combination was most apparent against biofilm cultures and *L. plantarum*. The combination of cinnamon and chlorhexidine resulted in at least a 10-fold reduction in chlorhexidine concentration needed for equivalent inhibition of both biofilm cultures of *S. mutans* and *L. plantarum*. Although manuka oil demonstrated a lower level of growth inhibition, ~45% against the biofilm cultures, a potential for synergistic interactive effects

Table 2. The inhibitory concentrations of essential oils (EO), Cool Mint Listerine® and chlorhexidine digluconate (CHX) against biofilm cultures of *S. mutans* and *L. plantarum*

	S. mutans			*L. plantarum*		
		MIC combination			MIC combination	
Test agent (unit)	MIC alone	EO	CHX	MIC alone	EO	CHX
Chlorhexidine (μg/ml)	13.3 ± 2.8	–	–	16.7 ± 2.9 [20]	–	–
Cinnamon oil (mg/ml)	2.1 ± 0.4	1.0 ± 0.2 [2.5]	1.0 ± 0.2***** [2.5]	2.5	1.25 [2.5]	1.25***** [2.5]
Manuka oil (mg/ml)	4.2 ± 0.7	1.6 ± 0.4 [1.25]	1.6 ± 0.4 [1.25]	20 [20]	3.3 ± 0.7 [5]	3.3 ± 0.7***** [5]
L. morrisonii oil (mg/ml)	5	2.5	2.5*****	20	3.3 ± 0.7	3.3 ± 0.7*****
Tea-tree oil (mg/ml)	> 20	10	10	> 20	10	10
Arnica oil (mg/ml)	> 20	10	10	> 20	10	10
Eucalyptus oil (mg/ml)	> 20	10	10	> 20	10	10
Grapefruit oil (mg/ml)	> 20	10	10	> 10	10	10
Listerine® (%)	50	12.5	5	50	12.5	5*****
Thymol (mg/ml)	1.28	0.64	10	1.28	0.3 ± 0.05	4.2 ± 0.7*****
Menthol (mg/ml)	> 0.84	10	10	> 0.84	10	10

The values represent data obtained from three independent assays after 48 h incubation and where appropriate the mean ± SE is given.
*****Indicates a reduction in the amount of chlorhexidine required to achieve the same or a greater level of growth inhibition compared to chlorhexidine used alone ($P < 0.05$). Values in parentheses represent the bactericidal concentration. Chlorhexidine alone was bacteriostatic only against biofilm cultures of *S. mutans*.

with chlorhexidine was demonstrated. An eightfold reduction in the chlorhexidine concentration was observed when manuka oil was combined with chlorhexidine. Although growth inhibition was lower than with chlorhexidine alone (50–60%), it yielded the benefit of increased bactericidal effects, since chlorhexidine at the test concentrations was not bactericidal against biofilm cultures of *S. mutans*. Chlorhexidine activity *in vivo* is reported as bacteriostatic (2), and an essential oil–chlorhexidine combination may prove more beneficial in terms of its bactericidal activity.

There have been no reports describing the antimicrobial effects of combining the essential oils cinnamon or manuka oils with chlorhexidine or Listerine® with chlorhexidine. A combination of thymol (a component of Cool Mint Listerine®) with chlorhexidine has been developed as a commercially available varnish, Cervitec® (Vivadent, Schaan, Liechtenstein), which contains 1% w/w chlorhexidine diacetate and 1% w/w thymol. It has been suggested that Cervitec® is specifically active against *S. mutans* and has demonstrated varying degrees of success at treating caries *in vivo* (1, 12). The results presented here indicate that *L. plantarum* is more sensitive to the thymol–chlorhexidine combination than is *S. mutans*.

The antimicrobial effects of cinnamon oil have been demonstrated against important food-borne pathogens including *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enteritidis*, with inhibition concentrations of 30–50 μg/ml against planktonic cultures (32). The antibacterial activity of cinnamon has been attributed to the presence of ~65–75% cinnamaldehyde (22, 23). It has been reported recently that cinnamaldehyde is bactericidal to *Listeria monocytogenes* but, in contrast to our findings with *L. plantarum*, not inhibitory to *Lactobacillus sakei* (10). However, little is known about its antibacterial properties against oral bacteria. The use of honey, in particular *L. scoparium* (manuka), to treat recalcitrant burns and wounds has been documented (4, 20), and its success attributed to the triketone phytochemical components (24). It has been proposed that manuka honey may be effective in the treatment of gingivitis and periodontal disease (9, 21). Preliminary research from our laboratory has indicated that manuka honey demonstrated a species-specific antimicrobial effect against *Candida albicans* in dental plaque microcosms (31). Manuka oil has been used for respiratory ailments and its activity linked to the presence of terpenoid compounds, such as α-terpineol and cineole (3, 8). Manuka oil has exhibited *in vitro* antimicrobial effects against selected oral pathogens (33), although the inhibitory concentration against planktonic cultures of *Streptococcus mutans* JC2 was higher (2.5 mg/ml) than that reported in this study (0.62 mg/ml). This may be linked to growth conditions, strain variation, and the source of the essential oil (8).

