

## REFERENCES

- Amornchat, C., Krivaphan, V., and Triratana, T. The antibacterial activities of *Andrographis paniculata* crude extract on oral bacteria. J Thai Dent Assoc 41 (1991): 178-85.
- Bennett, G.J., and Lee, H.H. Review article. Xanthenes from *Guttiferae*. Phytochemistry 28 (1989): 967-98.
- Brooks, G.F., Butel, G.S. and Morse, S.A. Jawetz, Melnick, & Adellberg's. Medical Microbiology. 23 ed. Singapore: McGraw-Hills Company, 2004.
- Budavari, S., O'Neil, M.J., Smith, A., Heckelman, P.E., and Kinneary, J.F. Merk Index. Vol. 12. New Jersey: Merks Research Laboratory Division of Merck & Co, 1996.
- Cai, L., and Wu, C.D. Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. J Nat Prod 59 (1996): 987-90.
- Chairungrilerd, N., Furukawa, K., Ohta, T., Nozoe, S., and Ohizumi, Y. Pharmacological properties of alpha-mangostin, a novel histamine H1 receptor antagonist. Eur J Pharmacol 314 (1996): 351-6.
- Chen, S.X., Wan, M., and Loh, B.N. Active constituent against HIV-1 protease from *Garcinia mangostana*. Planta Med 62 (1996): 381-382.
- Chynoweth, D.P. Microbial Growth Phase. Gainesville, Florida: University of Florida, 2004. Available from [http://www.gen.ufl.edu/~chyn/age4660/lect/lect\\_09](http://www.gen.ufl.edu/~chyn/age4660/lect/lect_09).
- Ciancio, S.G. Antiseptics and antibiotics as chemotherapeutic agents for periodontitis management. Compend Contin Educ Dent 21 (2000): 59-62, 64, 66.
- Cowan, M.M. Plant products as antimicrobial agents. Clin Microbiol Rev 12 (1999): 564-82.
- Doan, N., Contreras, A., Flynn, J., Morrison, J. and Slots, J. Proficiencies of three anaerobic culture systems for recovering periodontal pathogenic bacteria. J Clin Microbiol 37 (1999): 171-4.
- Dzink, J.L., and Socransky, S.S. Comparative in vitro activity of sanguinarine against oral microbial isolates. Antimicrob Agents Chemother 27 (1985): 663-665.

- Fives-Taylor, P.M., Meyer, D.H., Mintz, K.P. and Brissette, C. Virulence factors of *Actinobacillus actinomycetemcomitans*. Periodontol 2000 20 (1999) :136-67.
- Goldstein, E. J., Citron, D.M., Warren, Y., Merriam, C.V., Tyrrell, K. and Fernandez, H. In vitro activities of iodonium salts against oral and dental anaerobes. Antimicrob Agents Chemother 48 (2004): 2766-70.
- Goodson, J.M., and Tanner, A. Antibiotic resistance of the subgingival microbiota following local tetracycline therapy. Oral Microbiol Immunol 7 (1992): 113-7.
- Gopalakrishnan, G., Banumathi, B., and Suresh, G. Evaluation of the antifungal activity of natural xanthenes from *Garcinia mangostana* and their synthetic derivatives. J Nat Prod 60 (1997): 519-24.
- Greenstein, G., and Polson, A. The role of local drug delivery in the management of periodontal diseases: a comprehensive review. J Periodontol 69 (1998): 507-20.
- Hannah, J.J., Johnson, J.D., and Kuflinec, M.M. Long-term clinical evaluation of toothpaste and oral rinse containing sanguinaria extract in controlling plaque, gingival inflammation, and sulcular bleeding during orthodontic treatment. Am J Orthod Dentofacial Orthop 96 (1989): 199-207.
- Hiranras, P. Formulation of *Garcinia mangostana* Linn. extract buccal mucoadhesive film. Master's Thesis, Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 2001.
- Hirasawa, M., Shouji, N., Neta, T., Fukushima, K. and Takada, K. Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (Shiitake, an edible mushroom). Int J Antimicrob Agents 11 (1999): 151-7.
- Hirasawa, M., Takada, K., Makimura, M. and Otake, S. Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. J Periodontal Res 37 (2002): 433-8.
- Holt, S.C., Kesavalu, L., Walker, S., and Genco, C.A. Virulence factors of *Porphyromonas gingivalis*. Periodontol 2000 20 (1999): 168-238.
- Hwang, J.K., Chung, J.Y., Baek, N.I. and Park, J.H. Isopanduratin A from *Kaempferia pandurata* as an active antibacterial agent against cariogenic *Streptococcus mutans*. Int J Antimicrob Agents 23 (2004): 377-381.

