Anticariogenic Activity of Garcinone B from *Garcinia mangostana*

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Abstract:

Objectives: The aim of the present study was to evaluate anticariogenic activity of garcinone B isolated from *Garcinia mangostana* Linn. pericarp extract.

Materials and Methods: Garcinone B was evaluated for their antibacterial activity against cariogenic bacteria by Kirby-Bauer disk diffusion method. Cariogenic bacteria tested in this study were *Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus, Lacticaseibacillus casei*and standard strains *(S. mutans* ATCC 25175*).* Time-killed assay and hydrophobicity were also determined.

Results: We found that garcinone B was effective for all bacterial tests. It exhibited strong antibacterial activity against cariogenic bacteria with the range of MIC value of 0.25–0.5 μg/ml and zone of inhibition of 6-14 mm. The garcinone B exhibited strong antibacterial activity against mutans Streptococci *and Lactobacillus* with MIC value of 0.25 μg/ml and 0.5 μg/ml, respectively. In the time-kill curve, garcinone B was bactericidal against *S. mutans* ATCC 25175 at the concentration of 1 μg/ml, and completely killed the bacteria within 1 h at the concentration of 2 μg/ml. A decreased cell surface hydrophobicity of *S. mutans* ATCC 25175 induced by garcinone B was found.

Conclusions: These findings reveal that garcinone B has potential for antibacterial activity against cariogenic microorganisms. In addition, garcinone B has potential candidate for alternative chemotherapeutic approaches to dental caries.

Keyword: Antibacteria, Garcinone B, *Lactobacillus,* Mangosteen, *S. mutans*

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Introduction

Dental caries constitute a multifactorial complex disease, essentially a demineralization process affecting tooth tissue. Cariogenic bacteria, such as *Streptococcus mutans, Streptococcus sobrinus or Lactobacillus* spp., are a considerable factor (salivary pH, buffer capacity, flow rate and composition) causing such caries (1). The prevalence of dental caries among 151 Thai children aged 9-18 months was 32.5%, 15.9% had at least one cavity (cavitated caries) and 16.6% had white lesions (non-cavitated caries) (2). Their oral hygiene was usually poor, indicated by high levels of dental biofilm and high numbers of mutans streptococci (*S. mutans*, *S. sobrinus* and standard strain) (3). *S. mutans* is a cariogenic bacterium that plays an important role in the beginning of dental caries, both in fissures and on smooth enamel surfaces, while *S. sobrinus* is isolated more specifically from the proximal sites of posterior teeth (4). Their aciduric and acidogenic properties cause a demineralization process affecting tooth tissue. *Lactobacillus* is considered as a secondary invader involved in the progression of carious lesions and carious dentin (5). Mangosteen (*Garcinia mangostana L.*) is a tropical tree in some Southeast Asia countries such as Indonesia, Malaysia and Thailand with a long history of use in traditional medicine for the treatment of chronic diarrhea, infected wounds, skin infections and dysentery (6). The major bioactive secondary metabolites of mangosteen are xanthone derivatives, which have potent pharmacological activities including antibacterial, antifungal, antioxidant, anticancer (7,8) and anti-inflammatory properties (9). Garcinone B, one of the derivatives, has been demonstrated to reduce prostaglandin E_2 release and nuclear factor kappa-B mediated transcription, central regulators of inflammatory genes in C6 rat glioma cells (10). Garcinone B exhibited strong inhibitory effect against *Mycobacterium tuberculosis* (11). In this study, we demonstrated that garcinone B from mangosteen extract has anticariogenic properties such as inhibition of bacterial growth against cariogenic bacteria and cell-surface hydrophobicity of *S. mutans.*

