

# Antibacterial Activity of Mangosteen Pericarp Extract against Periodontal Pathogens

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**Abstract**

Extract from mangosteen pericarp has been known for its antibacterial activity against several pathogens that cause skin infection, diarrhea, tuberculosis, acne and dental caries. The purpose of this study was to examine the activity of mangosteen pericarp extract against bacteria associated with periodontal disease. Results showed that the extract was effective against *Porphyromonas gingivalis* and *Tannerella forsythia*, but not *Actinobacillus actinomycetemcomitans*. The minimum inhibitory concentration and minimum bactericidal concentration (MBC) for *P. gingivalis* were 20 and 40 µg/ml, respectively. The respective values for *T. forsythia* were 10 and 20 µg/ml. Time-kill assays for *P. gingivalis* showed that treatment with the extract at 2x MBC caused decreases in viable count of almost 2 orders of magnitude after 15 minutes, and viable organisms were not detected after 30 minutes. When the extract concentration was increased to 4x MBC, the bacteria were completely inactivated in only 15 minutes. The rate of bacterial killing was comparable to that of chlorhexidine, an antiseptic commonly used in periodontal therapy. The strong antibacterial activity of mangosteen pericarp extract against periodontal pathogens makes it a promising new agent for the prevention and treatment of periodontal disease.

**Key words:** antibacterial activity; *Garcinia mangostana*; periodontal pathogens; *Porphyromonas gingivalis*

**Introduction**

Periodontal disease is an infectious disease caused by a group of Gram-negative anaerobic bacteria in dental plaque. According to the Consensus Report of the 1996 World Workshop on Clinical Periodontics, *Porphyromonas gingivalis*, *Tannerella forsythia* (formerly *Bacteroides forsythus*) and *Actinobacillus actinomycetemcomitans*, have been implicated in the etiology of periodontal disease. These bacteria are capable of producing a number of virulence factors, which are directly toxic to host tissues or immune cells,

or indirectly damage host tissues via induction of inflammatory cytokines.<sup>1</sup>

Numerous antibiotics and antiseptics like chlorhexidine, tetracycline, amoxicillin, metronidazole, etc. have been used to reduce dental plaque bacteria associated with periodontal disease. However, they can cause undesirable side effects such as allergic reaction, nausea, vomiting, diarrhea, unpleasant taste or tooth staining.<sup>2</sup> The excessive use of these chemicals also results in the development of drug resistance.<sup>3</sup> In addition, the cost for research and development of new drugs is quite high, making them too expensive for developing countries. These problems justify further search for a new source of antimicrobial agents. Medicinal plants have recently attracted attention because they confer natural antibacterial activity, and have been traditionally used in folk medicine to treat infectious diseases. They also have less tendency to acquire resistance, and can be produced at low cost.<sup>4</sup>

*Garcinia mangostana* Linn., commonly known as mangosteen, is a fruit tree found in Southeast Asia and South India. It has been used in Thai traditional medicine for treatment of diarrhea, skin infection and chronic wound.<sup>5</sup> Extract from its pericarp has demonstrated antibacterial activity against a wide variety of microorganisms including *Streptococcus mutans*, *Staphylococcus aureus* (both normal and methicillin-resistant), *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterococcus* species, *Mycobacterium tuberculosis* and *Propionibacterium acnes*.<sup>6-13</sup> Phytochemical studies have shown that its active components belong to a group of xanthone derivatives such as  $\alpha$ -,  $\beta$ - and  $\gamma$ -mangostin, gartinin, 1- and 3-isomangostin, etc.<sup>14</sup> Among these,  $\alpha$ -mangostin has the most potent antibacterial activity.<sup>7-11,13</sup>

Previous studies, both *in vitro* and *in vivo*, have demonstrated low toxicity of the mangosteen pericarp extract and its active components.<sup>15-19</sup> Alpha-mangostin, an active component of the extract, was administered orally to rats at a high dose (1.5 g/kg body weight) to test its hepatotoxicity. It was found that after 12 hours, increases in serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities were much less than those of paracetamol given at the same dose.<sup>15</sup> Another study used xanthenes isolated from mangosteen pericarp given to rats at an oral dose of 100 mg/kg body weight/day, and did not observe any toxicities after 7 days of treatment.<sup>16</sup> In human clinical trials, 1.5%  $\alpha$ -mangostin cream was locally applied on skin of patients with chronic ulcers for up to 3 weeks. No local irritation or side effects were observed.<sup>17,18</sup>

From its broad-spectrum antibacterial activity and low toxicity, the mangosteen pericarp extract may have potential for periodontal therapy. However, its actions on periodontal pathogens have never

been demonstrated. The purpose of this study was to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and kinetics of killing of crude extract from mangosteen pericarp against *P. gingivalis*, *T. forsythia* and *A. actinomycetemcomitans*. Its activity was also compared to those of  $\alpha$ -mangostin, an active component of the extract, and chlorhexidine, an antiseptic commonly used in periodontal treatment.

