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Short communication

A comparison of the antibacterial efficacies of essential oils against oral pathogens

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Cariogenic bacteria and periodontopathic bacteria are present in dental plaque as biofilms. In this study, we investigated the antibacterial effects of essential oils on the following oral bacteria: *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Streptococcus mutans*, and *Streptococcus sobrinus*. We tested manuka oil, tea tree oil, eucalyptus oil, lavandula oil, and romarinus oil and determined their minimum inhibitory concentration and minimum bactericidal concentration. The essential oils inhibited the growth of the bacteria tested, manuka oil being the most effective. Minimum bactericidal concentration values showed that lavandula oil acts bacteriostatically, and the remaining oils, bactericidally. Periodontopathic bacterial strains tested were killed completely by exposure for 30 s to 0.2% manuka oil, tea tree oil or eucalyptus oil. Tea tree oil and manuka oil showed significant adhesion-inhibiting activity against *P. gingivalis*. All the essential oils tested inhibited the adhesion of *S. mutans*. This study showed that, among the essential oils tested, manuka oil and tea tree oil in particular had strong antibacterial activity against periodontopathic and cariogenic bacteria. From the viewpoint of safety, we also examined the effects of these essential oils on cultured human umbilical vein endothelial cells and found that, at a concentration of 0.2%, they had little effect on cultured cells.

Key words: antibacterial effect; cariogenic bacteria; essential oil; periodontopathic bacteria

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The human oral cavity is inhabited by more than 500 species of bacteria at 10^8–10^9 bacteria per ml saliva or mg dental plaque (19). Caries is a disease caused by the plaque bacteria such as *Streptococcus mutans* and *Streptococcus sobrinus* (5, 10). Gram-negative bacilli such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Fusobacterium nucleatum* have frequently been isolated from periodontal lesions and have been shown to be related to the onset and progression of periodontal disease (12, 23–25, 27, 30). Furthermore, it has been suggested in recent years that oral bacteria are associated with many systemic diseases such as pneumonia and cardiovascular disease (2, 9, 17); therefore, the need for oral care in a systemic health regimen has also been emphasized. Dental plaques that have been deposited firmly as biofilms must be removed mechanically, but antibacterial mouthrinses are effective in decreasing tooth surface plaque. In general, mouthrinses contain fluorides, alcohols, and detergents or antibacterial substances. Ideal antibacterial substances must be effective against more microorganisms, act rapidly, maintain activity at low concentrations, have no side effects, and be usable without causing discomfort. Frequently used antibacterial chemicals include povidone iodine products, chlorhexidine, and cetylpyridinium chloride; in addition, natural antibacterial substances have attracted attention (8, 13, 20). In this study, we compared the antibacterial activities of phytochemical essential oils against oral bacteria.

Leptospermum scoparium (manuka) oil, *Melaleuca alternifolia* (tea tree) oil, *Eucalyptus radiata* (eucalyptus) oil, *Lavandula officinalis* (lavandula) oil, and *Romarinus officilalis* (romarinus) oil were obtained from Laboratoire PhytoSun'Aroms (Ance, France), and used in this study. An oil containing ω 3, 6, & 9 fatty acids was used as a control. The cariogenic bacteria *S. mutans* JC-2, and *S. sobrinus* 6715 and B13, and the periodontopathic bacteria *A. actinomycetemcomitans* strains Y4, ATCC 29523, ATCC 29524 and ATCC 33384, *P. gingivalis* strains ATCC 33277, ATCC 53977, Su63, and W50, and *F. nucleatum*

strains ATCC 25586, #2 and #20, were used in this study. These strains were maintained anaerobically on blood agar plates containing trypticase soy agar (Becton Dickinson Microbiology System, Cockeysville, MD) supplemented with 10% defibrinated horse blood, hemin (5 μg/ml; Sigma Chemical Co., St. Louis, MO) and menadione (0.5 μg/ml; Wako Pure Chemical Industries, Osaka, Japan).

