

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of mangosteen (*Garcinia mangostana* Linn.) rind extract against *Aggregatibacter actinomycetemcomitans*

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ABSTRACT

Introduction: The main contents of mangosteen (*Garcinia mangostana* Linn.) is xanthone with antibacterial activity. *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) was a gram-negative, facultative anaerobic bacillus that causes periodontal diseases such as localised aggressive periodontitis (LAP). Mangosteen rind extract could inhibit the growth of *Porphyromonas gingivalis* and *A. actinomycetemcomitans*. This study was aimed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of mangosteen rind extract against *A. actinomycetemcomitans*. **Methods:** This study was an experimental and in-vitro study of antimicrobial properties of mangosteen (*Garcinia mangostana* Linn.). The extract of mangosteen was made by the maceration method using 70% ethanol solvent. *A. actinomycetemcomitans* (ATCC 43718) obtained from Laboratory of Microbiology Airlangga University Surabaya. This study used a microdilution method and analysed using the Enzyme Linked Immune Sorbent Assay (ELISA). The determination of the MIC was measured using the formula for cell inhibition percentage. Three concentrations higher than the MIC standard were cultured on the Mueller Hinton agar. The lowest concentration on agar which was not found any growth of the bacterial colonies was determined as the MBC. **Results:** The results of this study indicated that the MIC and MBC of mangosteen rind extract against *A. actinomycetemcomitans* were 195.3 ppm and 1562.5 ppm, respectively. **Conclusion:** Mangosteen rind extract can inhibit and bactericide the growth of *A. actinomycetemcomitans*.

Keywords: Antibacterial, *Aggregatibacter actinomycetemcomitans*, mangosteen rind extract, xanthone.

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INTRODUCTION

Mangosteen (*Garcinia mangostana* Linn.) is a fruit plant that grows in tropical rainforest country such as Southeast Asia.¹ Mangosteen fruit is round, dark purple or reddish, and has a juicy white pulp possessing a slightly acidic and sweet flavour that is enjoyed by many, and has resulted in it being referred to as the “queen of fruits”. The pericarp of mangosteen fruit has been used in traditional medicine in Southeast Asia for centuries to treat infection, wounds, inflammation and diarrhea.²

The content of mangosteen fruit, in general, are chrysanthemin, garcinone A, B, and C, sesquiterpenoids, gartanin, fructose, sucrose, tannins, and xanthone as the main content of mangosteen rind.^{1,3} Xanthone is an antioxidant, antitumor, antiallergic, anti-inflammatory, antiviral, and antibacterial.⁴ One of component xanthone that had antibacterial activity in the oral cavity is α -mangostin.⁵ Study showed that extract of mangosteen rind was effective on inhibiting the growth of gram-positive bacteria and gram-negative bacteria in vitro.^{5,6}

Mangosteen extract has been commonly used to improve dental health by eradicating oral pathogens.⁷ Research by Pribadi et al.⁸ showed that pericarp mangosteen ethanol extract could inhibit the activity of the glucosyltransferase enzyme from *Streptococcus mutans*, which is essential for the development of dental caries. Furthermore, mangosteen extract including α -mangostin has been used as an anti-bacterial component in an adhesive paste to prevent dental caries.^{8,9}

Aggregatibacter actinomycetemcomitans (*A. actinomycetemcomitans*) is a gram-negative, facultative anaerobic bacillus that causes periodontal diseases such as localized aggressive periodontitis (LAP) and consequently bone resorption. The potential virulence factor of this organism are factors that modulate the promotion of colonization and inflammation, factors that induce host tissue destruction, and factors that inhibit host tissue repair.¹⁰

Studies on the antibacterial ability of mangosteen have been carried out by several researchers. The pericarp gel when applied topically as an adjunct to periodontal treatment was found to enhance the clinical effects of periodontal treatment.¹¹

Hendiani et al.¹² conducted a clinical study that mangosteen rind gel as adjunctive therapy for scaling and root planing was able to reduce pockets depth, gingival index, and gingival bleeding, and improve clinical epithelial attachment. In vitro study showed that mangosteen rind extract inhibited growth *P. gingivalis* and *A. actinomycetemcomitans* with macrodilution method.¹³ There has been no study about antibacterial of mangosteen rind extract against *A. actinomycetemcomitans* with microdilution method. Therefore, this study was aimed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of mangosteen rind extract against *A. actinomycetemcomitans*.

