CHAPTER I

INTRODUCTION

1. Background and Significance

Dental caries and periodontal disease are the most common oral health problems worldwide. They are chronic infectious diseases caused by bacteria in dental plaque. Dental caries is caused by *Streptococcus mutans*, (Loesche, 1986; Zambon and Kasprzak, 1995) while periodontal disease is associated with a group of anaerobic bacteria, i.e. *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans* and *Tannerella forsythia* (formerly *Bacteroides forsythus*). (Zambon, 1996)

Elimination of dental plaque is a key for the successful treatment of dental caries and periodontal disease. Antibiotics and antiseptics have been used in adjunct to mechanical cleaning devices for dental plaque control. These agents have been used as systemic therapy or incorporated into various formulations for local applications such as toothpaste, mouthrinse and subgingival local delivery devices. (Mombelli and Samaranayake, 2004) However, the use of chemical plaque control has faced the problems with global emergence of drug resistance, unwanted side effects, and high expense in developing new agents. (Ciancio, 2000; Goodson and Tanner, 1992; Greenstein and Polson, 1998)

Natural substances from medicinal plants or from the animals' shell, skin or secretion have been used as a folk medicine in the treatment of infectious diseases. Some substances have been demonstrated for their antimicrobial activities against oral pathogens, (Amornchat, Krivaphan and Triratana, 1991; Taweechaisupapong, et al., 2000) and some have been proven in clinical trials for their effectiveness in the prevention of dental plaque formation and dental caries, and in the treatment of periodontal disease. (Hannah, Johnson and Kuftinec, 1989; Hirasawa, et al., 2002) These natural antimicrobial

substances have advantages over conventional antibiotics and antiseptics in that they have low potential for toxicity, lack of resistance and can be produced at low cost. (Cowan, 1999) Therefore, they have recently attracted attention for the development of a new antimicrobial agent for chemical plaque control.

Garcinia mangostana Linn., commonly known as mangosteen, is a fruit tree widespread in South-East Asian countries. The fruit hull or pericarp of this plant has been used in Thai indigenous medicine for the treatment of skin infections, wounds and diarrhea. (Mahabusarakum, et al., 1983) Phytochemical studies have shown that the active ingredients from mangosteen pericarp extract belong to a group of xanthones such as α-mangostin, β-mangostin and γ-mangostin, etc. (Mahabusarakam, Iriyachitra and Taylor, 1987) Among these, α-mangostin is the major component, (Bennett and Lee, 1989) and exerts the strongest antimicrobial activity against a wide range of both gram-positive and gram-negative bacteria. (Iinuma, et al., 1996; Sundaram, et al., 1983) Therefore, mangosteen pericarp extract may have potentials to be developed as mouthrinse or local delivery agents for chemical plaque control.

The antimicrobial activity of mangosteen pericarp extract against oral pathogens has never been tested. In this study, we will examine the antimicrobial activity of this extract against selected oral pathogens including *S. mutans*, *P. gingivalis* and *A. actinomycetemcomitans*. Its antimicrobial activity will be compared with purified α-mangostin and chlorhexidine, a commonly used antiseptic.

2. Objectives

2.1 To identify the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of mangosteen crude extract against *S. mutans*, *A. actinomycetemcomitans* and *P. gingivalis*.

2.2 To compare the antimicrobial activity of mangosteen crude extract with purified α-mangostin and chlorhexidine.

2.3 To determine the time-kill kinetics of mangosteen crude extract.

2.4 To compare the time-kill kinetics of mangosteen crude extract with chlorhexidine.

3. Scope of Study

3.1 MIC is identified by a broth dilution method, while MBC is identified by comparing the number of remaining viable bacteria with initial number of bacteria.

3.2 The number of viable bactena in colony forming unit (CFU) / ml is determined by counting the number of bacterial colonies that grow in agar plates using a spread plate method.

3.3 The time-kill kinetics is determined by the number of remaining viable bacteria at varying time from 5 to 90 min after exposed to the mangosteen extract at the concentrations of two or four times of MBC.

4. Inclusion Criteria

Bacterial strains used in this study include the followings: *S. mutans* ATCC 25175 and $KPSK_2$, *A. actinomycetemcomitans* ATCC 43718 (Y4) and *P. gingivalis* ATCC 53978 (W50).

5. Definitions Used in this Study

5.1 MIC is defined as the lowest concentration of a testing material in the broth that limits the turbidity of the broth to < 0.05 at the absorbance at 600 nm when compared with the broth containing the testing material without bacterial inoculum.

5.2 MBC is defined as the lowest concentration of a testing material that kills microorganism at least 99.99% of initial number of bacteria.

5.3 CFU/ml is the average number of bacterial colonies in the duplicate plates multiplied by a dilution factor.

5.4 Log_{10} reduction in CFU/ml is defined as logarithm of the ratio of the number of bacterial colonies in the absence of a testing material to the number in its presence.

6. Contributions of this Study

6.1 New knowledge on antimicrobial activity of mangosteen extract against oral pathogens.

6.2 Further development of mangosteen extract in the forms of mouthrinse or local delivery agents, based on the knowledge of its MIC, MBC and time-kill kinetics.