- linuma, M., Tosa, H., Tanaka, T., Asai, F., Kobayashi, Y., Shimano, R., et al.  
Antibacterial activity of xanthenes from guttiferaceous plants against methicillin-resistant *Staphylococcus aureus*. J Pharm Pharmacol 48 (1996): 861-5.
- Ingraham, J.L., and Ingraham, C.A. Introduction to Microbiology. A Case History Approach. 3 ed. California: Peter Marshall, 2004.
- Jones, C.G. Chlorhexidine: is it still the gold standard?. Periodontol 2000 15 (1997): 55-62
- Karnjanachotdamrong, P. Mangostin gel from *Garcinia mangostana* fruit. Senior Project, Pharmacy, Faculty of Pharmaceutical Science, Chulalongkorn University, 2000.
- Kongchunmitkul, W. Preparation and evaluation of antibacterial activity of mangosteen rind extract throat spray. Master's Thesis, Pharmaceutical Technology, Faculty of Pharmaceutical Science, Chiangmai University, 2002.
- Kusuma, K. A comparison study of the foot ulcer healing between dressing with mangostin cream and normal saline wet dressing in diabetic patient. Master's Thesis, Adult Nursing, Faculty of Nursing, Mahidol University, 2003.
- Loesche, W.J. Oxygen sensitivity of various anaerobic bacteria. Appl Microbiol 18 (1969): 723-7.
- Loesche, W.J. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 50 (1986): 353-80.
- Mahabusarakam, W., Iriyachitra, P., and Taylor, W.C. Chemical constituents of *Garcinia mangostana*. J Nat Prod 50 (1987): 474-8.
- Mahabusarakum, W., Phongpaichit, S., Jansakul, C., and Wiriyaichitra, P. Screening of antibacterial activity of chemicals from *Garcinia mangostana*. Songkhla Nakkharin J 5 (1983): 337-9.
- Mahabusarakum, W., Phongpaichit, S., and Wiriyaichitra, P. Antibacterial activities of chemicals constituent from *Garcinia mangostana*. Linn. J Sci Soc Thailand 12 (1986): 239-242.

- Meurman, J. H., Jousimies-Somer, H., Suomala, P., Alaluusua, S., Torkko, H. and Asikainen, S. Activity of amine-stannous fluoride combination and chlorhexidine against some aerobic and anaerobic oral bacteria. Oral Microbiol Immunol 4 (1989):117-9.
- Mombelli, A., and Samaranayake, L.P. Topical and systemic antibiotics in the management of periodontal disease. Int Dent J 54 (2004): 3-14.
- Moongkarndi, P., Kosem, N., Kaslungks, S., Luanratana, O., Pongpan, N., and Neungton, N. Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. J Ethnopharm 90 (2004): 161-6.
- Mueller, M., de la Pena, A. and Derendorf, H. Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: kill curves versus MIC. Antimicrob Agents Chemother 48 (2004): 369-77
- Murray, P.R. and Niles, A.C. Effect of incubation conditions on anaerobic susceptibility testing results. J Clin Microbiol 16 (1982): 1152-54.
- Nakatani, K., Arakawa, T., Oosawa, K., Shimura, S., Atsumi, M., and Ohizumi, Y. Inhibitions of histamine release and prostaglandin E<sub>2</sub> synthesis by mangosteen, a Thai medicinal plant. Biol Pharm Bull 25 (2002b): 1137-41.
- Nakatani, K., Arakawa, T., Yasuda, H., and Ohizumi, Y. Inhibition of cyclooxygenase and prostaglandin E<sub>2</sub> synthesis by gamma-mangostin, a xanthone derivative in mangosteen in C6 rat glioma cells. Biochem Pharmacol 63 (2002a): 73-9.
- Olsen, I., Shah, H.N., and Gharbia, S.E. Taxonomy and biochemical characteristics of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. Periodontol 2000 20 (1999): 14-52.
- Overholser, CD.Jr. Longitudinal clinical studies with antimicrobial mouthrinses. J Clin Periodontol 15 (1988): 517-9.
- Pfaller, M.A., Sheehan, D.J. and Rex, J.H. Determination of fungicidal activities against yeasts and molds: lessons learned from bactericidal testing and the need for standardization. Clin Microbiol Rev. 17 (2004): 268-80.