Materials and methods

Crude extracts and purified garcinone B

The fruits of *G. mangostana* were collected from Bahnkai District, Chanthaburi Province, Thailand, in April 1999. A voucher specimen [voucher #0032(RU)] of this plant is deposited at the Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand. The garcinone B was isolated from the fruit mangosteen as described previously (12). Briefly, the fruits of *G. mangostana* were collected from Bahnkai Destrict, Chantaburi Province, Thailand, in April 1999. The pericarps were extracted throroughly with MeOH and evaporation of the solvent gave crude extract. The crude extract was partitioned between $CHCl_3$ and H_2O to efford $CHCl_3$ extract. Then repeated column chromatography using $CHCL_3$, acetone and MeOH yielded garcinone B. The purified garcinone B (dried-form) was dissolved in absolute dimethyl sulfoxide (DMSO, Merck, Germany) to a concentration of 10 mg/ml and used as stock solution.

Cariogenic bacterial strains and culture condition

A collection of bacteria known to cause tooth decay (*S. mutans, S. sobrinus, L. casei, and L. acidophilus*) were collection from Department

of Medical Sciences, Ministry of Public Health, Thailand. The organisms were identified to the species level by standard microbiological techniques including colonial characteristics, morphological characteristics and biochemical characteristics. Laboratory control strains *S. mutans* (ATCC 25175) were purchased from American Type Culture Collection (Rockville, Md., USA) to serve as control. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to Mueller-Hinton broth (MHB, Oxoid, Nepean, ON) that were incubated for 24 h at 37°C. The cultures were diluted with fresh MHB to achieve optical densities corresponding to 2.0 x 10^6 colony-forming units (CFU)/ml.

Kirby-Bauer Disc Diffusion Method

The agar disc diffusion method was used for screening antibacterial activity according to the Clinical and Laboratory Standard Institute (13). Briefly, garcinone B was prepared at a limit amount of 0.75 mg/ml in dimethyl sulfoxide (DMSO). 10 µl of the solution was pipetted onto a sterile filter paper discs (diameter of 6 mm) and placed onto Mueller Hilton (MH) agar surface spreaded with 0.1 ml bacteria [10^5-10^6 CFU/ml]. The plates were then incubated for 16-18 h at 37°C for bacterial growth. Inhibition zone values were measured including the diameter of the disc. For each disc, the inhibitory zone diameter was measured in at least three dimensions using a standard ruler, whose smallest division was 1 mm. A diameter of less than 8 mm was omitted for clarity of presentation. A disc prepared with only the coresponding volume of DMSO was served as negative control. Indeed, DMSO has never given clear zone over 1 mm (i.e., about 0.5 mm from each side of the paper disc), and usually gave none). Antibiotic discs (chloramphenicol and vancomycin) (Oxoid) were used as positive controls. Antimicrobial activity was expressed as the inhibitory diameters (mm) produced by the tested compounds.

MIC determination

As the garcinone B is naturally dark in nature, it was designed for MIC determination using the disk diffusion method (14). Briefly, initial candidate garcinone B was prepared in DMSO, and subsequent two-fold dilution was performed with 0.5 ml of DMSO. 10 µl of each solutions was pipette onto a sterile filter paper discs (diameter of 6 mm) and placed onto MH agar surface spread with 0.1 ml bacteria [10^5-10^6 CFU/ml]. The plates were then incubated for 16-18 h at 37°C. At the end of incubation period the diameter of the inhibition zone was measured. The endpoint MIC is the lowest concentration of compounds at which the inhibition zone was less than 8 mm. DMSO was used as negative control. E-test strips of vancomycin and chloramphenicol (AB Biodisk, USA) were used as positive control.

Time-Kill assay

Time-kill curve experiments were performed to evaluate the bactericidal activities of the garcinone B against cariogenic bacteria. Time-kill curved was assessed at the garcinone B concentrations of 1 µg/ml and 2 µg/ml. Colonies of *S. mutans* ATCC 25175 grown on Brain Heart Infuxion (BHI) agar (Difco, Franklin Lakes, NJ, USA) were picked and resuspended in 300 ml of

BHI broth to a bacterial cell suspension of 1 x 10^6 CFU/ml. 1 μg/ml or 2 μg/ml of garcinone B was added to the cell suspensions and incubated at 37°C. Aliquots of the cultures (0.1 ml) were taken at 0, 1, 3, 7, 12 and 24 h and serially diluted in BHI broth and plated on BHI agar. Following 48 h of incubation, the number of colonies grown on the plates was counted to determine the total viable cell count. Cell suspension that had not been treated with garcinone B was assayed as a negative control.

