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Antibacterial activity of mangosteen pericarp extract against cariogenic 
*Streptococcus mutans*

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Abstract

**Objective** The purpose of this study was to examine the antibacterial activity of extract from mangosteen pericarp against *Streptococcus mutans*, bacteria associated with dental plaque formation and caries development.

**Materials and methods** Bacterial strains used in this study were *S. mutans* ATCC 25175 and KPSK. Antibacterial activity was expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The rates at which bacteria were killed were also determined by exposure to the extract at 2x and 4x MBC for varying time periods.

**Results** The extract was effective against both strains of *S. mutans*. The MIC and MBC for the strain ATCC 25175 were both equal to 0.625 µg/ml. The respective values for the strain KPSK were 0.625 and 1.25 µg/ml. The MIC and MBC were comparable to those of chlorhexidine, an antiseptic commonly used in dental plaque control. Time–kill assays for the strain ATCC 25175 showed that treatment with the extract at 2x MBC caused a significant decrease in viable count (log₁₀ colony forming units/ml) of almost one order of magnitude after 90 minutes. When the extract concentration was increased to 4x MBC, the viable bacterial count was reduced by almost two orders of magnitude in 60 minutes, and was further decreased to an undetectable level in 90 minutes.
Introduction

Dental plaque is a film of oral microorganisms on the tooth surface that plays an important part in the development of dental caries. Among bacteria in dental plaque, *Streptococcus mutans* is considered the most significant cariogenic bacteria. It is able to change dietary sugars to water-insoluble glucans, which promote bacterial adhesion and plaque development. It is also capable of fermenting dietary sugars into organic acids, resulting in tooth demineralization and caries formation. Therefore, inhibition or elimination of *S. mutans* is important for dental plaque control and caries prevention.

Several antiseptics and natural substances like chlorhexidine, triclosan, essential oils, sanguinarine, green tea polyphenols, etc. have been shown to exhibit antibacterial activity against *S. mutans*. These agents are incorporated into various dental formulations such as toothpaste, mouthrinse, chewing gum, gel and vanish. They are used in adjunct to mechanical cleaning devices, and have been shown to have varying degrees of clinical success for dental plaque control. However, they can cause undesirable side effects. Chlorhexidine, one of the most efficient antimicrobial agents, can cause tooth staining, unpleasant taste and increased calculus formation. Sanguinarine, an alkaloid isolated from *Sanguinaria canadensis*, was reported to be associated with oral leukoplakia. These problems justify further research and development of new antibacterial agents that are strongly active against *S. mutans*, with minimal side effects on the oral tissues.

*Garcinia mangostana* Linn., commonly known as mangosteen, is a fruit tree found in Southeast Asia and South India. It has been used in Thai folk medicine for treatment of diarrhea, skin infection and chronic wound. Extract from its pericarp has demonstrated antibacterial activity against a wide variety of microorganisms including *Staphylococcus aereus* (both normal and methicillin-resistant), *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterococcus* species, *Mycobacterium tuberculosis* and *Propionibacterium acnes*. Phytochemical studies have shown that its active components belong to a group of xanthone derivatives such as α-, β- and γ-mangostin, gartinin, 1- and 3-isomangostin, etc. Among these, α-mangostin has the most potent antibacterial activity.

Previous studies have demonstrated that mangosteen extract has low toxicity when given orally or applied topically. 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. Another study

**Conclusion** Mangosteen pericarp extract possessed strong antibacterial activity against *S. mutans*. These results suggest that the extract may be developed as a new agent for dental plaque control and caries prevention.

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Key words: antibacterial; dental caries; mangosteen; *Streptococcus mutans*
used xanthones isolated from mangosteen pericarps
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. In human clinical trials, 1.5% α-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.

Although mangosteen extract has a broad-
spectrum antibacterial activity, its actions on cariogenic
bacteria 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 S. mutans
ATCC 25175 and KPSK. Its MIC and MBC were
also compared to those of α-mangostin, an active
component of the extract, and chlorhexidine, an
antiseptic commonly used in dental plaque control.