METHODS

This research was an experimental in-vitro study to determine the MIC and MBC extract of mangosteen rind (*Garcinia mangostana* L.) on the growth of *A. actinomycetemcomitans* ATCC 43718 by microdilution method. This research was conducted in February 2018 until June 2018 at the Central Laboratory of Biopharmaca Study, IPB Bogor. *A. actinomycetemcomitans* (ATCC 43718) bacteria were cultured from Laboratory of Microbiology Research Center UNAIR Surabaya. Mangosteen rind extract was carried out at Central Laboratory of Biopharmaca Study, IPB University Bogor.¹⁴

Mangosteen rind extract

Mangosteen rind (*Garcinia mangostana* L.) was extracted by maceration method. It was soaked with 500 mL of 70% ethanol solvent for 24 hours. The immersion was filtered and immersed with ethanol again three times until a clear filtrate was obtained. Then the filtrate was concentrated using a rotary evaporator so that a thick extract without ethanol were obtained.^{12,14,15}

Preparation of test bacteria

Gram-negative bacterial cultures of *A. actinomycetemcomitans* ATCC 43718 were inoculated into Muller Hinton Broth (MHB) media. Subsequently, it was incubated for 48 hours at 37°C. Bacteria were inoculated into 0.9 ml NaCl solution as much as 3 ml. Then the turbidity was

measured using a UV-Vis spectrophotometer at λ 600 nm. Ten mixtures with specific ratios that produce transmittance close to 25% were used for antibacterial, MIC and MBC testing.

Calculation of MIC and MBC by microdilution method

The testing material was diluted on a microplate containing 96 microwells. To the A and C microwell columns, 100 μ l of Mueller Hinton broth (MHB) and 100 μ l of solvent (dimethyl sulfoxide) were added to a concentration of 100,000 ppm. Microwell columns B and D were added 100 μ l of Mueller Hinton broth (MHB) media and 100 μ l of mangosteen rind extract which had been dissolved to obtain a concentration of 100,000 ppm. After that, a serial dilution of row 1 to 12 microwell was carried out. Then the suspension of *A. actinomycetemcomitans* ATCC 43718 (5 μ l) was put into all microwells in columns C and D. The microplate was then incubated at 37°C for 24 hours and measured turbidity level by using ELISA

Reader (Epoch™ Microplate Spectrophotometer, United States). The determination of the MIC was measured using the formula for cell inhibition percentage¹⁵, with: Optical Density (OD) control obtained from OD(media, solvent, bacteria) reduced by OD(media, solvent); OD sample obtained from OD(media, testing material, bacteria) reduced by OD(media, testing material).

MBC was determined by testing three concentrations higher than MIC carried out subculture on Mueller Hinton agar medium and incubated at 37 °C for 48 hours. Media without bacterial colonies was determined as MBC.

RESULTS

Three concentrations above the minimum inhibitory concentration were regenerated on the Mueller Hinton agar medium at 195.3 ppm. The minimum bactericidal concentration showed agar plates showed no bacterial growth at a concentration 1562.5 ppm (Figure 1).

Table 1. The measurements of optical density using ELISA reader after incubation

Well	Concentration (ppm)											
	100000	50000	25000	12500	6250	3125	1562.5	781.25	390.6	195.3	97.6	48.5
MS	0.096	0.098	0.097	0.168	0.330	0.097	0.097	0.096	0.185	0.927	0.111	0.247
MT	2.122	1.065	0.616	0.431	0.300	0.136	0.134	0.117	0.178	0.337	0.303	0.352
MSB	0.611	0.469	0.480	0.586	0.374	0.566	0.608	0.282	0.071	0.369	0.406	0.355
MTB	2.221	1.371	0.843	0.371	0.680	0.429	0.421	0.133	0.285	0.305	0.614	0.703

ppm (part per million); MS (Media + Solvent); MT (Media + Testing Material); MSB (Media + Solvent + Bacteria); MTB (Media + Testing Material + Bacteria)