The minimum inhibitory concentrations of the respective essential oils against oral bacteria were determined with liquid cultures in 96-well cell culture plates according to a modification of the method described by Shapiro et al. (22). Todd Hewitt broth (Becton Dickinson Microbiology System) was used for mutans streptococci and *F. nucleatum*. For *A. actinomycetemcomitans*, Todd Hewitt broth supplemented with 1% yeast extract (Difco Laboratories, Detroit, MI) was used. For *P. gingivalis*, trypticase soy broth (Becton Dickinson Microbiology System) containing hemin and menadione was used. Serial dilutions (1.0–0.002%) of each essential oil were prepared in each culture medium. Aliquots (200 μl) of each dilution were dispensed in 96-well cell culture plates (Nunc, Naperville, IL). Subsequently, 10^5–10^6 test bacteria that had been cultured overnight in each culture medium were inoculated into each well, and cultured for 1–2 days under anaerobic conditions. Then the absorbance was measured at 595 nm. The highest dilution at which no growth ($OD_{595} \leq 0.05$) was observed, was defined as the minimum inhibitory concentration. As shown in Table 1, manuka oil effectively inhibited the growth of oral bacteria. The minimum inhibitory concentrations were 0.25% and 0.13% against oral streptococci, and 0.03% against the gram-negative bacteria tested. Tea tree oil and eucalyptus oil had an minimum inhibitory concentration of 1% against oral streptococci, and 0.06–0.5% against the gram-negative bacteria tested. Lavandula oil and romarinus oil inhibited the growth of gram-negative bacteria but did not inhibit the growth of oral streptococci even at 1%. Control oil did not inhibit the growth of any of the bacteria tested in this study at a concentration of 1%. After the measurement of minimum inhibitory concentration, 50-μl aliquots of cultures were taken from wells showing no bacterial growth, inoculated onto blood agar plates, and cultured for 1 week under anaerobic conditions. The concentration at which no bacterial growth was observed was defined as the minimum bactericidal concentration. The minimum bactericidal concentrations of the essential oils except lavandula oil were the same as or 2–4 times the minimum inhibitory concentrations of the respective essential oils (Table 2). However, lavandula oil did not show any bactericidal activity at its minimum inhibitory concentration, suggesting that it acts bacteriostatically. Exposure for 30 s to 0.5% manuka oil, tea tree oil and eucalyptus oil killed *S. mutans*, *A. actinomycetemcomitans*, *P. gingivalis* or *F. nucleatum* completely (data not shown). These three essential oils completely killed gram-negative bacterial strains tested, even at 0.2% concentration. Romarinus oil also exhibited bactericidal activity against tested bacteria. Lavandula oil was not effectively bactericidal against any bacterial strain tested.

Dental plaques are understood to be biofilms composed of many species of bacteria, and the adhesive ability of these bacteria seems to be an important pathogenic factor. Therefore, we investigated the inhibitory effect of essential oils on the adhesion of *P. gingivalis* and *S. mutans* to the bottom of cell culture wells. The inhibitory effect of essential oils on the adhesion of *P. gingivalis* ATCC 33277 and *S. mutans* JC-2 to the bottom of cell culture plates

Table 1. Minimum inhibitory concentration values (%) for essential oils towards oral bacteria

	Essential oils				
Strains	Manuka	Tea tree	Eucalyptus	Lavandula	Romarinus
S. sobrinus					
6715	0.13	1.0	1.0	>1.0	>1.0
B13	0.25	1.0	1.0	>1.0	>1.0
S. mutans					
JC-2	0.25	1.0	1.0	>1.0	>1.0
A. actinomycetemcomitans					
Y4	0.03	0.5	0.5	0.5	0.5
ATCC 29523	0.03	0.5	0.5	0.5	0.5
ATCC 29524	0.03	0.5	0.5	0.5	0.5
ATCC 33384	0.03	0.25	0.5	0.5	0.5
P. gingivalis					
ATCC 33277	0.03	0.13	0.5	0.5	1.0
ATCC 53977	0.03	0.13	0.25	0.5	0.5
W50	0.03	0.25	0.5	1.0	1.0
Su63	0.03	0.13	0.5	0.5	1.0
F. nucleatum					
ATCC 25586*	0.03	0.06	0.13	0.25	0.5
#2*	0.03	0.06	0.25	0.25	0.5
#20*	0.03	0.06	0.25	0.25	0.5

Each essential oil was tested at concentrations of 1.0%, 0.5%, 0.25%, 0.13%, 0.06%, 0.03%, 0.016%, 0.008%, 0.004%, and 0.002%.
Optical density (OD) at 595 nm was measured on the day after inoculation.
*: measured after 2 days.
Each assay was repeated on at least 3 different days.

