Antimicrobial Effect of Tea Tree Oil (*Melaleuca alternifolia*) on Oral Microorganisms

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Summary

The oil of *Melaleuca alternifolia* (tea tree) has an antimicrobial effect on a wide range of gram-positive and gram-negative bacteria, yeasts, and fungi. In the present study, the bacteriostatic or bactericidal / fungicidal effects, respectively, of a tea tree oil solution, a newly developed tea tree oil gel for oral use (Tebodont®), the corresponding carrier gel, a chlorhexidine digluconate solution and PlakOut® against ten different oral microorganisms were studied in vitro.

The minimum inhibitory concentration (MIC) values ranged from 0.0293 % to 1.25 % for the tea tree oil solution, and from 0.0082 % to 1.25 % for the tea tree oil gel. The minimum bactericidal / fungicidal concentration (MBFC) values ranged from 0.0521 % to 2.5 % for the tea tree oil solution, and from < 0.0098 % to 3.33 % for the tea tree oil gel. The most sensitive germs were *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, while *Streptococcus mutans* and *Prevotella intermedia were* the least sensitive. Both for chlorhexidine digluconate solution and for PlakOut®, the values for the minimum inhibitory concentration and for the minimum bactericidal / fungicidal concentration ranged from < 0.0002 % to 0.0125 %.

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Introduction

Tea tree oil is extracted from the leaves of *Melaleuca alternifolia*, a bush-like tree of the myrtle family that is found in Australia. As a natural and efficient microbicide, it enjoys some popularity in pharmaceutical and cosmetic applications (for reviews see Carson & Riley 1993, Carson et al 1998, Galle-Hoffman & König 1999). Tea tree oil consists of about 100 terpinenes and their alcohols, of which terpinene-4-ol, α-pinene, linalool and α-terpineol are considered the most important components having an antimicrobial effect (Carson & Riley 1995, Raman et al 1995).

In vitro studies have demonstrated a good antimicrobial effect of tea tree oil or individual components towards a broad range of gram-positive and gram-negative bacteria, yeasts and other fungi (Carson & Riley 1993, Raman et al 1995, Hammer et al 1996, Hammer et al 1998, Maudsley & Kerr 1999, Hammer et al 2000). In recent years, tea tree oil has gained additional interest, as it shows growth-inhibiting effects even against (multi-)resistant microbes. Thus, tea tree oil was effective in vitro against problematic pathogens such as methicillin-resistant *Staphylococcus aureus*, glycopeptide-resistant enterococci, gentamicin-resistant and extended-spectrum-β-lactamase-producing *Klebsiella* sp. and certain multidrug-resistant pseudomonads, and in the clinical treatment of infections with fluconazole-resistant *Candida albicans* in AIDS patients (Carson et al 1995, Nenoff et al 1996, Chan & Loudon 1998, Jandourek et al 1998, Elsom & Hide 1999, Harkenthal et al. 1999, May et al 2000). Antiviral effects against human viruses have not been demonstrated so far, but effects against plant viruses have (Bishop 1995, Bourne et al 1999).

Promising clinical applications of tea tree oil in the literature have comprised local therapies for mild forms of acne, onychomycosis, and bacterial vaginitis (Bassett et al 1990, Blackwell 1991, Hammer et al 1999a, Tong et al 1999).

In dentistry, too, use of this essential oil as an antibiotic agent was proposed already in the early 20th century (MacDonald 1930). However, there are only two studies addressing the antimicrobial effect of tea tree oil on oral microorganisms (Shapiro et al 1994, Walsh & Longstaff 1987).

Chlorhexidine is a commonly used agent for the prevention and treatment of oral infections. It is effective, but has undesirable side effects (Denton 1991). Therefore, alternatives are being sought, these days increasingly among natural products as well.

The aim of this study was to investigate the bacteriostatic or bactericidal / fungicidal properties of tea tree oil in an orally applicable gel form (Tebodont®, Dr. Wild & Co. AG, Basel) against oral microorganisms, and to compare these results to those obtained with chlorhexidine. For this purpose, the macro-dilution test was used, which allows determining the minimum inhibitory concentration (MIC) and the minimum bactericidal / fungicidal concentration (MBFC) of substances against microorganisms in a liquid medium.