## Materials and methods

### Preparation of mangosteen pericarp crude extract and $\alpha$ -mangostin

Pericarps of mangosteen were collected from Thewate market in Bangkok in July 2003. Crude extract and purified  $\alpha$ -mangostin were prepared as previously described.<sup>20</sup> Briefly, dried and ground pericarps were macerated in hexane for 24 hours to remove non-polar substances. The resulting marc was subsequently macerated in ethyl acetate for 24 hours. The ethyl acetate extract was then recrystallized, and ground into powder. The yield of mangosteen crude extract from the dried pericarp was approximately 3% (w/w).

To obtain  $\alpha$ -mangostin, the crude extract was chromatographed on a silica gel column, and eluted with increasing percentages of ethyl acetate in hexane (0-25%). An hexane-ethyl acetate (4:1) eluate was selected based on the thin layer chromatography profile. The selected fraction was further identified as  $\alpha$ -mangostin by using mass spectrometry, nuclear magnetic resonance spectroscopy and a Gallenkamp melting point apparatus. The yield of  $\alpha$ -mangostin from the dried pericarp was approximately 0.4% (w/w).

### Bacterial culture

Bacterial strains used in this study were *P. gingivalis* ATCC 53978 (W50), *T. forsythia* ATCC 43037 and *A. actinomycetemcomitans* ATCC 43718 (Y4). *A. actinomycetemcomitans* was cultured in an incubator containing 5-7% carbon dioxide at 37°C. *P. gingivalis* and *T. forsythia* were grown anaerobically in a GasPak system (BBL Microbiology Systems, Cockeysville, MD, USA) at 37°C.

Growth in liquid media: All bacteria were cultured in trypticase soy broth (BBL Microbiology Systems). The broth for *P. gingivalis* was supplemented with 5% fetal bovine serum (Life Technology, Paisley, Scotland), 5 mg/l hemin (Sigma Chemical Co., St. Louis, MO, USA) and 0.1 mg/l vitamin K (Atlantic Laboratories, Corp., Ltd., Bangkok, Thailand). The broth for *T. forsythia* was prepared in a similar fashion, but also supplemented with 15 mg/l N-acetyl muramic acid (NAM; Sigma Chemical Co.).

Growth on solid media: *P. gingivalis* was grown on Brucella blood agar (BBL Microbiology Systems) supplemented with 5% human whole blood, 5 mg/l hemin and 0.1 mg/l vitamin K. The agar for *T. forsythia* was the same as that for *P. gingivalis*, but also supplemented with 15 mg/l NAM. *A. actinomycetemcomitans* was grown on brain heart infusion agar (Difco Laboratories, Detroit, MI, USA).

#### **MIC determination**

MIC was determined by a broth dilution method. Mangosteen pericarp crude extract or  $\alpha$ -mangostin was dissolved in dimethyl sulfoxide (DMSO), and subsequent two-fold serial dilutions were performed in the culture medium. Chlorhexidine digluconate was used as a positive control, and was serially diluted in a similar fashion. Medium without extract served as a control for bacterial growth. Each tube was inoculated with bacteria obtained during the logarithmic phase of growth. The initial density of bacteria was approximately  $2.5 \times 10^6$  colony forming units (CFU)/ml. After 24-hour incubation, MIC was recorded as the lowest concentration that limited the turbidity of the broth to  $< .05$  at the absorbance of 600 nm. Solvent controls were also included, though no significant effect on bacterial growth was observed at the highest concentration employed.

#### **MBC determination**

MBC was determined by comparing the number of remaining viable bacteria with the initial number of bacteria. All tubes from the MIC experiments that showed no visible turbidity were serially diluted and spread onto agar plates for viable cell counting. The plates were incubated for 24-48 hours for *A. actinomycetemcomitans* and 72-96 hours for *P. gingivalis* and *T. forsythia*. MBC was then recorded as the lowest concentration that killed at least 99.99% of the initial number of bacteria. All MIC and MBC experiments were repeated three times.