Table 2. Minimum bactericidal concentration values (%) for essential oils towards oral bacteria

	Essential oils				
Strains	Manuka	Tea tree	Eucalyptus	Lavandula	Romarinus
S. sobrinus					
6715	0.25	1.0	1.0	>1.0	>1.0
B13	0.25	1.0	1.0	>1.0	>1.0
S. mutans					
JC-2	0.25	1.0	1.0	>1.0	>1.0
A. actinomycetemcomitans					
Y4	0.13	0.5	0.5	>1.0	1.0
ATCC 29523	0.13	0.5	0.5	>1.0	1.0
ATCC 29524	0.13	0.5	0.5	>1.0	1.0
ATCC 33384	0.13	0.5	0.5	>1.0	1.0
P. gingivalis					
ATCC 33277	0.06	0.5	0.5	>1.0	1.0
ATCC 53977	0.03	0.13	0.25	>1.0	0.5
W50	0.06	0.25	0.5	>1.0	1.0
Su63	0.06	0.25	0.5	>1.0	1.0
F. nucleatum					
ATCC 25586*	0.03	0.25	0.5	>1.0	0.5
#2*	0.03	0.25	0.5	>1.0	0.5
#20*	0.03	0.25	0.5	>1.0	0.5

Each assay was repeated on at least 3 different days.

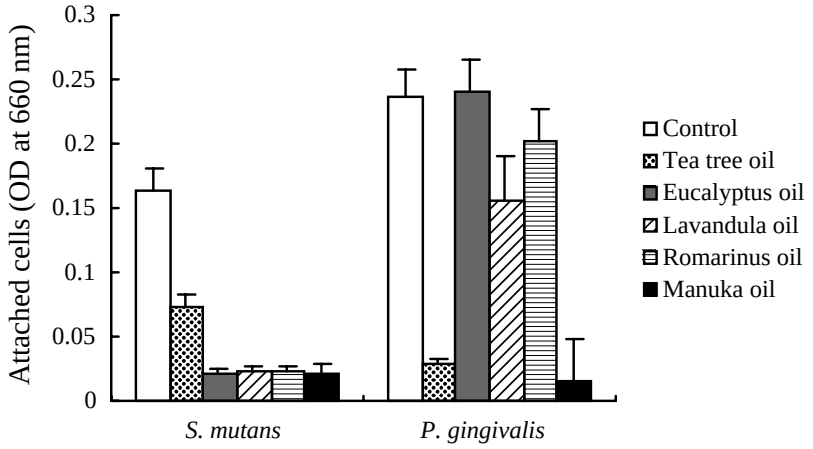

Fig. 1. Inhibitory effect of essential oils on adhesion of *P. gingivalis* and *S. mutans*. Bacteria attached to culture dishes were stripped off with a cell scraper, and cell suspensions were measured for absorbance. All essential oils significantly inhibited adhesion of *S. mutans* (*vs*. control; $P < 0.05$). Tea tree oil and manuka oil showed significant adhesion-inhibiting effects on *P. gingivalis* (vs. control; $P < 0.05$). Data are means ± standard deviation from three duplicate independent assays.

(Nunc) was also examined. Bacterial cells were cultured with 0.1% of each essential oil for 4 days under anaerobic conditions. After culture, the culture medium and the floating bacterial cells were removed, and the wells were rinsed twice with PBS. Subsequently, PBS was added, and the attached bacteria were stripped off with a cell scraper, and the turbidity of the bacterial suspension was measured to examine the adhesion-inhibiting effect. The Mann-Whitney *U*-test was used to identify statistically significant differences. As shown in Fig. 1, all the essential oils had marked adhesion-inhibiting effects on *S. mutans* ($P < 0.05$). Tea tree oil and manuka oil showed a significant adhesion-inhibiting effect on *P. gingivalis* ($P < 0.05$). The inhibitory effect on adhesion found suggests that the phytochemical oils suppress the biofilm formation.

It might be suspected that strongly antibacterial essential oils have side effects on the host; however, a cytotoxicity test on human epithelial cells and fibroblasts showed that tea tree oil had a low toxicity (26). In the present study, the effect of essential oils on the host was examined using human umbilical vein endothelial cells (HUVEC) 8715 (BioWhittaker Inc., Walkersville, MD). Essential oils were added to precultured, confluent HUVEC to final concentrations of 0.2% or 0.5%, and each culture was continued to examine the effect of essential oils on the cells under a microscope or with a Cell Titer 96^{TM} AQ Assay Kit (Promega, Madison, WI). Figure 2 shows the relative activity (%) of HUVEC at each essential oil concentration, defining the activity of HUVEC measured using a Cell Titer 96^{TM} AQ Assay Kit in the absence of essential oils as 100%. Essential oils at 0.5% increased the number of dying cells and decreased the activity of cells, but had little effect on these cells at 0.2%. In particular, lavandula oil had little effect on these cells, which showed virtually no differences from controls. Microscopic examination also showed that at an essential oil concentration of 0.5%, considerable numbers of cells became detached from the bottom of culture wells, but at an essential oil concentration of 0.2%, only a few cells became detached.