Materials and Methods

Microorganisms and growth conditions

The oral microorganisms studied in this study are: *Streptococcus mutans* ATCC 25175, *Streptococcus sanguis* ATCC 10556, *Streptococcus anginosus* ZIB 6006 (clinical isolate from the Centre for Dentistry [ZfZ] Basel), *Actinobacillus actinomycetemcomitans* ZIB 1001 (clinical isolate ZfZ Basel), *Lactobacillus salivarius* ssp. *salivarius* DSM 20555, *Actinomyces naeslundii* ATCC 12104, *Fusobacterium nucleatum* ZIB 2001 (clinical isolate ZfZ Basel), *Prevotella intermedia* ATCC 25611, *Porphyromonas gingivalis* ZIB 3071 (clinical isolate ZfZ Basel) and *Candida albicans* ZIB 9100 (clinical isolate ZfZ Basel). The microorganisms were cultured at 36 °C on human blood plates (Columbia agar base [BBL Becton Dickinson] supplemented with 4 mg / l of haemin, 1 mg / l of menadione, and 50 ml / l of human blood) under the following conditions: Facultatively anaerobic bacteria in air +10 % CO_2 for 3–5 days, anaerobic bacteria in 10 % CO_2, 10 % H_2, 80 % N_2 for ten days, and *C. albicans* under aerobic conditions for two days.

The microorganisms were grown in the following liquid media: Todd-Hewitt's Broth (BBL Becton Dickinson) for facultatively anaerobic bacteria, thioglycolate broth (CM391, Oxoid) supplemented with 4 mg / l haemin and 1 mg / l menadione for most anaerobic bacteria, Cooked Meat Medium (Oxoid) supplemented with 5 g / l yeast extract (Difco), 5 g / l H_2HPO_4, 1 mg / l resazurin and 0.5 g / l yeast extract (Difco), 5 g / l H_2HPO_4, 1 mg / l resazurin and 0.5 g / l cysteine hydrochloride for *P. intermedia*; Columbia Broth (BBL Becton Dickinson) supplemented with 4 mg / l haemin, 1 mg / l menadione and 50 ml / l human blood for *P. gingivalis*; Sabouraud Liquid Medium (Oxoid) for *C. albicans*. These media were also used in tests to determine the MIC and MBFC.

Active substances

The tea tree oil (Lot № 7115, Producer: Main Camp Tea Tree Oil, Ballina, Australia) was provided by the company Dr. Wild & Co. AG and contained, inter alia, 37–45 % terpinene-4-ol and a maximum of 5 % 1,8-cineole. To improve solubility in aqueous media, an 80-% solution was prepared in Tween 80 (Sigma-Aldrich Chemie) (Walsh & Longstaff 1987, Carson & Riley 1995).

In addition, a gel containing 10 % tea tree oil (with the oil of lot № 7115) and the corresponding carrier gel (Dr. Wild & Co. AG; containing water, glycerine, sodium, methyl cellulose, sodium saccharin, hydrogenated castor oil, menthol, and orange oil) was tested without the active ingredient.

10 % chlorhexidine digluconate (Spitalapotheke Basel) and PlakOut® Gel (Hawe-Neos Dental, Switzerland) were used as controls.

The substances to be tested were primarily examined for their sterility.

Minimum inhibitory concentration (MIC) and minimum bactericidal / fungicidal concentration (MBFC)

The growth inhibition of the individual active substances towards the oral microorganisms was determined using the macro-dilution test (NCCLS 1997a, NCCLS 1997b, von Graevenitz et al 1987). To this end, stock solutions of 1 g of gel in 1 ml of doubly concentrated liquid nutrient medium were prepared from each of the tea tree oil gel, the placebo gel, and the PlakOut® gel, resulting in a tea tree oil concentration of 5 % for the tea tree oil gel and a chlorhexidine concentration of 0.1 % for the PlakOut® gel. Liquid tea tree oil and chlorhexidine were diluted in the appropriate nutrient broth to produce stock solutions of 5 % and 0.2 %, respectively. All substances were further diluted sequentially at steps of 1:2 in liquid broth. For each test substance, ten dilution steps were examined.