**Materials and methods**

**Preparation of mangosteen crude extract and α-
mangostin**

Pericarps of mangosteen were collected from
Thewate market in Bangkok in July 2003. Crude
extract and purified α-mangostin were prepared as
previously described. 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 α-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
α-mangostin by using mass spectrometry, nuclear
magnetic resonance spectroscopy and a Gallenkamp
melting point apparatus. The yield of α-mangostin
from the dried pericarp was approximately 0.4% (w/w).

**Bacterial culture**

Bacterial strains used in this study were S. mutans
ATCC 25175 and KPSK. They were cultured in
trypticase soy broth and agar (BBL Microbiology
Systems, Cockeysville, MD, USA), and maintained
in an incubator containing 5–7% carbon dioxide at 37°C.

**Identifying MIC**

MIC was determined by a broth dilution method.
Mangosteen extract or α-mangostin was dissolved in
dimethy 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 x 10⁶ colony forming units (CFU)/ml. After 24–hour
incubation, MIC was recorded as the lowest
concentration that limited the turbidity of the broth to
<0.05 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.

**Identifying MBC**

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. 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 exposed to the mangosteen extract at the concentrations of two or four times of MBC, and at a very high concentration (256x MBC). After exposure for the specified times, 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. The sensitivity limit of detection was 10$^3$ CFU/ml. All assays were performed three to five times.

Statistical analysis

All statistical computations were performed by SPSS 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 were determined by independent t test or one-way analysis of variance (ANOVA), followed by Dunnett’s post hoc test. The chosen level of significance was $P < 0.05$.

Results

MIC and MBC of mangosteen extract

The mangosteen pericarp extract was active against both strains of $S$. mutans (Table 1). Both MIC and MBC for the strain ATCC 25175 were equal to 0.625 µg/ml. Those values for the strain KPSK$_2$ were 0.625 and 1.25 µg/ml, respectively. The MIC and MBC of the crude extract were comparable to those of $\alpha$-mangostin, an active component of the extract, and chlorhexidine, an antiseptic commonly used in plaque control.

<table>
<thead>
<tr>
<th>S. mutans</th>
<th>crude extract</th>
<th>$\alpha$-mangostin</th>
<th>chlorhexidine</th>
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<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>ATCC 25175</td>
<td>0.625</td>
<td>0.625</td>
<td>0.625</td>
</tr>
<tr>
<td>KPSK$_2$</td>
<td>0.625</td>
<td>1.25</td>
<td>0.625</td>
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</tbody>
</table>

Time-kill assays

To determine the rates at which bacteria were killed, $S$. mutans strain ATCC 25175 was exposed to the extract at the concentrations of 2x MBC (1.25 µg/ml) and 4x MBC (2.5 µg/ml) for 30, 60 and 90 minutes (Fig. 1). At 30 minutes, the groups treated with the extract at both concentrations showed no significant changes in viable cell count (log$_{10}$ CFU/ml) as compared to the control. At 60 minutes, the group treated with the extract at 2x MBC showed a slight
decrease in viable cell count, while the extract at 4x MBC decreased viable cell count by almost two orders of magnitude. Only the latter group reached statistical significance. At 90 minutes, the extract 2x MBC significantly decreased viable count by almost one order, while the extract at 4x MBC completely killed the bacteria.

To test whether the extract at a very high concentration could reduce the treatment time to within minutes of exposure, the time–kill kinetics was performed using the extract at 256x MBC (160 µg/ml) (Fig. 2). At this concentration, the extract significantly reduced the viable count by one order of magnitude in 5 minutes and completely killed the bacteria within 15 minutes.

Fig. 1 Time–kill curve for *S. mutans* ATCC 25175 treated with mangosteen pericarp extract at 2x and 4x MBC. The graph is plotted as logarithm of the number of remaining viable cells (log₁₀ CFU/ml) against time. The results are presented as means ± standard deviations of four to five independent experiments.
Fig. 2 Time-kill curve for *S. mutans* ATCC 25175 treated with mangosteen pericarp extract at 256x MBC.

