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The antimicrobial activity of alpha-bisabolol and tea tree oil against *Solobacterium moorei*, a Gram-positive bacterium associated with halitosis

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ABSTRACT

Objective: To investigate the antimicrobial effect of alpha-bisabolol and tea tree oil alone and in combination against the halitosis-associated Gram-positive bacillus *Solobacterium moorei*.
Design: The inhibitory activity of alpha-bisabolol and tea tree oil against the reference strain *S. moorei* CCUG39336 and four clinical *S. moorei* isolates was investigated by a direct exposure test. Additionally, the ability of alpha-bisabolol to increase the sensitivity of *S. moorei* was tested by pretreating the bacteria with sublethal concentrations prior to the administration of tea tree oil.
Results: A dose-dependent killing was observed for the antimicrobial agents in a direct exposure test with the reference strain *S. moorei* CCUG39336. Concentrations of ≥0.5% tea tree oil caused decreases in viability of >5 log colony forming units/ml even after short incubation periods, while bacterial viability was less affected by alpha-bisabolol. The combination of 0.1% alpha-bisabolol plus 0.05% tea tree oil showed a synergistic effect on *S. moorei* strain CCUG39336 and on two of the four clinical *S. moorei* isolates tested. However, incubation of *S. moorei* with a sublethal concentration of 0.1% alpha-bisabolol for three days prior to the administration of 0.05% tea tree oil did not enhance the antibacterial effect of tea tree oil.
Conclusion: Halitosis-associated bacterium *S. moorei* is susceptible to the antimicrobial agents tea tree oil and alpha-bisabolol, suggesting that these compounds might be beneficial in oral healthcare products.

1. Introduction

Halitosis is in most cases of oral origin and is usually associated with bacterial overgrowth on the dorsum of the tongue, but may include other oral causes like gingivitis, periodontitis and dental caries. Extra-oral or systemic causes of halitosis are rare in a generally healthy population and can involve rhinopharyngological, oropharyngological, gastrointestinal and other systemic pathologies.[1] Anaerobic bacteria, residing in various oral niches, degrade organic substances to volatile sulphur compounds (VSC), diamines and phenyl compounds, which are responsible for the malodour.[2] *Treponema denticola*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Prevotella intermedia* and Actinobacilli are commonly isolated from halitosis patients

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and well-characterized as VSC-producing bacteria.[3,4] Recent studies suggest a close association between halitosis and *Solobacterium moorei*, a Gram-positive, non-sporeforming, VSC-producing, anaerobic bacillus.[3,5,6]

To reduce the amount of bacteria in the oral cavity and in particular on the dorsal tongue, mechanical tongue cleaners and chemical products such as tongue gels are available. Antibacterial agents in gels include essential oils, lipophilic liquids containing volatile compounds, from plants such as *Salvia officinalis*, *Chamomilla recutita*, *Calendula officinalis*, or *Melaleuca alternifolia*, but also metal ions (e.g. zinc), oxidizing agents like chlorine dioxide or sodium chlorite, sodium bicarbonate or agents such as triclosan.[4,7–10]

The monocyclic sesquiterpenoid alpha-bisabolol is the active ingredient of a tongue gel and several toothpastes. It can be isolated from chamomile (*Matricaria chamomilla*) and has anti-inflammatory and antimicrobial properties.[11–13] Additionally, alpha-bisabolol has been shown to enhance the permeability of bacterial cells thereby increasing their susceptibility to antimicrobials.[14] Another essential oil used in tongue gels is tea tree oil, which is isolated mainly from the Australian native plant *M. alternifolia*. Tea tree oil has been shown to exhibit antibacterial, antifungal, antiviral and antiprotozoal activities.[15–21]

The aim of this study was to analyse the antibacterial potential of tea tree oil and alpha-bisabolol, alone or in combination, against the halitosis-associated bacterium *S. moorei*. Additionally, the potential of alpha-bisabolol as a membrane-sensitizing agent of *S. moorei* was investigated.