To each 2-ml sample of the dilution series, 100 µl of a microorganism culture containing approximately 5×10^6 CFU / ml was added, and the mixture was incubated under the appropriate conditions for 24 hours for aerobic and facultatively anaerobic microbes, and for 5–10 days for anaerobic microbes. The germ count actually used for inoculation was precisely determined by plating on human blood plates. The MIC corresponded to the lowest concentration of active ingredient at which no growth was visually observed at the end of the observation period. To determine the MBFC, from the areas where no microbial growth was visible, 100 µl of the liquid was spread on a human blood plate. The MBFC corresponded to the lowest drug concentration at which a maximum of 1 ‰ of the inoculated germs remained viable. Although with the yeast *Candida albicans* only one oral fungus was tested, the term fungicidal concentration is used in this

study, because it is the term commonly used in the literature. The experiments were carried out three times, with a few exceptions.

Results

The MIC and MBFC values obtained are summarised in Tables I and II. The tea tree oil solution as well as the tea tree oil gel had a bacteriostatic or bactericidal effect, respectively, on the tested microorganisms. Here, the MIC values for the tea tree oil solution were in the range from 0.0293 % to 1.25 %, and those for the tea tree oil gel from 0.082 % to 1.25 %, while the MBFC values were in the range from 0.0521 % to 2.5 % (tea tree oil solution) and from <0.098 % to 3.3 % (tea tree oil).

Tab. I: Mean minimum inhibitory concentration in %

	Substance				
Microorganism	**Tea tree oil solution**	**Tea tree oil gel [a)]**	**Gel [b)]**	**PlakOut® [c)]**	**Chlorhexidine**
S. mutans	0.2604	0.2084	N	< 0.0002	< 0.0004
S. sanguis	0.1563	0.2604	N	< 0.0003	< 0.0005
S. anginosus	0.1563	0.2084	N	< 0.0002	< 0.0004
A. actinomycetemcomitans	0.0293	< 0.0130	0.1302	< 0.0002	< 0.0004
L. salivarius	0.2084	0.2604	N	< 0.0002	< 0.0004
A. naeslundii	0.1302	1.25	N	< 0.0002	< 0.0004
F. nucleatum	0.0846	0.0912	< 0.0358	< 0.0002	< 0.0004
P. intermedia	1.25	1.25	N	0.0125	0.0125
P. gingivalis	0.0651	0.0082	0.0911	0.0027	0.0016
C. albicans	0.1302	0.0456	1.0417	0.0013	0.0009

a) relative to the tea tree oil concentration
b) relative to the gel concentration; N: ≥ 5 %
c) relative to the chlorhexidine concentration

Tab. II Mean minimum bactericidal / fungicidal concentrations in percent

	Substance				
Microorganism	**Tea tree oil solution**	**Tea tree oil gel [a)]**	**Gel [b)]**	**PlakOut® [c)]**	**Chlorhexidine**
S. mutans	1.0417	3.33	N	0.0006	0.0016
S. sanguis	0.4167	0.6250	N	< 0.0005	0.0011
S. anginosus	0.4167	0.5208	N	< 0.0005	0.0008
A. actinomycetemcomitans	0.0521	< 0.0098	0.5208	< 0.0002	< 0.0004
L. salivarius	1.5625	0.7297	N	< 0.0002	< 0.0007
A. naeslundii	0.5208	1.25	N	< 0.0002	< 0.0004
F. nucleatum	0.1693	0.1172	0.1693	< 0.0002	< 0.0005
P. intermedia	2.5	1.8750	N	0.0125	0.0125
P. gingivalis	0.0651	0.0130	0.1170	0.0027	0.0016
C. albicans	0.3125	0.2084	N	0.0018	0.0032

a) relative to the tea tree oil concentration
b) relative to the gel concentration; N: ≥ 5 %
c) relative to the chlorhexidine concentration

Interestingly, the placebo gel also had microbiostatic or microbicidal effects on some microorganisms, such as *A. actinomycetemcomitans, F. nucleatum, P. gingivalis*, and *C. albicans*. Accordingly, at the same concentration of the active ingredient, in these cases the tea tree oil gel was also more efficient than the tea tree oil solution (synergy effect).