#### **Time-kill kinetics**

Time-kill kinetics was determined by the number of remaining viable bacteria at varying time points after exposure to the mangosteen pericarp extract at the concentrations of two or four times of MBC. After exposure for 5, 15 or 30 minutes, the samples were diluted at least 10 folds to arrest antibacterial activity and to reduce a carry-over. The suspensions were then transferred onto agar plates for viable cell counting. The control broth without extract was served as a control for bacterial growth at each time point. Time-kill curve was plotted as logarithm of the number of remaining viable bacteria ( $\log_{10}$  CFU/ml) against time. Chlorhexidine digluconate at the same

concentrations was used as a positive control. The sensitivity limit of detection was  $10^3$  CFU/ml. All assays were performed four to five times.

#### **Statistical analysis**

All statistical computations were performed by SPSS for Windows software (version 10.0; SPSS Inc., Chicago, IL). Data from time-kill kinetics were presented as means and standard deviations. Differences in viable bacterial count at each time point were analyzed by one-way analysis of variance (ANOVA). To compare the differences between groups, Tukey's post hoc test was used. The chosen level of significance was  $p < .05$ .

## **Results**

#### **MIC and MBC of mangosteen pericarp extract**

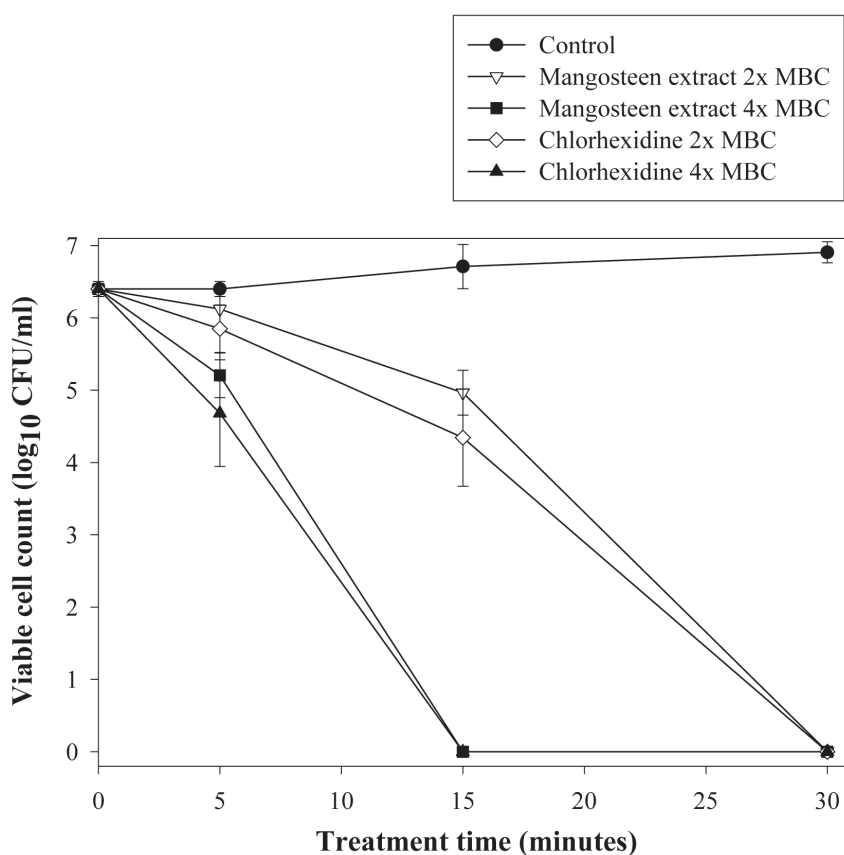
The mangosteen pericarp extract was active against *P. gingivalis* and *T. forsythia*, but not *A. actinomycetemcomitans* (Table 1). The MIC and MBC for *P. gingivalis* were 20 and 40  $\mu$ g/ml, respectively. Those values for *T. forsythia* were 10 and 20  $\mu$ g/ml, respectively. Alpha-mangostin, an active component of the extract, was also effective against the same microorganisms, with the MIC and MBC comparable to those of the crude extract. Chlorhexidine, a positive control used in this study, was strongly active against all tested microorganisms including *A. actinomycetemcomitans*. Its MIC and MBC for *P. gingivalis* and *T. forsythia* were lower than those of the mangosteen pericarp extract.