Cell surface hydrophobicity

Cell surface hydrophobicity of *S. mutans* ATCC 25175 was measured as described by Rosenberg et al., 1983 (15). The microorganisms were cultured in 300 ml of BHI broth and collected by centrifugation. The cells were washed and suspended in PUM buffer, pH 7.1 (22.2 g $K_2HPO_4.3H_2O$, 7.26 g KH_2PO_4, 1.8 g urea, 0.2 g $MgSO_4.7H_2O$ in 1 L of distilled water) to an optical density of 1.2 at 550 nm. The cell suspension (1.5 ml) was mixed with an equal volume of 2-fold serial dilution of the garcinone B (concentration 1 μg/ml) in test tubes. After standing for 10 min at room temperature, 0.2 ml of n-hexadecane was added and agitated uniformly on a vortex mixer for 1 min. After the n-hexadecane phase was separated from the aqueous phase, the optimal density of the aqueous phase was determined at 550 nm. Hydrophobicity was expressed as the percent decrease in turbidity as compared with that of the corresponding sample mixed without added hydrocarbon.

Statistical analysis

Besides disc diffusion tests, all experiments were set up in triplicate. Data are presented as means ± SEM. Data were analyzed and processed using Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA). One-way repeated-measurement analysis of variance (ANOVA) was followed by post hoc testing using the Holm-Sidak method when appropriate. Differences between means of the experimental and control groups were evaluated by paired t-test as indicated in the figure legends. The level of statistical significance was set at $p < 0.05$.

Results

Our previous screening study, we evaluated the antibacterial activities of isolated xanthones (such as demethylcalabaxanthone, dimethylpyranoxanthone, 11-hydroxy-1-isomangostin, trapezifolixanthone, 8-deoxygartanin, gartanin, garcinone B, garcinone C, garcinone D, mangostenol, mangostinone, α-mangostin, β-mangostin, γ-mangostin) from mangosteen *(Garcinia mangostana L.)* extract (data not shown). All xanthones except garcinone B, garcinone C, α-mangostin and γ-mangostin had no any antibacterial activity against cariogenic bacteria (*S. mutans, S. sobrinus, L. casei, L. acidophilus*) (data not shown). The strong antimicrobial activity of garcinone B was noted with most gram positive bacteria tests (data not shown). Garcinone B had strong anti-bacterial activity against axenic laboratory isolates of *S. mutans, S. sobrinus, L. acidophilus, L. casei as well as reference S. mutans* ATCC 25175. The MIC values obtained

by antimicrobial susceptibility tests are shown in Table 1. The anti-cariogenic activity of garcinone B was variable in the range of 0.25-0.5 μg/ml. Garcinone B had anti-bacterial activity on cariogenic bacteria; *S. mutans, S mutans* ATCC 27175, *S. sobrinus* (MIC value of 0.25 μg/mL), *L. acidophilus* and *L. casei* (MIC value of 0.5 μg/ml). Garcinone B had higher anti-bacterial activity against cariogenic bacteria than vancomycin (MIC value of 1.0 μg/ml) and chloramphenicol (MIC value of 4.0 μg/ml).

Table 1. MIC and zone of inhibition of garcinone B against cariogenic bacteria.