The graph is plotted as logarithm of the number of remaining viable cells (log_{10} CFU/ml) against time. The results are presented as means ± standard deviations of three independent experiments.

**Discussion**

The extract from mangosteen pericarp has been known for its broad-spectrum antibacterial activity against several Gram-positive and Gram-negative bacteria, especially those associated with skin infection, diarrhea, tuberculosis or acne. In present study, the extract exhibited strong antibacterial activity against cariogenic *S. mutans*. The MIC and MBC were comparable for both strains of *S. mutans*, and were in the same range as those for other microorganisms (1–50 µg/ml).10-15

Mangosteen pericarp extract contains several xanthones such as α-, β- and γ-mangostin, gartinin, 1- and 3- isomangostin, etc.17 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%. Another study using ethyl acetate as a solvent reported that the extract was composed of 77.8% α-mangostin and 15.9% γ-mangostin. The crude extract in this study used a similar extraction protocol as the latter study, and contained approximately 80% α-mangostin.

Among xanthone derivatives from mangosteen extract, α-mangostin has been shown by several studies to exert the most potent antibacterial activity. The method used in this study to prepare the extract yielded a high percentage of α-mangostin. Its MIC and MBC were also equivalent to those of α-mangostin, suggesting that its antibacterial activity is largely attributed to this purified compound. Since the time and cost for crude extract preparation was much less than that of α-mangostin, it is a more suitable candidate for development of a new plaque control agent.

Several antibacterial agents have been incorporated into dental products such as toothpaste, mouthrinse, chewing gum, gel or vanish for the control of dental plaque. Chlorhexidine is considered a gold standard for an anti-plaque agent. Its MIC values for S. mutans obtained in the present study resembled those previously published, and were comparable to those of the mangosteen extract. Sanguinarine, a natural substance isolated from Sanguinaria canadensis, was active against S. mutans with the MIC values of 1–16 μg/ml. The MIC ranges of essential oils including eucalyptol, menthol, thymol and methyl salicylate were 250–1,000 μg/ml. Green tea extracts contain various polyphenols such as catechin, epicatechin, gallocatechin, etc. Their MIC values against S. mutans were reported to be 250–1,000 μg/ml.

The MIC of the mangosteen extract was 0.625 μg/ml. Therefore, its antibacterial activity was stronger than or at least equivalent to those commercially available antibacterial substances.

An antibacterial agent is considered bactericidal if its MBC is equal or similar to the MIC. The MBC of the extract was not greater than two times the MIC, suggesting that it acted bactericidally against S. mutans. To determine the optimal treatment concentration and treatment time to obtain sufficient bactericidal effect, the time–kill assays were performed. Treatment with the extract at 2x MBC significantly decreased viable count (log₁₀ CFU/ml) of almost one order of magnitude after 90 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, the viable bacterial count was reduced by almost two orders of magnitude after exposed to the extract for 60 minutes, and was further decreased to an detectable level in 90 minutes.

The data from kinetics studies also provide an idea of the vehicle system that should be used to deliver an antibacterial agent to the oral cavity. An agent capable of killing S. mutans within minutes of exposure can be used in a product like toothpaste or mouthrinse since brushing or rinsing usually takes only a few minutes. The bactericidal effect of mangosteen extract at 2x MBC (2.5 μg/ml) required an exposure time of at least 90 minutes. When the concentration was increased to 256x MBC (160 μg/ml), it still required more than 5 minutes to completely kill the bacteria. Therefore, the extract should be formulated in a delivery vehicle such as chewing gum, gel or vanish that can sustain its release over a period of time. Further studies are required to determine the side effects and the antibacterial
activity of the extract when used over a period of time in the oral cavity.

**Conclusion**

The crude extract from mangosteen pericarp was effective against cariogenic *S. mutans*. Its MIC and MBC were comparable to those of chlorhexidine. The strong bactericidal activity of the extract suggests that it is a good candidate for further development as an anti-plaque agent for the prevention of dental caries.