- Prescott, L.M., Harley, J.P. and Klein, D.A. MICROBIOLOGY. 5 ed. Newyork : McGraw-Hill Companies, 2003.
- Sapwarobol, S. Effects of xanthones extracted from rind of *Garcinia mangostana* in isolated rat hepatocytes. Master's Thesis, Pharmacology, Faculty of Pharmaceutical Science, Chulalongkorn University, 1997.
- Seip, W.F., and Evans, G.L. Atmospheric analysis and redox potentials of culture media in the GasPak System. J Clin Microbiol 11 (1980): 226-33.
- Sindermsuk, J., and Deekijsermphonng, S. The antibacterial activities of crude extract from the fruit hull of *Garcinia Mangostana* on enteric pathogens and intestinal commensal. Bull Dept Med Serv 14 (1989): 421-26.
- Somprasit, A., Sripiyaratnanakul, K., Chuay-Yim, P., and Tanakittithum, P. Preliminary toxicological study of mangostin. Songklanakarin J Sci Technol 9 (1987): 51-7.
- Suksamrarn, S., Suwannapoch, N., Phakhodee, W., Thanuhiranlert, J., Ratananukul, P., Chimnoi, N., et al. Antimycobacterial activity of prenylated xanthones from the fruits of *Garcinia mangostana*. Chem Pharm Bull (Tokyo) 51 (2003): 857-9.
- Sundaram, B.M., Gopalakrishnan, C., Subramanian, S., Shankaranarayanan, D., and Kameswaran, L. Antimicrobial activities of *Garcinia mangostana*. Planta Med 48 (1983): 59-60.
- Taweechaisupapong, S., Wongkham, S., Chareonsuk, S., Suparee, S., Srilalai, P., and Chaiyarak, S. Selective activity of *Streblus asper* on Mutans streptococci. J Ethnopharmacol 70 (2000): 73-9.
- Talaro, K.P. and Taralo, A. Foundation in Microbiology international edition. 4 ed. Newyork: McGraw-Hill Companies, 2002.
- Twetman, S. Antimicrobials in future caries control? A review with special reference to chlorhexidine treatment. Caries Res 383 (2004):223-9.
- Williams. P., Ongsakul, M., Proudfoot, J., Croft, K., and Beilin, L. Mangostin inhibits the oxidative modification of human low density lipoprotein. Free Radic Res 23 (1995): 175-84.

Zambon, J.J. Periodontal diseases: microbial factors. Ann Periodontol 1 (1996): 879-925.

Zambon, J.J., and Kasprzak, S.A. The microbiology and histopathology of human root caries. Am J Dent 8 (1995): 323-8.

