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Oral spray containing plant-derived compounds is effective against common oral pathogens

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ABSTRACT

Objectives: Plant-derived compounds are a good source of therapeutic agents and inhibitors of inflammatory process. Dental caries, periodontal diseases and candidiasis are common oral infections caused by virulent biofilms. The objectives of this study were to develop oral spray containing plant-derived compounds; α-mangostin (α-MG) and/or lawsone methyl ether (2-methoxy-1,4-naphthoquinone) (LME) and determine its antimicrobial, anti-biofilm, and anti-inflammatory activities.

Design: Oral spray formulations were prepared containing α-MG (5 mg/ml) and/or LME (250 μg/ml). Antimicrobial activity against *Candida albicans, Streptococcus mutans,* and *Porphyromonas gingivalis* and anti-biofilm formation activities were determined as well as cytotoxicity and anti-inflammatory effects.

Results: The oral spray demonstrated antimicrobial activity against all three of the oral pathogens tested with stronger effects on *C. albicans* and *S. mutans* than *P. gingivalis*. The formulation containing α-MG (2.5 mg/ml) and LME (125 ug/ml) reduced growth of the microorganisms about 1–2 Log CFU/ml at 1–3 h and the killing effects were complete at 24 h. Based on biofilm assay, the oral spray containing both α-MG and LME showed greater inhibitory effects than those with α-MG or LME. In addition, the oral spray containing both α-MG and LME demonstrated more inhibition of nitric oxide production than α-MG alone. All the formulations were safe and demonstrated greater anti-inflammatory activity at lower concentration (< 6.25 μg/ml) than at a higher concentration.

Conclusion: Oral spray containing α-MG and/or LME is effective against common oral pathogens without significant cytotoxicity. Thus, it has the potential to prevent the infections and may serve as adjunctive treatment to conventional therapy.

1. Introduction

Dental caries, periodontal disease, and oral fungal infections caused by virulent biofilmsare global oral health problems (Hall-Stoodley & Stoodley, 2009). *Streptococcus mutans (S. mutans*) is the principal oral bacterium responsible for both the initiation and development of dental caries (Takahashi & Nyvad, 2011; Tanzer, Livingston, & Thompson, 2001). *Porphylomonas gingivalis (P. gingivalis),* a gram-negative anaerobe bacterium, is a keystone pathogen in the development of periodontal disease (Hajishengallis, Darveau, & Curtis, 2012). Oral candidiasis caused by *Candida* species, is frequently observed in HIV-infected subjects (Nittayananta, 2016), other immune suppressed patients, and denture wearers (Pereira-Cenci, Del Bel Cury, Crielaard, & Ten Cate, 2008).

Mechanical and chemical plaque controls are the methods in management of dental caries and periodontal diseases (Jepsen et al., 2017). Tooth-brushing with fluoridated toothpaste is recommended for self-performed oral hygiene care in order to prevent dental caries. Use of mechanical therapy along with antimicrobial therapy such as chlorhexidine mouthrinse is a biological rationale for the treatment of the periodontal disease. Various topical and systemic antifungal drugs including polyenes and azoles are available for the treatment of oral

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candidiasis. Although the disease respond well to conventional therapies, repeated courses of the current available medications only demonstrate reduction in clinical disease without a complete resolution of infection (Patton, Bonito, & Shugars, 2001), and often lead to drug resistance (Dos Santos Abrantes, McArthur, & Africa, 2014; Jeddy, Ranganathan, Devi, & Joshua, 2011; Jiang et al., 2014).

Current therapeutic approaches to control pathogenic oral biofilms are inadequate. It is well recognized that dental biofilm usually develops shortly after regular tooth-brushing, and is considered to be the crucial step of the development of the diseases. The complex nature of biofilm formations emphasizes the importance of finding new agents that possess anti-biofilm activity. Given these challenges, novel oral products with anti-caries, anti-periodontal, and antifungal activities along with anti-biofilm formation are urgently needed.

Plants are valuable sources of novel bioactive compounds as they produce a wide variety of secondary metabolites with biological properties against oral pathogens (Hall-Stoodley & Stoodley, 2009). Mangosteen is a widely cultivated fruit tree in Southeast Asian nations, including Thailand, Sri Lanka, The Philippines, and Vietnam (Ee, Daud, Taufiq-Yap, Ismail, & Rahmani, 2006). Mangosteen extract has been shown to demonstrate bactericidal activity against cariogenic bacteria (Juntavee et al., 2014), In addition, the pericarp of *G. mangostana* has been used in traditional medicine to treat a variety of infections. α-mangostin (α-MG) is a pure compound derived from pericarp of mangosteen (*Garcinia mangostana* L.). Nguyen et al. (2014) reported that α-MG disrupted the development of *S. mutans* biofilms and gel containing α-MG improved periodontal health of patients (Rassameemasmaung et al., 2008). *Impatiens balsamina* L. is a medicinal plant native to Southern Asian countries, including India, Bangladesh, and Burma. Different parts of the plant are used to treat infections and skin diseases. Lawsone methyl ether (2-methoxy-1,4-naphthoquinone) (LME) is a pure compound isolated from flowers of *Impatiens balsamina* L. (Panichayupakaranant & Reanmongkol, 2002). Previous studies detected potent antifungal and antibacterial activities in LME and LME containing mouthwash (Nittayananta et al., 2013; Yang et al., 2001).