	Zone of Inhibition			MIC		
	Garcinone B	Vancomycin	Chloramphenicol	Garcinone B*	Vancomycin**	Chloramphenicol**
Disk content (μg)	7.5	32	32			
Microorganisms						
Cariogenic bacteria						
S. mutans	11	9	13	0.25	1	4
S. sobrinus	9	8	12	0.25	1	4
L. acidophilus	6	0	22	0.5	0	8
L. casei	6	0	22	0.5	0	8
Reference strains						
S. mutans ATCC 25175	14	9	12	0.25	1	4

*Disc diffusion method

**E-test strips protocol

Table 1 shows results of disc diffusion tests on the cariogenic bacteria. Garcinone B produced significant growth inhibition zones against all of the cariogenic bacterial tested. The diameter of the growth inhibition zone was directly proportional to the concentration of xanthone. The zone of inhibition produced by garcinone B at a concentration of 7.5 μg for *S. mutans* ATCC 25175, *S. mutans, S. sobrinus, Lactobacillus* was about 14, 11, 9 and 6 mm, respectively. Susceptibility tests were also performed for antibiotics frequently used such as vancomycin and chloramphenicol. All laboratory isolated cariogenic bacterial strains were all

sensitive to chloramphenicol and vancomycin at concentration of 30 μg while *Lactobacillus* strains were resistant to vancomycin.

Time-kill assay

Data from time-kill assay reveal that a garcinone B at the concentration of 1 μg/ml and 2 μg/ml had bactericidal effects on *S. mutans* ATCC 25175 [Fig 1]. The reductions of cell viability were noted within 1 h of incubation. The amount was reduced to 1.34 x 10^2 CFU/ml and 9.2 CFU/ml for garcinone B (1 μg/ml), at 1 and 3 h, respectively. The cariogenic bacteria were reduced significantly by both concentrations of garcinone B at 3 h of incubation. The bactericidal effect of garcinone B 2 μg/ml was very effective by completely killed the bacteria within 3 h. The sharply reduction curves were also effective in killing the other 4 cariogenic isolate strains (*S. mutans, S. sobrinus, L. casei and L. acidophilus*) by using garcinone B 2 μg/ml (data not shown). In addition, the cell viability of *S. mutans* after treatment with vancomycin, the bacteria was reduced to 3.44 x 10^3 CFU/ml and 1.67 x 10^2 CFU/ml, at 1 and 3 h, respectively. The bacteria was not completely killed after 24 h of incubation.