## APPENDICES

## APPENDIX A

### PHOTOGRAPHS OF EXPERIMENTAL PROCEDURES



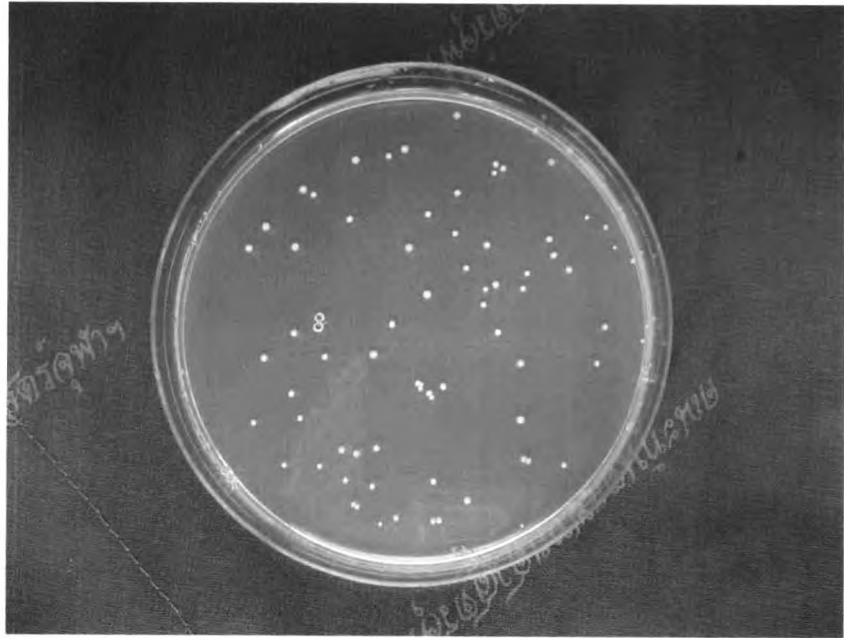


Figure 11. Photograph of mangosteen crude extract.



Figure 12. Photograph of a GasPak 100 system.

(A)



(B)

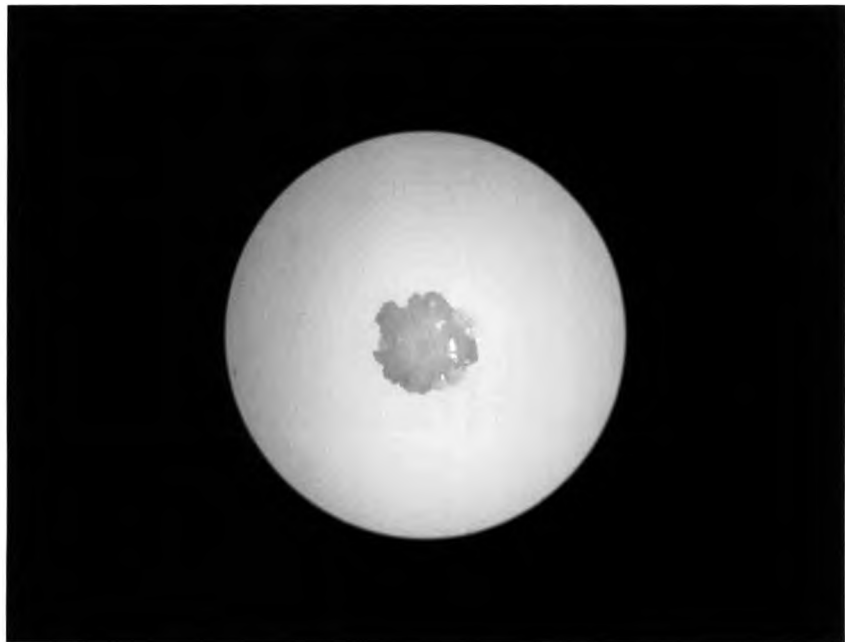


Figure 13. Colonies of *S. mutans* on trypticase soy agar.

(A) Countable bacterial colony on agar plate.

(B) *S. mutans* colony has yellowish-white color, irregular margin with rough and shiny surface. The photograph was inspected by stereoscope with a power of 40x.

(A)



(B)