As yet, there is a limited number of studies describing the mode of action of many essential oils. Although current *in vitro* data suggests that there is a role for essential oils in treating infections, their efficacy and toxicity need to be evaluated first (16). All of the essential oils used in this study have been incorporated into a range of over-the-counter remedies and for well known essential oils such tea-tree (*Melaleuca alternifolia*) there are reports describing its mode of action (6, 30), and antibacterial activity against select oral pathogens (33). In this study, the inhibitory concentration of tea-tree oil (10 mg/ml) was comparable to activity reported against *S. mutans* and *Lactobacillus* sp. isolated from the human oral cavity (11) and planktonic cultures of *S. mutans* JC2 (33). The antibacterial properties of tea-tree oil (*Melaleuca alternifolia*) have been attributed to the monoterpenoid, terpinen-4-ol, and since monoterpenoids are lipophilic, it is thought to diffuse into and damage cell membrane structures, causing increased fluidity or disordering membrane structure and inhibition of membrane-bound enzymes (30). The principle target of chlorhexidine is the bacterial cytoplasmic membrane, which results in the loss of structural organization and integrity, and coagulation and precipitation of cytoplasmic constituents usually occurs (17). The possible interactive effects observed in this study between the essential oils and chlorhexidine may be linked to the antibacterial activity of each being targeted at cell membranes. Identifying the active components of the essential oils and generating structural analogs may further exploit such activity.

An anticaries product that contained less chlorhexidine but still achieved the same level of antimicrobial potency would be highly desirable. Lowering the effective dose of chlorhexidine with the addition of essential oils may prolong its clinical efficacy and reduce its associated side-effects. This study has indicated a potential role of essential oils in the development of novel anticaries treatments, either alone or in combination with chlorhexidine, and

warrants further investigation. Although the main focus of this study was to assess the antimicrobial effects of essential oils in combination with chlorhexidine against oral pathogens, there may be a role for essential oil–chlorhexidine combinations in inhibiting nonoral microbes, especially given the widespread use of chlorhexidine and the increasing appeal of natural antimicrobials.