#### **Time-kill assays**

To determine the rates at which bacteria were killed, they were exposed to the extract at the concentrations of 2x and 4x MBC for 5, 15 and 30 minutes (Fig. 1). Only *P. gingivalis* was tested because *T. forsythia* was difficult to grow in broth culture. At 5 minutes, the group treated with the extract at 2x MBC showed a slight decrease in viable cell count ( $\log_{10}$  CFU/ml), while the extract at 4x MBC decreased viable cell count by one order of magnitude. Only the latter group reached statistical significance. At 15 minutes, the group treated with the extract at 2x MBC showed a significant decrease in viable cell count by almost two orders, while the extract at 4x MBC completely killed the bacteria. At 30 minutes or longer, the extract at both concentrations completely killed the bacteria. The time-kill kinetics of chlorhexidine was similar to that of mangosteen pericarp extract. When compared at the same concentration and at the same time point, no significant difference in the number of remaining viable bacteria was observed between groups.

**Table 1** The MIC and MBC ( $\mu\text{g/ml}$ ) of mangosteen pericarp extract against periodontal pathogens compared to those of  $\alpha$ -mangostin and chlorhexidine

Periodontal pathogens	Crude extract		$\alpha$ -mangostin		Chlorhexidine	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>P. gingivalis</i>	20	40	20	40	1.25	2.5
<i>T. forsythia</i>	10	20	10	20	5	10
<i>A. actinomycetemcomitans</i>	>640	>640	>640	>640	1.25	2.5



**Fig. 1** Time-kill curve for *P. gingivalis* plotted as logarithm of the number of remaining viable cells ( $\log_{10}$  CFU/ml) against time. The bacteria were treated with mangosteen pericarp extract at 2x and 4x MBC compared to chlorhexidine at the same concentrations. The results are presented as means  $\pm$  standard deviations of four to five independent experiments.

## Discussion

The extract from mangosteen pericarp has been known for its strong antibacterial activity against a wide variety of Gram-positive and Gram-negative bacteria, especially those associated with skin infection, diarrhea, tuberculosis, acne and dental caries.<sup>6-12</sup> In the present study, the extract was also effective against selected periodontal pathogens including *P. gingivalis* and *T. forsythia*. However, no appreciable activity was observed against *A. actinomycetemcomitans*. The MIC values for these periodontal pathogens were 10-20 µg/ml, which were in the same range as those values obtained for other microorganisms in previous studies (1-50 µg/ml).<sup>6-12</sup>

When compared to crude extracts from other known medicinal plants, the mangosteen pericarp extract conferred much stronger antibacterial activity. A crude methanol extract from clove (*Syzygium aromaticum*), for example, was active against *P. gingivalis* at MIC of 625 µg/ml. The MIC of its purified compounds, kaempferol and myricetin, was 20 µg/ml.<sup>21</sup> A crude methanol extract from the native American plant *Ceanothus americanus* was effective against *P. gingivalis* at MIC of 1,250 µg/ml. The MIC of its purified compound, ceanothic acid, was 62 µg/ml.<sup>22</sup> Therefore, the antibacterial activity of the mangosteen pericarp crude extract was comparable to those observed in the purified compounds from other plant extracts.

Mangosteen pericarp extract contains several xanthenes such as α-, β- and γ-mangostin, gartinin, 1- and 3-isomangostin, etc.<sup>14</sup> The chemical components of the crude extract often vary depending upon the extraction protocol. When using 40% ethanol as a solvent, the extract contained 10% α-mangostin and 12% γ-mangostin. When ethanol concentration was increased to 100%, the yield of γ-mangostin was increased to 55%.<sup>23</sup> Another study using ethyl acetate as a solvent reported that the extract was composed of 77.8% α-mangostin and 15.9% γ-mangostin.<sup>24</sup> The crude extract in this study used a similar extraction protocol as the latter study, and contained approximately 80% α-mangostin.<sup>20</sup>

Among xanthone derivatives from mangosteen pericarp extract, α-mangostin has been shown by several studies to exert the most potent antibacterial activity.<sup>7-11,13</sup> In the present study, the mangosteen pericarp crude extract contained a high percentage of α-mangostin.<sup>20</sup> Its MIC and MBC were also equivalent to those of α-mangostin, suggesting that its antibacterial activity was largely attributed to this purified compound. The crude extract can be produced at lower cost and shorter preparation time than α-mangostin.<sup>20</sup> Therefore, it is more suitable to be commercially

developed as a new antibacterial agent.