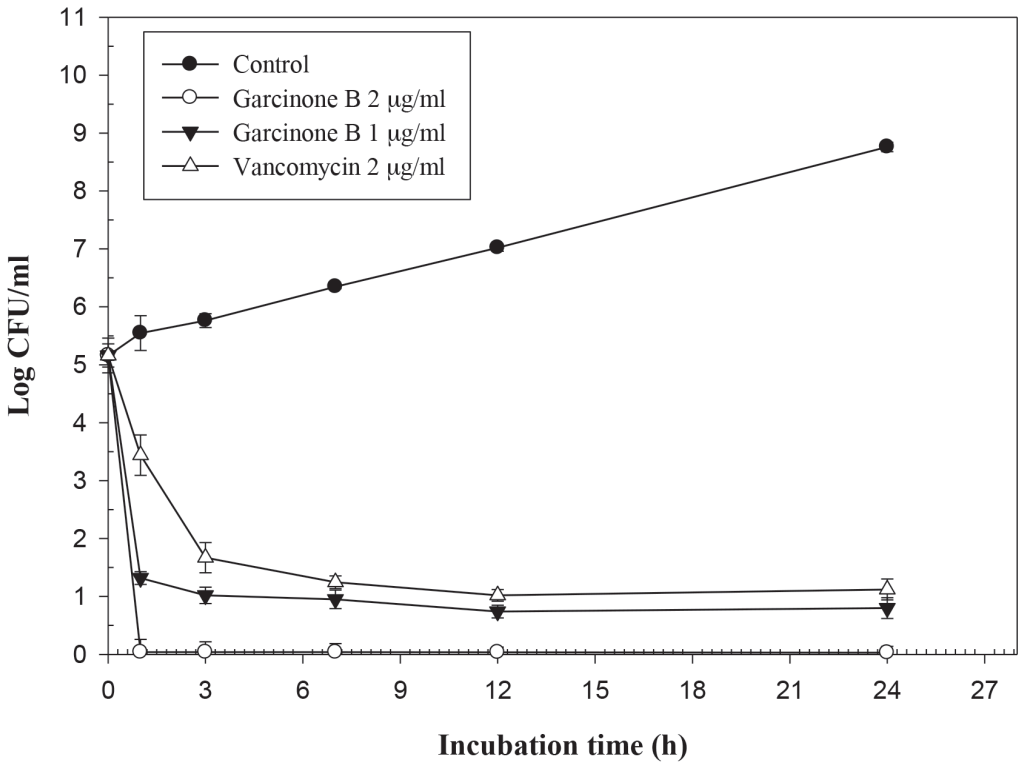

Fig 1. Time-kill curve for *S. mutans* ATCC 25175 obtained using garcinone B. *S. mutans* ATCC 25175 were incubated with garcinone B 1 μg/ml (closed triangle), garcinone B 2 μg/ml (open circle) and vancomycin 2 μg/ml (opened triangle) for 24-h incubation. Control bacteria were incubated in the absence of substance (closed circle). At indicated time, 0.1 ml of the media cultured was scooped for bacterial counts and the log CFU/ml is shown. This experiment was repeated three times, and a representative experiment is shown.

Surface hydrophobicity

Cell surface hydrophobicity of *S. mutans* ATCC 25175 was decreased by the addition of garcinone B. It showed a pronounced effect on the reduction of cell surface hydrophobicity from 90 to 23% after addition of garcinone B 0.2-2 μg/ml in the cariogenic bacterial culture [Fig 2].