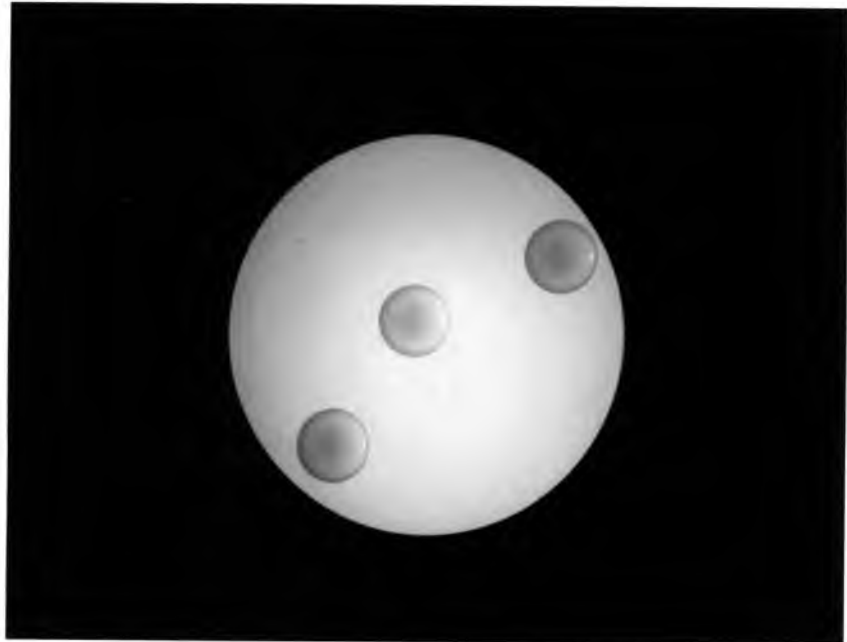
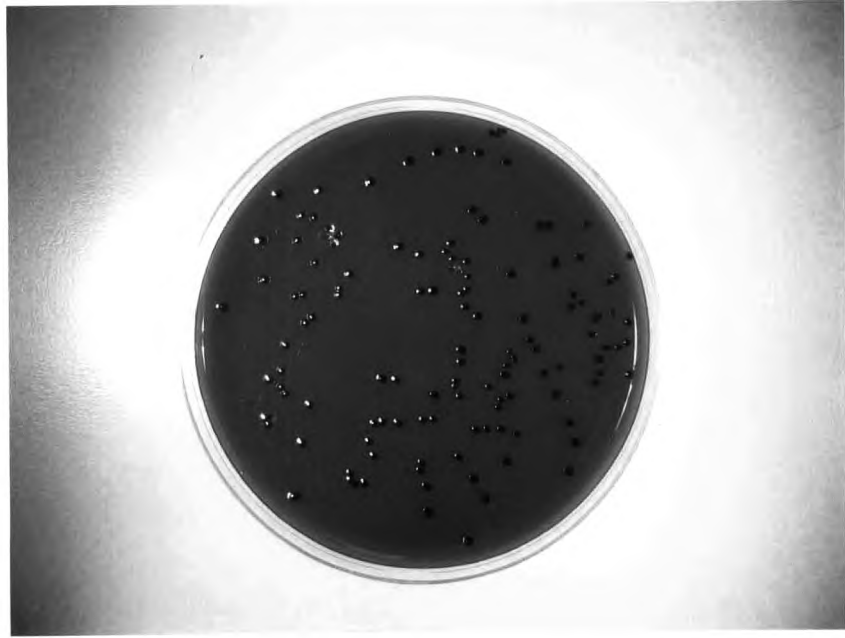


Figure 14. Colonies of *A. actinomycetemcomitans* on brain heart infusion agar.

(A) Countable bacterial colony on agar plate.

(B) *A. actinomycetemcomitans* colony has yellowish-white color, smooth colony with a star shaped inner colony. The photograph was inspected by stereoscope with a power of 40x.

(A)



(B)



Figure 15. Colonies of *P. gingivalis* on brucella blood agar.

(A) Countable bacterial colony on agar plate. *P. gingivalis* has brown to black color, smooth and shiny surface.

(B) *P. gingivalis* shows complete hemolysis on blood agar.

## APPENDIX B

### TABLES OF EXPERIMENTAL RESULTS

**Table 5. The growth pattern of *S. mutans* ATCC 25175 at varying time points after inoculated in the liquid media.**

Time (Hour)	Absorbance at 600 nm	Number of viable bacteria (CFU/ml)	Log <sub>10</sub> CFU/ml
0	0.022	$1.71 \times 10^6$	6.233
1	0.035	$2.07 \times 10^6$	6.316
2	0.061	$1.84 \times 10^7$	7.265
3	0.122	$2.04 \times 10^8$	8.310
4	0.220	$1.60 \times 10^9$	9.203
5	0.480	$1.0 \times 10^9$	9.000
6	0.652	$2.95 \times 10^{10}$	10.470
7	0.790	$9.25 \times 10^{10}$	10.966
8	0.788	$1.03 \times 10^9$	9.013
9	0.748	$3.5 \times 10^7$	7.544

**Table 6. The growth pattern of *A. actinomycetemcomitans* at varying time points after inoculated in the liquid media.**

Time (Hour)	Absorbance at 600 nm	Number of viable bacteria (CFU/ml)	Log <sub>10</sub> CFU/ml
0	0.004	$1.84 \times 10^7$	7.265
1	0.005	$5.70 \times 10^6$	6.756
2	0.006	$4.55 \times 10^7$	7.658
3	0.030	$7.72 \times 10^7$	7.888
4	0.042	$2.48 \times 10^8$	8.394
5	0.082	$3.14 \times 10^8$	8.497
6	0.143	$9.48 \times 10^8$	8.977
7	0.221	$8.36 \times 10^9$	9.922
8	0.318	$5.00 \times 10^9$	9.699
9	0.458	$5.90 \times 10^9$	9.771



**Table 7. Effect of DMSO on the growth of *S. mutans* ATCC 25175.** Initial inoculum of bacteria is  $6.87 \times 10^6$  CFU/ml. Data are presented as means  $\pm$  standard deviations.