Table 2. Percentage of inhibition of bacterial cells of *A. actinomycetemcomitans* in mangosteen rind extract

Concentration (ppm)	% Inhibition <i>A. actinomycetemcomitans</i>
100000	80.78
50000	17.52
25000	40.73
12500	114.35
6250	-763.64
3125	37.53
1562.5	43.84
781.25	91.39
390,6	193.86
195.3	94.26
97.6	-5.42
48.5	-225

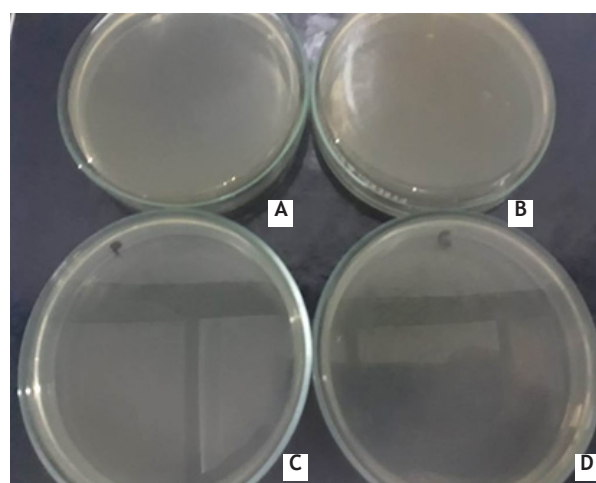


Figure 1. (A) and (B) culture results at a concentration of 781.25 ppm; (C) and (D) culture results at a concentration of 1562.5 ppm

DISCUSSION

This study showed that mangosteen extract could inhibit and kill *A. actinomycetemcomitans* with MIC value of 195.3 ppm and MBC value of 1562.5 ppm. Although with different concentrations, these findings were following the previous study by Hendiani et al. that showed inhibitory and bactericidal power of mangosteen rind extract against *A. actinomycetemcomitans*.¹³ This study uses the microdilution method that is more sensitive than macrodilution in the previous study. Mangosteen rind extract has an antibacterial activity which is similar to the research conducted by Suhartati et al.¹⁶, which is suggested that the antibacterial effect of mangosteen rind extract is having a difference on the gram-negative and anaerobic bacteria. Phytochemical screening test of mangosteen rind showed that the ethanol extract of mangosteen rind contained active compounds, such as chrysanthemin, garcinone A, B, and C, sesquiterpenoids, gartanin, fructose, sucrose, tannins, and xanthone as the main content of mangosteen rind. Xanthone compounds can inhibit growth bacterial^{3,5}, as also found in the study conducted by Dharmaratne¹⁷ on xanthone from mangosteen rind extract against *S.aureus* and *Enterococcus* bacteria.³

Xanthenes are the most important chemical content in mangosteen rind. The main compounds of xanthenes include α -mangostin, β -mangostin, γ -mangostin, and methoxy- β -mangostin. One of component xanthone that has antibacterial activity in the oral cavity is α -mangostin. The antibacterial activity of xanthone has complex compounds against extracellular proteins that interfere the integrity of the bacterial cell membrane. Also, xanthenes are capable of coagulating proteins that cause the bacterial cell membrane to become lysis.^{5,16,18}

Tannins will coagulate bacterial cells and eliminate toxins. Tannins can shrink and interfere with the permeability of bacterial cell membrane. Permeability disruption of bacterial cells causes their growth is stunted or dead.^{5,16}

This study was still in vitro tests; therefore, it did not reflect the real condition found in periodontal pockets. It did not consider the factors such as the interaction of host, agent, and environment. The crude form of mangosteen

was used in our study, and the primary active compound in mangosteen was not isolated and identified. Further studies shall be done to determine active antibacterial compounds against periodontal disease bacteria. In vivo studies should be conducted to prove its antibacterial activity against periodontal microorganisms without causing significant local or systemic adverse effects.

CONCLUSION

Mangosteen rind extract can inhibit and bactericide the growth of *A. actinomycetemcomitans*.

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