As a comparison, the MIC and MBFC values of a chlorhexidine gluconate solution and of PlakOut®, a product containing chlorhexidine, were determined. The values for both the MIC and the MBC *[sic; recte: MBFC]* were between < 0.0002 % and 0.0125 %, well below those for tea tree oil.

Discussion

Tea tree oil is used in many cosmetic products at concentrations of 2–5 % (Carson et al 1995). At a concentration of 2 %, in vitro both the tea tree oil solution and the tea tree gel inhibited all of the ten investigated oral germs, and for nine out of the ten, this concentration was higher than the MBFC, too. A tea tree oil concentration of 5 % was above the MBFC for all germs. As references, the MIC and MBC values of a chlorhexidine solution and of PlakOut® (containing 0.2 % chlorhexidine), two commonly used products for bacterial reduction in the oral area, were determined. A concentration of 0.2 % chlorhexidine was expected to be above the MBFC for all the oral bacteria studied. The chlorhexidine MIC values were usually lower by a factor of 100–1000 than those for tea tree oil were, and are broadly consistent with those found in the literature (Denton 1991). Interestingly, the carrier gel also had microbiostatic effects on some microorganisms, such as

A. actinomycetemcomitans, C. albicans, and *F. nucleatum*. The base underlying this effect is unclear. This result could explain some of the differences between the effects of tea tree oil solution and tea tree oil gel.

The mode of action of the tea tree oil is explained with the lipophilic terpenes (especially terpinene-4-ol) it comprises. These penetrate into the cell membranes of the microorganisms and exert a toxic effect on the membrane structure and its function, so that the cytoplasmic membrane can no longer maintain its function as permeability barriers (Cox et al 1998, Gustafson et al 1998, Cox et al 2000, Mann et al 2000). No resistance to tea tree oil has been described so far, although there are no broadly designed studies on this issue.

Tea tree oil is not mutagenic, and toxicity from oral ingestion in rats is considered moderate, with an LD_{50} of 1.9 g / kilogram body weight (Carson & Riley 1993, Ford 1988). Contact dermatitis and allergies following local application have been reported in the literature (Knight & Hansen 1994, Selvaag et al 1994, Carson et al 1998, Hausen et al 1999).

Tea tree oil obtained from Clone 88, a new cultivar with increased relative content of the active substance terpinen-4-ol and reduced 1,8-cineol content, showed an increased microbial effect in vitro, especially against problematic bacteria such as *Pseudomonas aeruginosa* and methicillin-resistant *S. aureus* (May et al 2000). Further research can help to produce plants with even better antimicrobial effects and less tendency to skin irritation.

The effect of tea tree oil on oral microorganisms was studied by Walsh & Longstaff (1987) and Shapiro et al (1994). A compilation is shown in Table III. For comparable bacterial strains, the MIC values in the present study were mostly between those of Shapiro et al (1994) and Walsh & Longstaff (1987). Since in each of these studies partially different bacterial isolates, media, and incubation conditions were used, variations in MICs could be caused thereby. This is especially true for the study by Walsh & Longstaff (1987), in which suboptimal growth media may have contributed to the lower MIC values found there.

MIC values of tea tree oil against non-oral microorganisms likewise vary between different publications. This could be explained by the use of other strains, by differing test arrangements, or differing tea tree oil preparations (Carson & Riley 1993, Carson & Riley 1995, Hammer et al 1998, Galle-Hoffman & König 1999, May et al 2000). In vitro studies with tea tree oil have the inherent problem that tea tree oil is insoluble in water and must therefore be solubilised with the aid of detergents (Carson & Riley 1995, May et al 2000). The highest Tween-80 concentration used in this study was 1.25 % in the cultures. Although Tween-80 alone has no significant inhibitory effects on microorganisms, synergistic or antagonistic effects cannot be excluded (Carson & Riley 1995, Hammer et al 1999b). Detergents and the presence of organic material can increase the MBC and MIC levels for certain microorganisms (Hammer et al 1999b). Whether such interactions between locally applied tea tree oil and organic material of the skin or mucosa alter the clinical efficacy of tea tree oil as well, has not yet been studied.