DMSO concentration (g/L)	Log <sub>10</sub> CFU/ml
0	9.55 $\pm$ 0.569
3.3	9.67 $\pm$ 0.197
6.6	9.28 $\pm$ 0.297

The differences in number of viable cells were analyzed by analysis of variance. No significant differences in viable cell count (log<sub>10</sub>CFU/ml) in the DMSO groups as compared to the broth control. (P> 0.05 )

**Table 8. Effect of DMSO on the growth of *P. gingivalis*.** Initial inoculum of bacteria is  $2.0 \times 10^8$  CFU/ml. Data are presented as means  $\pm$  standard deviations.

DMSO concentration (g/L)	Log <sub>10</sub> CFU/ml
0	9.55 $\pm$ 0.008
3.3	9.63 $\pm$ 0.056
6.6	9.33 $\pm$ 0.163

The differences in number of viable cells were analyzed by Kruskal- Wallis test. No significant differences in viable cell count (log<sub>10</sub>CFU/ml) in the DMSO groups as compared to the broth control. (P> 0.05 )

**Table 9. Identifying MIC and MBC of mangosteen crude extract, chlorhexidine and DMSO against *A. actinomycetemcomitans*.**

The table presented absorbance at 600 nm (OD.) and viable cell count ( $\log_{10}$ CFU/ml) of broth containing mangosteen crude extract, chlorhexidine and DMSO.

The number of initial bacteria inoculum was  $1.91 \times 10^6$  CFU/ml (=6.28  $\log_{10}$ CFU/ml).

Mangosteen conc. ( $\mu$ g/ml)	Conc. of DMSO (g/l)	OD. of blank	OD. of mangosteen crude extract	$\log_{10}$ CFU/ml
640	26.4	1.994	> 3	8.48
320	13.2	1.136	1.171	ND
160	6.6	0.765	0.779	ND
80	3.3	0.342	0.535	ND
40	1.65	0.184	0.448	ND
Conc. of Chlorhexidine	-	OD. of blank	OD. of Chlorhexidine	$\log_{10}$ CFU/ml
5	-	0	0	0
2.5	-	0	0	0
1.25	-	0	0	> 5
0.625	-	0	0.240	> 5
0.312	-	0	0.231	ND
Control	-	0.303	-	8.05

**Table 10. Identifying MIC and MBC of mangosteen crude extract and chlorhexidine against *S. mutans* ATCC 25175.**

The table presented absorbance at 600 nm(OD.) and viable cell count ( $\log_{10}$ CFU/ml) of broth containing mangosteen crude extract or chlorhexidine.

The number of initial bacterial inoculum was  $1.885 \times 10^6$  cfu/ml (=6.27  $\log_{10}$ CFU/ml).

Mangosteen conc. ( $\mu\text{g/ml}$ )	OD. of mangosteen crude extract	$\text{Log}_{10}$ CFU/ml
5	0.013	0
2.5	0.015	0
1.25	0.018	0
0.625	0.016	0
0.312	0.820	ND
alpha-mangostin conc. ( $\mu\text{g/ml}$ )	OD. of alpha-mangostin	$\text{Log}_{10}$ CFU/ml
5	0.004	0
2.5	0.002	0
1.25	0.002	0
0.625	0.001	0
0.312	0.451	> 5
Chlorhexidine conc. ( $\mu\text{g/ml}$ )	OD. of chlorhexidine	$\text{Log}_{10}$ CFU/ml
5	0.000	0
2.5	0.018	0
1.25	0.006	0
0.625	0.005	0
0.312	0.014	0
0.156	0.886	ND
Control	1.015	8.04

**Table 11. Identifying MIC and MBC of mangosteen crude extract and chlorhexidine against *S. mutans* KPSK<sub>2</sub>.**

The table presented absorbance at 600 nm(OD.) and viable cell count ( $\log_{10}$ CFU/ml) of broth containing mangosteen crude extract or chlorhexidine.