At present, chlorhexidine and povidone iodine products are generally used as antibacterial agents for cleaning the oral cavity (11). Natural antibacterial substances have attracted attention as being safer (14–16, 28). Studies of the antibacterial activity of essential oils, mainly tea tree oil, have pointed out their usefulness (1, 3, 4, 6, 7, 18, 22). In this study, we compared several essential oils with regard to their antibacterial activity against cariogenic and periodontopathic bacteria. Among the essential oils used, tea tree oil and manuka oil, in particular the latter, showed strong antibacterial activity. It has been reported that the antimicrobial activity of manuka oil is associated with a fraction containing several triketones including leptospermone (18, 29). Among the constituents of tea tree oil, the main constituents with antibacterial activity are terpinen-4-ol and γ-terpinene. Tea tree oil has been reported to have a several times stronger effect than these constituents alone (3). In addition to these ingredients, tea tree oil is known to contain about 100 different molecules, which presumably synergistically increase the effect on the oral bacteria studied. Although it has been indicated that lipophilic terpenens act on the phospholipid layer of the cell membrane and can destroy its normal structure and function (4), the antibacterial

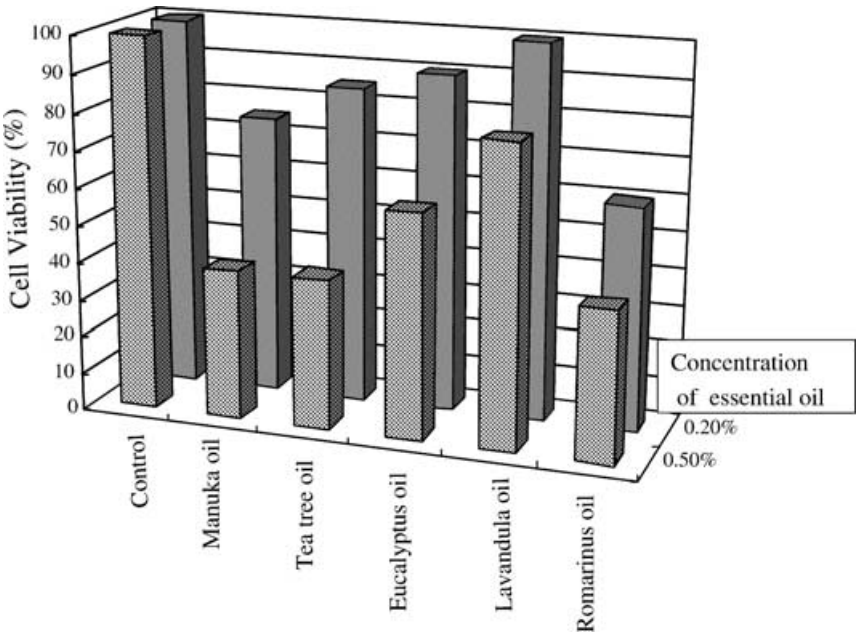

Fig. 2. Effect of essential oils on the viability of HUVEC. Essential oils were added to precultured, confluent HUVEC to final concentrations of 0.2% or 0.5%. Defining the activity of HUVEC measured using a Cell Titer 96^{TM} AQ Assay Kit in the absence of essential oils as 100%, the relative activity of HUVEC at each essential oil concentration was expressed as a percentage. Data are means from three duplicate independent assays with standard deviations of less than 15%.

mechanisms of essential oils still need to be elucidated.

We were thus able to show that essential oils, particularly manuka oil and tea tree oil, exhibited growth-inhibiting and bactericidal effects on periodontopathic and cariogenic bacteria, and also adhesion-inhibiting effects on *P. gingivalis* and *S. mutans*. From the viewpoint of safety, these essential oils seem to be promising antibacterial substances for oral care at concentrations of 0.2% or lower, at which they had little effect on human cells.

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