The macro-dilution test selected in this study cannot provide information about the in vivo efficacy of the tea tree oil gel at the selected concentrations. To date, there are only a few studies that confirm the antimicrobial effect of tea tree oil in vivo (Bassett et al 1990, Buck et al 1994, Jandourek et al 1998, Tong et al 1999). Although a five-percent tea tree oil gel was not quite as effective against slight forms of acne as a five-percent benzoyl peroxide gel was, the tea tree oil was superior in terms of skin tolerability (Bassett et al 1990). In another clinical study, the toenails of 112 onychomycosis patients were treated with either 100 % tea tree oil or 1 % clotrimazole. After six months, there was no significant difference in clinical assessment between the two treatments (Buck et al 1994). In eight out of twelve patients with HIV-associated fluconazole-refractory candidiasis, treatment with tea tree oil helped to cure or improve the situation (Jandourek et al 1998).

In two studies, tea tree oil has shown better activity against transiently present bacteria than against commensal flora – a welcome situation, as the commensal flora is considered to have a barrier function against colonisation with pathogens (Hammer et al 1996, Hammer et al 1999a). Whether this applies to the application in the oral area as well, is a question unanswered by the present work. With *S. sanguis* and *S. anginosus*, only two oral bacteria were studied that can be classified as members of an inconspicuous oral commensal flora (Slots 1979, Moore & Moore 1994).

In this study, MIC / MBFC levels were determined on planktonic monocultures. The effect of tea tree oil on an oral biofilm, such as plaque, may be different. Clinical studies could answer this question and determine the further potential of the natural product tea tree oil in the treatment of oral infections and periodontitis.

Conclusion

This in-vitro study has shown that a 2 % tea tree oil solution and a 2 % tea tree oil gel (Tebodont®) developed for oral use are able to inhibit all of the 10 studied oral germs in their growth as planktonic monocultures. For nine out of ten of these germs, this concentration was also above the minimum bactericidal / fungicidal concentration, i.e. it had a microbicidal effect.

With its antimicrobial properties, the tea tree oil gel could be considered as a natural alternative product for treatment of the infected oral mucosa and periodontium. Efficacy in a clinical application would have to be demonstrated by clinical studies.

Table III: Comparison of the MIC values (in %) for the tea tree oil solution of the present study with those from the studies by Shapiro et al (1994) and Walsh & Longstaff (1987)

Microorganism	**Kulik et al**	**Shapiro et al**	**Walsh & Longstaff**
Streptococcus sp.	0.19 (n = 3)	0.6	0.04 (n = 2)
A. actinomycetemcomitans	0.03	0.11	0.02
Actinomyces sp.	0.13	0.6	0.05 (n = 2)
F. nucleatum	0.08	> 0.6	0.02
P. intermedia	1.25	N/D a)	0.02
P. gingivalis	0.07	0.11	0.02

a) N/D = not determined

Summary

KULIK E, LENKEIT K, MEYER J: **Antimicrobial activity of tea tree oil (Melaleuca alternifolia) against oral microorganisms (in German).** Acta Med Dent Helv 5: 125–130 (2000)

The essential oil of *Melaleuca alternifolia* (tea tree oil) exhibits antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria, yeasts and fungi. In this study the bacteriostatic and bacteriocidal/fungicidal activity of a tea tree oil solution, of a new tea tree oil containing gel (Tebodont®) and the respective placebo-gel, of a chlorhexidindigluconate-solution and of PlakOut® was tested in vitro against ten different oral microorganisms.

Minimum inhibitory concentrations were in the range from 0.0293% to 1.25% for the tea tree oil solution and from 0.0082% to 1.25% for the tea tree oil gel. The values for minimum bactericidal/fungicidal concentrations were in the range from 0.0521% to 2.5% for the tea tree oil solution and from < 0.0098% to 3.33% for the tea tree oil gel. The most susceptible microorganisms were *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*, whereas *Streptococcus mutans* and *Prevotella intermedia* were the least susceptible ones. Both for the chlorhexidindigluconate solution and for PlakOut® the values for the minimal inhibitory concentration and for the minimal cidal concentration were between <0.0002% und 0.0125%.

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