The number of initial bacterial inoculum was  $1.825 \times 10^6$  cfu/ml (=6.26  $\log_{10}$ CFU/ml).

Mangosteen conc. ( $\mu$ g/ml)	OD. of mangosteen crude extract	$\log_{10}$ CFU/ml
5	0	0
2.5	0	0
1.25	0.002	0
0.625	0.009	> 5
0.312	0.765	ND
Chlorhexidine conc. ( $\mu$ g/ml)	OD. of chlorhexidine	$\log_{10}$ CFU/ml
2.5	0	0
1.25	0	0
0.625	0.016	0
0.312	0.074	4.20
0.156	0.580	ND
Control	0.811	8.92

**Table 12. Identifying MIC and MBC of mangosteen crude extract and chlorhexidine against *P. gingivalis* W 50.**

The table presented absorbance at 600 nm(OD.) and viable cell count ( $\log_{10}$ CFU/ml) of broth containing mangosteen crude extract and chlorhexidine.

Initial number of bacteria  $1.589 \times 10^8$  cfu/ml (=8.2  $\log_{10}$ CFU/ml).

Mangosteen conc. ( $\mu\text{g/ml}$ )	OD. of blank	OD. of mangosteen extract	$\text{Log}_{10}$ CFU/ml
80	.081	.068	0
40	.044	.031	0
20	.031	.013	5.92
10	.026	.118	> 5
5	.010	1.300	ND
Chlorhexidine conc. ( $\mu\text{g/ml}$ )	OD. of blank	OD. of Chlorhexidine	$\text{Log}_{10}$ CFU/ml
5	0	0	0
2.	0	0	0
1.25	0	.008	> 5
0.625	0	.031	ND
Control	1.889	-	9.18

**Table 13.** Time-kill kinetics of *S. mutans* ATCC 25175 when treated with mangosteen crude extract at 2x and 4x MBC compared to chlorhexidine at the same concentrations. The data are presented as  $\log_{10}$  reduction in CFU/ml compared to the broth control at each time point (means  $\pm$  standard deviations).

Time (minutes)	2xMBC (1.25 $\mu$ g/ml)		4xMBC ( 2.5 $\mu$ g/ml)	
	Mangosteen	Chlorhexidine	Mangosteen	Chlorhexidine
30	0.06 $\pm$ 0.030	0.05 $\pm$ 0.149	0.39 $\pm$ 0.203	0.20 $\pm$ 0.246
60	0.66 $\pm$ 0.356	0.20 $\pm$ 0.112	1.85 $\pm$ 0.643	0.31 $\pm$ 0.088
90	0.89 $\pm$ 0.267	0.32 $\pm$ 0.238	6.94 $\pm$ 0.292	0.47 $\pm$ 0.109



**Table 14.** Time-kill kinetics of *P. gingivalis* when treated with mangosteen crude extract at 2x and 4x MBC compared to chlorhexidine at the same concentrations. The data are presented as log<sub>10</sub> reduction in CFU/ml compared to the broth control at each time point (means  $\pm$  standard deviations).

Time (minutes)	2xMBC (80 $\mu$ g/ml)		4xMBC ( 160 $\mu$ g/ml)	
	Mangosteen	Chlorhexidine	Mangosteen	Chlorhexidine
5	0.28 $\pm$ 0.389	0.55 $\pm$ 0.341	1.20 $\pm$ 0.220	1.72 $\pm$ 0.699
15	1.74 $\pm$ 0.099	2.37 $\pm$ 0.975	6.71 $\pm$ 0.306	6.71 $\pm$ 0.306
30	6.91 $\pm$ 0.147	6.91 $\pm$ 0.147	6.91 $\pm$ 0.147	6.91 $\pm$ 0.147

## BIOGRAPHY

Miss Piraporn Vichienroj was born on the third of February, 1977. She is a graduate of the Faculty of Dentistry, Chulalongkorn University since 2000. She worked at Sakaew Hospital from 2000 to 2002. She has studied in a Master degree program in Periodontology at Graduate School, Chulalongkorn University in 2002. Now she works in private hospital and private clinic.

