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Magnolia bark extract increases oral bacterial cell surface hydrophobicity and improves self-perceived breath freshness when added to chewing gum

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ABSTRACT

Magnolia bark extract (MBE) is a natural product used as an anti-inflammatory, anti-platelet, and chemo-preventive agent. Here, we investigate the effects of MBE on the self-perceived freshness of breath evaluated in ten human volunteers, who chewed gum with and without MBE added, as a functional food. Furthermore, the effects of exposing oral bacteria to MBE on cell-surface hydrophobicity were determined and bactericidal effects of MBE in human saliva were assessed. Volunteers perceived freshness of breath similar directly after chewing a gum with or without MBE added, but 60 min after chewing MBE gum, freshness was perceived significantly better. Also, cell-surface hydrophobicity of Gram-negative, but not of Gram-positive bacteria, increased dose-responsively after exposure to MBE, while spiking of saliva with MBE did not affect bacterial viability. Taken together, MBE gum improves self-perceived freshness of breath likely due to an increase in cell-surface hydrophobicity of Gram-negative bacteria, instead of a bactericidal mechanism.

1. Introduction

There is a rising interest worldwide in natural products for the maintenance of health. Magnolia bark extract (MBE) has been widely used in medicine for 2000 years, and lately MBE received scientific attention as an anti-inflammatory, anti-platelet, and even chemo-preventive agent (Chao et al., 2010; Fuentes & Palomo, 2014; Lai et al., 2011; Liou, Hua, Hsu, & Weng, 2015; Zhang & Wang, 2010). MBE is harvested from the *Magnolia officinalis* tree and its two active components, magnolol and honokiol, are both hydrophobic (William Wrigley Jr. Company, 2009; Zhang & Wang, 2010). Maintenance of oral health is largely achieved by regular toothbrushing, the use of mouthrinses, dental floss or other interdental cleaning devices. In addition, chewing of gum has been promoted as an adjunct to regular oral hygiene (Crocombe, Brennan, Slade, & Loc, 2012; Imfeld, 1999). Ever since the introduction of sugar-free gum,

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chewing gum has developed from candy towards a functional food, providing an additional function to increase oral health or prevent disease, especially through the addition of xylitol (Remacle & Reusens, 2004; Van Loveren, Broukal, & Oganessian, 2012).

The oral cavity is comprised of more than 700 bacterial species (Aas, Paster, & Stokes, 2005) many of which contribute to oral health (Zarco, Vess, & Ginsburg, 2012) rather than to disease. Yet, conventional maintenance of oral health is geared towards removal of as many bacteria as possible, irrespective of whether they are known contributors to oral health or disease. The composition of the oral microbiome is of importance in the balance between health and disease. Specific bacteria in the oral microbiome are associated with specific oral diseases. For instance, acid producing bacteria are associated with caries, while Gram-negative bacteria are often associated with oral malodour or periodontal disease. Also the occurrence of several other more general diseases such as diabetes mellitus, cardiovascular disease, preterm birth, and obesity can be related to the composition of the oral microbiome (Han, 2011; He, Li, Cao, Xue, & Zhou, 2014; Zarco et al., 2012). Key to prevention of disease is symbiosis of bacteria with the host (Marsh, Head, & Devine, 2015) and preventing overgrowth by specific oral pathogens. Accordingly, alternative oral hygiene measures that aim towards the removal of specific oral pathogens from the oral cavity rather than untargeted bacterial removal are currently looked for more than ever (Allaker & Ian Douglas, 2015; Eckert, Sullivan, & Shi, 2012; Li et al., 2010; Sullivan et al., 2011). As early as 1967, it was already demonstrated that the composition of the oral microbiome could be shifted towards a composition of solely Gram-negative bacteria by rinsing with vancomycin (Loe, Theilade, & Jensen, 1967).

Inhibitory effects of MBE on the growth of specific oral bacterial strains have been reported (Cheng, Li, He, & Zhou, 2015) and, *in vitro* experiments have shown effectiveness against bacteria responsible for oral malodour (Greenberg, Urnezis, & Tian, 2007), although clinical effects on the freshness of breath have never been demonstrated. When applied in a chewing gum, MBE was shown to be possibly beneficial for gingival health by reducing gingival bleeding on probing (Campus et al., 2011; Greenberg et al., 2007).

