CHAPTER V

DISCUSSION AND CONCLUSIONS

1. Antimicrobial Activity of Mangosteen Crude Extract

Antimicrobial activity of mangosteen pericarp extract was determined against cariogenic *S. mutans* and periodontopathic *P. gingivalis* and *A. actinomycetemcomitans*. The results showed that the extract effectively inhibited *S. mutans* and *P. gingivalis*, but not *A. actinomycetemcomitans*. From the MIC values, *S. mutans* appeared to be more susceptible to the extract than *P. gingivalis*. However, differences in the initial density of bacteria used in this study should also be taken into considerations. *P. gingivalis* is an obligate anaerobe, which is difficult to grow upon subsequent inoculation into the broth media. Therefore, the initial density used in this study was about 100 times higher than that of *S. mutans*.

The mid-exponential phase of growth was selected for antimicrobial testing because the population is most uniform in terms of chemical and physiological properties. In general, the microbes in the exponential growth phase are more vulnerable to antimicrobial agents than those in the stationary phase. The growing and resting bacteria may response differently to an antimicrobial agent. (Prescott, Harley and Klein, 2003; Talaro and Talaro, 2002) Chloroform extract of shiitake mushroom equally killed growing and resting *S. mutans* and *P. intermedia*. Nevertheless the aqueous extract inhibited the growing bacteria but had no effect on the resting bacteria. (Hirasawa, et al., 1999)

When compared to other natural antimicrobial substances, the antimicrobial activity of mangosteen extract was much stronger. The MIC values of sanguanarine, *Streblus asper*, oil of clove and extract from tea leaves against *S. mutans* were 1-8 μg/ml, 2 mg/ml, 2.5 mg/ml, and 200-800 μg/ml, respectively. (Cai and Wu, 1996; Dzink and Socransky,

1985; Hirasawa, et al., 2002; Taweechaisupapong, et al., 2000) However, the mangosteen extract had disadvantage in that it was not effective against *A. actinomycetemcomitans*. To develop this extract for the treatment of mixed anaerobic infection in periodontal disease, it may need to combine with another antimicrobial agent to broaden its antimicrobial activity.

2. Antimicrobial Activity of Crude Extract vs. α-Mangostin

The mangosteen crude extract prepared by the method used in this study contained α-mangostin as high as 80%. When antimicrobial activity in terms of MIC and MBC was compared, the crude extract and the purified α-mangostin were equally effective.

The type and amount of xanthones in mangosteen pericarp extract may vary, depending upon the place of growing, freshness of mangosteen, the type of solvent and the method of extraction. (Nakatani, et al., 2002b) Different xanthones have different biological and pharmacological activities. Studies shown that α-mangostin exerted the strongest antimicrobial activity compared to other xanthones from mangosteen extract. (Mahabusarakum, et al., 1983, 1986) Its MIC values ranged from 1 to 50 μg/ml (Table 1). Our results suggested that the antimicrobial activity of the crude extract used in this study could be largely resulted from α-mangostin. Its antimicrobial activity against oral pathogens was also in the same range as MIC values against other organisms.

Preparing purified α-mangostin is expensive and time-consuming. The crude extract prepared in this study gave a high yield of α-mangostin, and exerted the same level of antimicrobial activity. Therefore, it is more suitable to commercially develop as a chemical plaque control agent.

3. Time-kill Kinetics of Mangosteen Extract

MIC and MBC measurements only examine the effect of an antimicrobial agent at a single concentration value at a single point of time. They do not provide information on the rate of antimicrobial activity and whether this rate can be enhanced by increasing

antimicrobial concentrations. On the other hand, time-kill kinetics represents the antimicrobial activity against the tested bacteria as a function of treatment time and drug concentration. Therefore, it provides more meaningful information that can be used to design dosages and dosing intervals or time for prolonged release of an antimicrobial agent. (Mueller, de la Pena and Derendrof, 2004; Pfaller, Sheehan and Rex, 2004)

Antimicrobial agents affect bacteria in two ways: bactericidal or bacteriostatic. Bactericidal is defined as an agent that exerts an irreversible and hence lethal action. The kinetics of bactericidal action is measured in minutes or hours, and the MBC value is not greater than one order above the MIC value. Bacteriostatic is defined as an agent that inhibits or retards bacterial growth reversibly. (Brooks, Butel and Morse, 2004) MBC values of mangosteen extract against *S. mutans* and *P. gingivalis* were equal or not greater than one order above MIC values. The time-kill kinetics also showed that the extract at 4x MBC could inactivate *S. mutans* and *P. gingivalis* in 90 and 15 minutes, respectively. Therefore, it was suggested that mangosteen pericarp extract was bactericidal against *S. mutans* and *P. gingivalis* .

The time-kill kinetics demonstrated that the mangosteen extract at 4x MBC was more effective in bacterial killing than the extract at 2x MBC. At 2x MBC, the extract slightly inhibited *S. mutans* at 90 minutes, but completely killed *P. gingivalis* in 30 minutes. At 4x MBC, it completely killed *S. mutans* in 90 minutes, and *P. gingivalis* in 15 minutes.