**Acknowledgement**

This work was supported by the Dental Faculty Research Fund, the Graduate Student Research Fund and the Ratchadaphisek Somphot Endowment Fund for the Development of New Faculty Staff from Chulalongkorn University. We thank Mr. Manop Pachantabut for technical assistance.

**References**


ถูกต้องด้านแบทที่เรียกของสารสกัดจากเปลือกม่วงคุคต่อเชื้อแอนิฟิคิดโคคลัส มีแพทเท็นส์ซึ่งเป็นสาเหตุของโรคพันธุ์

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บทคัดย่อ

วัตถุประสงค์: วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาถูกต้องด้านแบทที่เรียกของสารสกัดจากเปลือกม่วงคุคต่อเชื้อแอนิฟิคิดโคคลัส มีแพทเท็นส์ซึ่งเป็นสาเหตุของโรคพันธุ์

วัสดุและวิธีการ: เชื้อแอนิฟิคิดโคคลัส มีแพทเท็นส์ต้องใช้ในการทดลองมี 2 สายพันธุ์คือ ATCC 25175 และ KPSK, ถูกต้องด้านแบทที่เรียกโดยความเข้มข้นต่ำสุดในการยับยั้งการเจริญเติบโต และความเข้มข้นต่ำสุดในการยับยั้งการเจริญเติบโต นอกจากนี้ยังต้องวิเคราะห์เวลาที่ใช้ในการต้านเชื้อแอนิฟิคิดโคคลัส หลังจากได้รับสารสกัดที่ความเข้มข้น 2 และ 4 เท่าของความเข้มข้นต่ำสุดในการยับยั้งเชื้อ เป็นระยะเวลาต่างๆ กัน

ผลการศึกษา: สามารถดับเชื้อแอนิฟิคิดโคคลัส ถูกต้องด้านแบทที่เรียก 2 สายพันธุ์ โดยความเข้มข้นต่ำสุดในการยับยั้งการเจริญเติบโต และในการยับยั้งสายพันธุ์ ATCC 25175 มีค่าเท่ากันคือ 0.625 ในพอร์ตตันตัวมิลทิส สำหรับการวัดความเข้มข้นต่ำสุดในการยับยั้งเชื้อ KPSK, คือ 0.625 และ 1.25 ในพอร์ตตันตัวมิลทิส ตามลำดับ ถูกต้องด้านแบทที่เรียกเชื้อแอนิฟิคิดโคคลัส มีแพทเท็นส์ของสารสกัดจากเปลือกม่วงคุคต้องไล่ไม่ได้ถึงถ้ามีความเข้มข้น ซึ่งเป็นสาเหตุพันธุ์ที่ไม่สามารถยับยั้งได้ในการยับยั้งเชื้อแอนิฟิคิดโคคลัส จากการทดลองที่ใช้ในการต้านเชื้อแอนิฟิคิดโคคลัส สามารถดับเชื้อแอนิฟิคิดโคคลัส มีแพทเท็นส์สายพันธุ์ ATCC 25175 โดยใช้เวลาใกล้เคียงประมาณ 10 เท่าภายใน 90 นาที เมื่อมีความเข้มข้นของสารสกัดเป็น 4 เท่า จำนวนไม่ใกล้เคียงประมาณ 100 เท่าภายใน 60 นาที และสามารถยับยั้งเชื้อได้ทั้งหมดในเวลา 90 นาที

สรุป: การศึกษานี้แสดงให้เห็นว่าสารสกัดจากม่วงคุคต้มีประสิทธิภาพสูงในการต้านเชื้อแอนิฟิคิดโคคลัส มีแพทเท็นส์ซึ่งอาจนำไปสู่การพัฒนาสารตัวใหม่เพื่อใช้ในการควบคุมการเกิดควบคุมโรคพันธุ์ และป้องกันโรคพันธุ์ต่างๆไป

(ว. ทพน. จุฬาฯ 2550;30:1-10)

คำสั่งย่อ: ถูกต้องด้านแบทที่เรียก; ม่วงคุค; แอนิฟิคิดโคคลัส; มีแพทเท็นส์