An antibacterial agent is considered bactericidal if its MBC is equal or similar to the MIC.<sup>25</sup> The MBC of the mangosteen pericarp extract was only two times the MIC, suggesting that it acted bactericidally against periodontal pathogens. To determine the optimal treatment concentration and treatment time to obtain sufficient bactericidal effect, the time-kill assays for *P. gingivalis* were performed. Treatment with the extract at 2x MBC (80 µg/ml) significantly reduced the viable count (log<sub>10</sub> CFU/ml) of bacteria by almost two orders of magnitude in 15 minutes and completely killed the bacteria within 30 minutes. When the extract concentration was increased, its antibacterial activity increased as shown by the shorter contact time required to inactivate the bacteria. At 4x MBC (160 µg/ml), the viability of the bacteria was significantly decreased by one order of magnitude in 5 minutes, and was completely lost within only 15 minutes. These results suggest that the extract confers strong and rapid bactericidal activity against periodontal pathogens.

Chlorhexidine, a positive control used in this study, is one of the most effective antiseptics in periodontal therapy. However, it can cause undesirable side effects such as tooth staining, unpleasant taste and increased calculus formation.<sup>2</sup> The MIC values obtained for chlorhexidine in the present study resembled those previously published.<sup>26</sup> When MIC and MBC were compared, the mangosteen pericarp extract was less effective than chlorhexidine. However, at the concentrations of 80 and 160 µg/ml (2x and 4x MBC of the extract, respectively), their bacterial killing rates were comparable. Our previous study has shown that the mangosteen pericarp extract can be used at the concentrations up to 200 µg/ml without affecting cell viability.<sup>19</sup> Therefore, it may be used with safety as an antibacterial agent for periodontal therapy.

In conclusion, the crude extract from mangosteen pericarp was effective against periodontal pathogens including *P. gingivalis* and *T. forsythia*. The rate of bacterial killing was comparable to that of chlorhexidine. The strong and rapid bactericidal activity of the extract suggests that it is a good candidate for further development as an antibacterial agent in the prevention and treatment of periodontal disease.

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## บทวิทยากร

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### บทคัดย่อ

สารสกัดจากเปลือกมังคุดมีฤทธิ์ต้านแบคทีเรียหลายชนิดโดยเฉพาะที่เกี่ยวข้องกับการติดเชื้อของผิวหนัง ท้องร่วง วัณโรค การเกิดสิว และโรคฟันผุ วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาผลของสารสกัดต่อเชื้อซึ่งมีบทบาทสำคัญทำให้เกิดโรคปริทันต์อักเสบ ผลการศึกษาพบว่าสารสกัดจากเปลือกมังคุดมีฤทธิ์ต้านเชื้อพอร์ไฟโรโมแนส-จินจิवालิส และแทนเนอเรลลา ฟอร์ไซเทีย แต่ไม่มีผลต่อเชื้อแอกติโนแบซิลัส แอกติโนไมซีเตมโคมิแทนส์ โดยความเข้มข้นต่ำสุดในการยับยั้งการเจริญเติบโต และในการฆ่าเชื้อพอร์ไฟโรโมแนส จินจิवालิส มีค่าเท่ากับ 20 และ 40 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ ค่าดังกล่าวสำหรับเชื้อแทนเนอเรลลา ฟอร์ไซเทียคือ 10 และ 20 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ จากการประเมินระยะเวลาที่ใช้ในการฆ่าเชื้อพอร์ไฟโรโมแนส-จินจิवालิส พบว่า สารสกัดจากเปลือกมังคุดที่ความเข้มข้น 2 เท่าของความเข้มข้นต่ำสุดในการฆ่าเชื้อ สามารถฆ่าเชื้อโดยลดจำนวนโคโลนีลงประมาณ 100 เท่าภายใน 15 นาที และฆ่าเชื้อได้ทั้งหมดในเวลา 30 นาที เมื่อเพิ่มความเข้มข้นของสารสกัดเป็น 4 เท่าสามารถฆ่าเชื้อได้ทั้งหมดภายในเวลาเพียง 15 นาที ระยะเวลาที่ใช้ในการฆ่าเชื้อของสารสกัดจากเปลือกมังคุดมีค่าใกล้เคียงกับคลอร์เฮกซิดีน ซึ่งเป็นยาฆ่าเชื้อที่นิยมใช้ในการรักษาทางปริทันต์ การศึกษานี้แสดงให้เห็นว่าสารสกัดจากมังคุดมีประสิทธิภาพสูงในการฆ่าเชื้อก่อโรคปริทันต์อักเสบซึ่งเป็นอาจนำไปสู่การพัฒนาสารตัวใหม่เพื่อใช้ในการป้องกันและรักษาโรคต่อไป