Novel plant based agents may lead to efficacious anti-caries (Flemmig & Beikler, 2011; Jeon, Rosalen, Falsetta, & Koo, 2011), anti-periodontal, and antifungal therapies. As α-MG and LME have been shown to possess antimicrobial activities, we hypothesized that the combined activities of α-MG and LME would provide synergistic bioactivity against those oral pathogens. In addition, the compounds may have effects on oral biofilm formation. As such, a product containing α-MG and LME, would have significant advantages and could potentially protect against or prevent dental caries, periodontal disease and oral candidiasis development. In this *in vitro* study, three formulations of oral spray were developed containing α-MG and/or LME along with other edible ingredients. Antimicrobial, anti-biofilm, and anti-inflammatory activities of the spray were determined.

2. Materials and methods

2.1. Preparation of α-mangostin and lawsone methyl ether

10% α-MG was derived from pericarp of magosteen extract (food grade) and purchased from a local company in Thailand (Khemeephan Corporation, Bangkok, Thailand). LME was prepared by methylation of lawsone in acid conditions as previously described by Panichayupakaranant and Reanmongkol (2002). Briefly, 10.0 g lawsone was dissolved in 500 mL absolute methanol and 8.0 mL conc. hydrochloric acid. The mixture was heated under reflux conditions for 4 h before being cooled to room temperature. Then, the precipitate was separated by vacuum filtration and was re-crystallized in a mixture of ethyl acetate and methanol, to give yellow needles of LME (Panichayupakaranant & Reanmongkol, 2002)

2.2. Preparation of oral spray containing α-mangostin and lawsone methyl ether

Three formulas of oral spray containing active ingredients α-MG (5 mg/ml) and/or LME (250 μg/ml) were prepared by modifying the method used in a previously published paper (Syed, Gulrez, Al-Assaf, & Phillips, 2011). Other edible ingredients used in formulating base of the spray were the same for the three formulas. The physicochemical properties of the three formulations were recorded before and after stability study (Freeze-thaw 5 cycles) (Syed et al., 2011).

2.3. Microbial growth condition and inoculum preparation

S. mutans (ATCC 25175) was cultured on brain heart infusion (BHI) TYS20 B agar and incubated with 5% CO2 at 37 °C for 24 h *P. gingivalis* (ATCC 33277) was grown on Brucella agar supplemented with supplemented with 5 mg/L haemin and 5 mg/L vitamin. K and human anticoagulated whole blood, and incubated at 37 °C in an anaerobic jar for 5 days.

To establish ideal experimental conditions, inocula and bacterial growth curves were made from a few microbial colonies of *S. mutans* and *P. gingivalis* bacterial cultures. Isolated colonies were suspended in their corresponding culture media and turbidity of the inoculum was adjusted to reach 0.5 on the McFarland scale using a microplate reader.

2.4. Antibacterial activity assay

Antibacterial activity was assessed by obtaining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values using the microdilution technique with 96-well plates. All assays were performed in triplicate.

2.5. Determination of the bacterial and fungal minimum inhibitory concentration (MIC)

The ethanolic and oily phases were serially diluted with bacterial suspension in the 96-well plates and incubated at 37 °C under anaerobic conditions, for 24 h (*S. mutans* and *P. gingivalis*). Gentamicin was used as a negative control for bacterial growth, whereas a broth solution was used as control sterility. Microbial growth was noted as changes in optical density from bacterial inoculums, which were considered as positive controls. The MIC was identified as the lowest concentration that inhibited growth of the microroganisms. Similar techniques were applied to determine MIC of *C. albicans* by using nystatin suspension as a negative control for candidal growth.

2.6. Determination of the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The MBC was assessed by adding the suspensions from the wells without any growth of the bacterium during MIC assays in petri dishes with corresponding agar of the microorganisms, incubated at 37 °C under anaerobic conditions for 48 h (*S. mutans*) and for 5 days (*P. gingivalis*). After this incubation period, bacterial colonies were noted. Those in which bacterial colonies grew in petri dishes were designated as bacteriostatic, whereas those in which bacterial colonies did not grow were considered as bactericide. Similar techniques were applied to determine minimum fungicidal concentration (MFC) of *C. albicans*.

2.7. Time-kill assay

The bactericidal activity of the oral spray containing α-MG and LME was investigated by a time-kill assay. The bacteria in BHI broth cultures were adjusted to approximately 10^5 cfu/ml and then mixed (1:1) with the oral spray containing α-MG and LME at concentrations equivalent to 50% and incubated with 5%CO_2 at 37°. Bacterial surviving was