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References

1. Baca P, Munoz MJ, Bravo M, Junco P, Baca AP. Effectiveness of chlorhexidine-thymol varnish for caries reduction in permanent first molars of 6- to 7-year-old children: 24-month clinical trial. Community Dent Oral Epidemiol 2002: **5**: 363–368.
2. Beighton D, Decker J, Homer KA. Effects of chlorhexidine on proteolytic and glycosidic enzymes activities of dental plaque bacteria. J Clin Periodontol 1991: **18**: 85–89.
3. Brooker SG, Cambie RC, Cooper RC. Chemistry and pharmacology. In: New Zealand Medicinal Plants. Auckland: Reed 1998: 26–31.
4. Cooper RA, Molan PC, Harding KG. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J Appl Microbiol 2002: **93**: 857–863.
5. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999: **12**: 564–582.
6. Cox SD, Mann CM, Markham JL, Gustafson JE, Warmington JR, Wyllie SG. Determining the antimicrobial actions of tea-tree oil. Molecules 2001: **6**: 87–91.
7. de Soet JJ, Gruythuysen RJ, Bosch JA, van Amerongen WE. The effect of 6-monthly application of 40 % chlorhexidine varnish on the microflora and dental caries incidence in a population of children in Surinam. Caries Res 2002: **36**: 449–455.
8. Douglas MH, van Klink JW, Smallfield BM, Perry NB, Anderson RE, Johnstone P, et al. Essential oils from New Zealand manuka: triketone and other chemotypes of *Leptospermum scoparium*. Phytochemistry 2004: **65**: 1255–1264.
9. English HK, Pack ARP, Molan PC. The effects of manuka honey on plaque and gingivitis: a pilot study. J Int Acad Periodontol 2004: **2**: 63–67.
10. Gill OA, Holley RA. Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. App Environ Microbiol 2004: **70**: 5750–5755.
11. Hammer KA, Dry L, Johnson M, Michalak EM, Carson CF, Riley TV. Susceptibility of oral bacteria to *Melaleuca alternifolia* (tea-tree) oil *in vitro*. Oral Microbiol Immunol 2003: **18**: 389–392.
12. Haukali G, Poulsen S. Effect of a varnish containing chlorhexidine and thymol (Cervitec®) on approximal caries in 13- to 16-year-old schoolchildren in a low caries area. Caries Res 2003: **3**: 185–189.
13. Hwang BY, Roberts SK, Chadwick LR, Wu CD, Kinghorn AD. Antimicrobial constituents from Goldenseal (the rhizomes of *Hydrastis canedensis*) against selected oral pathogens. Planta Med 2003: **69**: 623–627.
14. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem 2003: **10**: 813–829.
15. Katsura H, Tsukiyama R-I, Suzuki A, Kobayashi M. *In vitro* antimicrobial activities against oral micro-organisms. Antimicrob Agents Chemother 2001: **45**: 3009–3013.
16. Martin KW, Ernst E. Herbal medicines for treatment of bacterial infections: review of controlled clinical trials. J Antimicrob Chemother 2003: **51**: 241–246.
17. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action and resistance. Clin Microbiol Rev 1999: **12**: 147–179.
18. Miller WD. The Micro-Organisms of the Human Mouth. Basel: S. Karger, 1973: 225–237.
19. Milner JL, Stohl EA, Handelsman J. Zwittermicin, a resistance gene from *Bacillus cereus*. J Bacteriol 1996: **178**: 4266–4272.
20. Molan PC. Potential of honey in the treatment of wounds and burns. Am J Clin Dermatol 2001: **2**: 13–19.
21. Molan PC. The potential of honey to promote oral wellness. Gen Dent 2001: **49**: 584–589.
22. Morozumi S. Isolation, purification and antibiotic activity of *o*-methoxycinnamaldehyde from cinnamon. Appl Environ Microbiol 1978: **36**: 577–583.
23. Ouattara B, Simard RE, Holley RA, Piette GJ, Begin A. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int J Food Microbiol 1997: **37**: 155–162.
24. Porter NG, Wilkins AL. Chemical, physical and antimicrobial properties of essential oils of *Leptospermum scoparium* and *Kunzea ericoides*. Phytochemistry 1998: **50**: 407–415.
25. Roberts SK, Wei G-X, Wu CD. Evaluating biofilm growth of two oral pathogens. Lett Appl Micobiol 2002: **35**: 552–556.
26. Ross ZM, O'Gara EA, Hill JD, Sleightholme HV, Maslin DJ. Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl Environ Microbiol 2001: **67**: 475–480.
27. Russell AD. Introduction of biocides into clinical practice and the impact on antibiotic resistant bacteria. Symp Ser Soc Appl Microbiol 2002: **31**: 121S–135S.
28. Scheie AA. The role of antimicrobials. In: Fejerskov O, Kidd E, eds. Dental Caries, the Disease and its Clinical Management. Oxford: Blackwell Munksgaard, 2003: 179–188.
29. Shapiro S, Meier A, Guggenheim B. The antimicrobial activity of essential oils and essential oil components towards oral bacteria. Oral Microbiol Immunol 1994: **1**: 202–208.
30. Sikkema J, de Bont JAM, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 1995: **59**: 201–222.
31. Sissons CH, Anderson SA, Molan PC. Suppression of *Candida albicans* in plaque microcosms by manuka honey. J Dent Res 2004: **83** (Spec Iss A): 0974.
32. Smith-Palmer A, Stewart J, Fyfe L. Antimicrobial properties of plant essential oils and essences against five food-borne pathogens. Lett Appl Micobiol 1998: **26**: 118–122.
33. Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antimicrobial efficacies of essential oils against oral pathogens. Oral Microbiol Immunol 2004: **19**: 61–64.
34. Tanner A, Maiden MFJ, Macuch PJ, Murray LL, Kent RL. Microbiota of health, gingivitis, and initial periodontitis. J Clin Periodontol 1998: **25**: 134–144.
35. White RL, Burgess DS, Manduru M, Bosso JA. Comparison of three different *in vitro* methods of detecting synergy. Time-Kill, Checkerboard, and E Test. Antimicrob Agents Chemother 1996: **40**: 1914–1918.