Unfortunately, the majority of studies on MBE investigated the general antimicrobial effect of MBE against oral bacteria and did not investigate a mechanism of action of MBE against specific oral bacteria. Although it was indicated that magnolol and honokiol are hydrophobic (Greenberg, Dodds, & Tian, 2008), the effects on bacterial surface hydrophobicity have not been investigated.

Cell surface hydrophobicity is an important parameter for bacterial aggregation and adhesion to oral surfaces (Nostro et al., 2004; Scannapieco, 1994; Weiss, Rosenberg, Judes, & Rosenberg, 1982). Exposure of oral bacteria to cetylpyridinium chloride and chlorhexidine (Goldberg, Konis, & Rosenberg, 1990) as well as low concentrations of amoxicillin, penicillin, metronidazole (Lee, Hong, & Cheong, 2003) were shown to change the cell surface hydrophobicity of oral bacterial strains such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Other research by Goldberg and Rosenberg described that changing bacterial cell surface hydrophobicity can effectively increase the binding and removal of oral bacteria from aqueous suspensions or desorb bacteria from solid surfaces by using a hydrophobic ligand (Goldberg et al., 1990; Goldberg & Rosenberg, 1991; Kozlovsky et al., 1996).

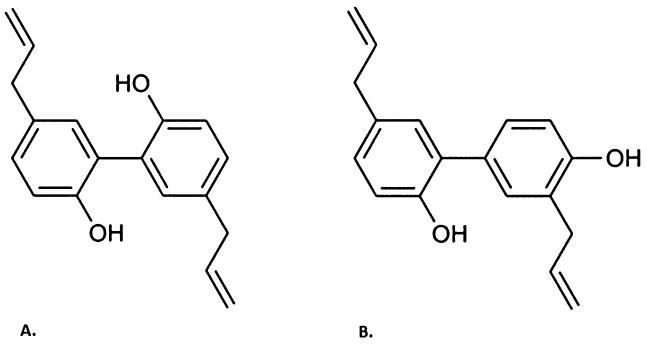

Fig. 1 – Chemical structures of (A) magnolol (5,5′-di-2-propenyl-(1,1′biphenyl)-2,2′-diol) and (B) honokiol (3′,5-di-2-propenyl-(1,1′-biphenyl)-2,4′diol).

In this study, we aim to demonstrate possible beneficial effects of MBE containing chewing gum on the self-perceived freshness of breath in a group of ten volunteers. Next, we explored the effect of exposing different oral Gram-positive and Gram-negative bacteria to MBE *in vitro* on their surface hydrophobicity using the MATH (Microbial Adhesion To Hydrocarbon) assay (Rosenberg, 1984) in its kinetic mode (Lichtenberg, Rosenberg, Sharfman, & Ofek, 1985). Bactericidal effects of MBE in saliva were assessed by determining the number of viable bacteria in human saliva, after collecting and spiking with MBE.

2. Materials and methods

2.1. *Chewing gum and magnolia bark extract*

Chewing gums were provided by Wm. Wrigley Jr. Company (Chicago, IL, USA). 3 mg of MBE (Honsea Sunshine Biotech Co., Ltd, Guangzhou, China), consisting of 95% magnolol (5,5′-di-2-propenyl-(1,1′biphenyl)-2,2′-diol) and 5% honokiol (3′,5-di-2-propenyl-(1,1′-biphenyl)-2,4′diol) (Fig. 1), was added to the coating of 1.5 g pellet shaped gums containing gumbase, sorbitol, flavours, sweeteners, and coolants. Gums without MBE added were employed as control gums. During the course of all experiments, MBE was stored at –20 °C.

2.2. *Freshness of breath in human volunteers*

Ten healthy volunteers (five females and five males, aged between 25 to 57 years participated in this double blind, cross-over study. Volunteers gave their written informed consent to the study design that was approved by the Medical Ethical Testing Committee of the University Medical Center Groningen (METc 2011/330). Inclusion criteria described that volunteers should consider themselves in good health and had a dentition with at least 16 natural elements. Volunteers were excluded from the study in case antibiotics were used up to three months prior to the study or a mouthrinse in the month preceding the study. Also, the use of mouthrinses, mints, or chewing gums other than the prescribed chewing gum was not permitted