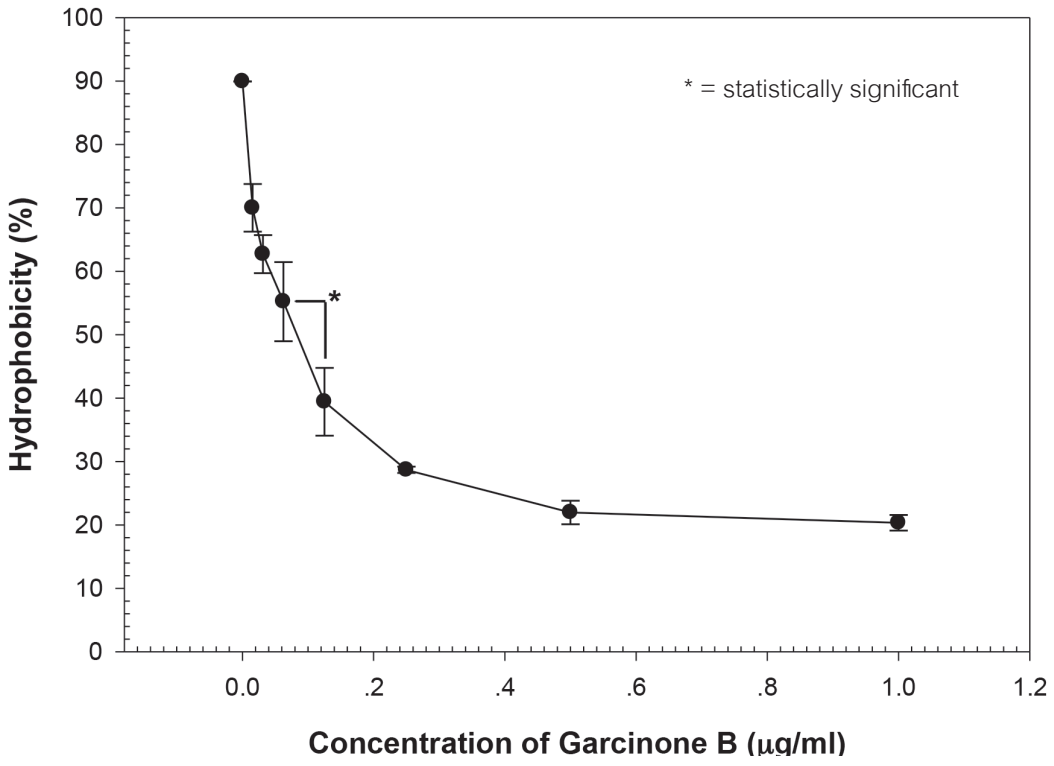

Fig 2. Effect of garcinone B on cell surface hydrophobicity of *S. mutans* ATCC 25175. Bacterial cells of *S. mutans* ATCC 25175 were mixed with garcinone B at the indicated concentration. The n-hexadecane was added to the bacterial cells and agitated uniformly. After phase seperation, the optical density of the aqueous phase was determined at 550 nm. Surface hydrophobicity was expressed as the percent decrease in turbidity as compared with that of the corresponding sample mixed without added hydrocarbon.
This experiment was repeated three times, and a representative experiment is shown.

Discussion

Hence the presence of the highly bioactive compounds in large quantities of *G. magnostana* the antibacterial properties may be one of the properties should be considered. Mangosteen extracts have previously been evaluated for their antibacterial activity on oral pathogens. It has been reported that mangosteen pericarp extract had broad-spectrum antibacterial activity against several gram-positive bacteria and gram-negative bacteria at MIC of 12.5-50 μg/ml. (16) Our screening study, all xanthones except garcinone B, garcinone C, α-mangostin and γ-mangostin had any antibacterial activity against all cariogenic bacteria. *S. mutans, S. sobrinus* and the standard strain, the main etiological agent of dental caries, were sensitive to garcinone B. While garcinone C, α-mangostin and γ-mangostin had antibacterial activity against mutans streptococci at the concentration higher than garcinone B. The antibacterial activity of mangosteen pericarp extract against cariogenic *S. mutans* has been demonstrated (17). This study we found that garcinone B had antibacterial activity against cariogenic bacteria. Garcinone B may have similar activity via the same mode of action as α-mangostin and β-mangostin as previous analysis of the antiinflammation activity (18). The xanthones nucleus contain tri-or tetra-oxygen function with either di-C_5 units or with a C_5 and a modified C_5 groups in ring A and C. Among these, 1,3,6,7-tetraoxygenated xanthones bearing the C_5 units at C-2 and C-8 in α-mangostin, the major constituent, β-mangostin and garcinone B exhibited the most potent antimycobacterial activities (12).

The data from time-killed studies provide an idea of the vehicle system that should be used to deliver an anticariogenic agent to the oral cavity. An agent capable of killing *S. mutans* within minutes of exposure can be used in a product like toothpaste since brushing usually lasts only a few minutes. The bactericidal effect of garinone B at 2 μg/ml required an exposure time within 60 minutes to completely kill. Therefore, the extract should be formulated in a delivery vehicle such as chewing gum, gel or varnish that can sustain its release over a period of time. Further studies are required to determine the period of time in the oral cavity.

Cell-surface hydrophobicity may play a major role in mediating adherence of certain oral species to tooth surface (15). Since *S. mutans* lost its surface hydrophobic could not adhere to hydroxyapatite (19). The activities should be attributed to mangostin constituents as shown with garcinone B in this study. The structural proteins of the bacteria bind to the mangosteen polyphenol, rapidly disrupting the integrity of the cytoplasmic membrane leading to cell lysis. Direct interactions of xanthone, such as α-mangostin with the bacterial membrane are responsible for a fast concentration-dependent membrane disruption and bactericidal action.

In conclusion, garcinone B isolated from mangosteen extract showed antibacterial activity against *S. mutans, S. sobrinus, L. acidophilus and L. casei,* related to dental caries. In addition, garcinone B has demonstrated anti-cariogenic characteristics that prove it to be a candidate for further studies into alternative chemotherapeutic approaches to dental caries.

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Conflicts of interest

There is no conflict of interest.

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