The mangosteen extract also exhibited time-dependent bactericidal activity. At 2x MBC, the antimicrobial activity of the extract against *S. mutans* slightly increased with time. On the contrary, the extract at 4x MBC significantly increased its antimicrobial activity when the exposure time increased from 30 minutes to 60 minutes, and reached its maximal activity at 90 minutes. At 2x MBC the antimicrobial activity of the extract against *P. gingivalis* significantly increased when the exposure time increased from 5 minutes to 15 minutes, and reached its maximal activity at 30 minutes. At 4x MBC, the extract reached it maximal activity after exposure for only 15 minutes.

The results from these experiments may be used in development of mangosteen extract for chemical plaque control. To use for anti-caries agent, the extract should be

maintained at the concentration of 2.5 μg/ml for at least 90 minutes to effectively kill *S. mutans.* To use for an anti-periodontitis agent, the extract should be maintained at the concentration of 80 μg/ml for at least 30 minutes or at the concentration of 160 μg/ml for at least 15 minutes to effectively inactivate *P. gingivalis*.

4. **Antimicrobial Activity of Mangosteen Extract vs. Chlorhexidine**

Chlorhexidine is a commonly used antiseptic, which exerts a broad-spectrum antibacterial activity against a wide range of both gram-positive and gram-negative bacteria. It was selected as a positive control in this study. The MIC and MBC data confirmed the results from previous study that chlorhexidine effectively inhibited all tested organisms at low concentrations. (Goldstein, et al., 2004; Hwang, et al.,2004; Meurman, et al., 1989) The mangosteen extract also effectively inhibited *S. mutans* and *P. gingivalis*, but not *A. actinomycetemcomitans*.

From the MIC and MBC experiments, chlorhexidine and mangosteen extract were equally effective against *S. mutans.* When both agents were further examined for time-kill kinetics at 2x and 4x MBC, chlorhexidine was less effective in killing this organism than the mangosteen extract at the same concentrations. At 4x MBC, the mangosteen extract completely killed the bacteria, while chlorhexidine slightly inhibited the bacteria by half an order. In contrast to mangosteen extract, the antimicrobial activity of chlorhexidine slightly increased with increasing concentration from 2x MBC to 4x MBC.

Chlorhexidine was superior in inhibiting *P. gingivalis*, as shown by the lower MIC and MBC values. When both agents were further examined for time-kill kinetics, the antimicrobial activity against this organism was comparable between these 2 agents. At 2x MBC, they completely killed the bacteria in 30 minutes. At 4x MBC, they completely killed the bacteria in only 15 minutes.

Chlorhexidine is one of the most effective antiseptics used for chemical plaque control. However, it has several unwanted side effects including bad taste, staining of teeth and tongue and taste alteration. (Ciancio, 2000; Jones, 1997) Our data from time-kill

kinetics demonstrated marked differences in the kinetics of antimicrobial activity between chlorhexidine and mangosteen extract. They also suggest that the mangosteen crude extract was equally effective or superior to chlorhexidine for killing of *S. mutans* and *P. gingivalis*. It also has certain advantages in that it has low potentials for unwanted side effects, lack of resistance, and can be produced at low cost. Therefore, it may be used to develop as a new antimicrobial agent for chemical plaque control.

5. Limitations of the Study

P. gingivalis was cultured anaerobically in a GasPak system. However, bacterial dilution, inoculation and plating were done in aerobic atmosphere. Therefore, some of bacteria might be killed while conducting the experiments. Anaerobic chamber has advantage over this system in that all experiments can be conducted in anaerobic atmosphere. However, when these two systems were compared, they were equally effective in isolating *P. gingivalis*. (Doan, et al., 1999) The effects of performing antimicrobial susceptibility tests between anaerobic chamber and GasPak system were also determined. The MIC results of testing 38 anaerobes including *P. gingivalis* against 11 antimicrobial agents were comparable for the two systems. (Murray and Niles, 1982)

Anaerobic bacteria vary in oxygen sensitivity. *P. gingivalis* belongs to a group of moderate anaerobes, which can be exposed to an atmospheric level of oxygen for 60 to 90 minutes without appreciable loss of viability. (Loesche, 1969) In this study, we tried to minimize the experiment time with *P. gingivalis* to less than 40 minutes.

6. Conclusions and Applications of the Results

Mangosteen pericarp extract effectively killed *S. mutans* and *P. gingivalis*, but not *A. actinomycetemcomitans*. Its antimicrobial activity appeared to be due to its high content of α-mangostin. It was also as effective as chlorhexidine in bacterial killing. Knowledge from this study will be useful for the future development of mangosteen extract as a new antimicrobial agent. Dosages and dosing intervals or time for prolonged drug release will be designed with reference to these data. The extract may be incorporated in the form of mouthrinse for the control of dental plaque and dental caries, or may be combined with another antimicrobial agent in the form of local delivery drugs for periodontal treatment.