2. Materials and methods

2.1. Bacteria and growth conditions

The reference strain of *S. moorei* CCUG39336 and the four oral isolates CH1#23, CH3#63, CH3A#109A and CH8#20 were grown anaerobically (AnaeroGen Compact, OXOID AG, Pratteln, Switzerland) on Columbia blood agar plates (Columbia Agar Base [BBL Becton Dickinson, Allschwil, Switzerland] supplemented with 4 mg/l hemin, 1 mg/l menadione, and 50 ml/l human blood) at 37 °C.

For the following tests, suspensions of *S. moorei* were prepared in 2 ml 0.9% prereduced NaCl (prereduced NaCl was obtained by storing the 0.9% NaCl solution in AnaeroGen-Compact for at least 24 h) to give a final concentration of about 6.4×10^6 CFU/ml, which corresponds to an optical density of $OD_{600} = 0.8$ (Spectrophotometer Ultrospec 2000, Amersham Pharmacia Biotech, Pharmacia Biotech Europe GmbH, Dübendorf, Switzerland). The correct bacterial counts were determined by plating dilutions of the suspensions onto Columbia blood agar plates. The plates were incubated anaerobically at 37 °C for 4 d and the colonies were counted.

2.2. Antimicrobial agents

Tea tree oil, chlorhexidine digluconate solution (stock solution 20% in water) and (−)-alpha-bisabolol were purchased from Sigma–Aldrich (Buchs, Switzerland), the solvents ethanol and Tween80 were obtained from Merck (VWR, Dietikon, Switzerland).

Table 1 shows the antimicrobial agents, the respective solvents as well as the stock solutions and the final concentrations used in the experiments. The purity of the antimicrobial agents was tested in every experiment by spotting 10 μl each of the stock solutions and the test concentrations onto a Columbia blood agar plate.

2.3. Direct exposure test

To determine the antimicrobial activity of the agents, 1 ml *S. moorei* suspension in 0.9% prereduced NaCl was mixed with 1 ml of the respective antimicrobial agent and incubated under anaerobic conditions at 37 °C. Aliquots of 100 μl were serially diluted in 0.9% NaCl immediately after mixing and after 1 and 10 min of incubation. Duplicate droplets of 10 μl of each dilution were spotted onto Columbia blood agar plates and incubated anaerobically at 37 °C for 3 d. Colonies were counted and expressed as colony-forming units per millilitre (CFU/ml) and the mean log 10 CFU reduction factor (log RF) was calculated.

For *S. moorei* CCUG39336 different concentrations of chlorhexidine, tea tree oil and alpha-bisabolol were tested as well as the antimicrobial effect of a combination of 0.1% alpha-bisabolol plus 0.05% tea tree oil. Additionally, a potential influence of the solvents Tween80 and ethanol was also examined. For the four clinical *S. moorei* isolates CH1#23, CH3#63, CH3A#109A and CH8#20 only 0.05% tea tree oil, 0.1% alpha-bisabolol and the combination of 0.05% tea tree oil plus 0.1% alpha-bisabolol were tested.

The experiments were repeated at least two times and 0.9% NaCl served in each experimental setup as control.

2.4. Pretreatment of *S. moorei* CCUG39336 with alpha-bisabolol

To test whether alpha-bisabolol can act as a sensitizing agent, 0.5 ml of a 3.2×10^6 CFU/ml *S. moorei* CCUG39336 suspension in 0.9% NaCl were added to 4.0 ml Trypticase-Soy-Broth (Becton Dickinson, Allschwil, Switzerland) enriched with 0.5 mg/l menadione, 5.0 mg/l hemin and 5% human blood containing either 0.1% alpha-bisabolol (a sublethal concentration of

Table 1 – Antimicrobial agents used in this study.

	Chlorhexidine digluconate (20%)	Alpha-bisabolol (95%)	Tea tree oil (34%)
Diluting agent	H_2O	3% Ethanol/0.9% NaCl	0.5% Tween80/0.9% NaCl
Stock concentration	2%	10%	5%
Test concentrations	0.2%, 0.02%, 0.002%	1%, 0.1%, 0.01%	5%, 0.5%, 0.05%