

Indonesian Mangosteen Fruit (*Garcinia mangostana* L.) Peel Extract Inhibits *Streptococcus mutans* and *Porphyromonas gingivalis* in Biofilms *In vitro*

Abstract

Background: *Streptococcus mutans* and *Porphyromonas gingivalis* are caries and periodontal disease-related bacteria. The mangosteen fruit (*Garcinia mangostana* L.) peel contains flavonoids, tannins, saponins, and xanthenes that have antibacterial properties. **Aims:** The aim of this study is to analyze mangosteen peel extracts' ability to inhibit *S. mutans* and *P. gingivalis* biofilms growth *in vitro*. **Materials and Methods:** Mangosteen peel extract effects on the *S. mutans* ATCC-3198 and *P. gingivalis* ATCC-3327 in biofilms growth were evaluated by a crystal violet biofilm assay. Each bacterium was inoculated into a brain–heart infusion broth for 24 h at 37°C anaerobic conditions. A volume of 200 µL (10⁷ colony-forming unit/mL) of bacterial suspension were distributed in microplate wells and incubated for 24 h. Mangosteen peel extracts with different concentrations were added into biofilm wells. Biofilm without treatment was used as negative control. Biofilm mass was calculated by 0.5% crystal violet staining, and optical density was measured at 600 nm using microplate reader. All obtained data were statistically analyzed using one-way analysis of variance test with $P < 0.05$ set as the level of significance. **Results:** The results showed that mangosteen peel extract could inhibit the growth of *S. mutans* and *P. gingivalis* in biofilms significantly compared to the negative control ($P < 0.05$). The most effective concentration and incubation time for inhibiting biofilm growth was 100% in 6 h for *S. mutans* and 100% in 24 h for *P. gingivalis*. **Conclusion:** Mangosteen peel extract is effective at inhibiting *S. mutans* and *P. gingivalis* biofilms, and this antibiofilm agent can be an alternative therapy in preventing caries and periodontal disease. Future studies are needed to explore this effect.

Keywords: Antibiofilm, biofilm, mangosteen peel extract, *Porphyromonas gingivalis*, *Streptococcus mutans*

Introduction

The national prevalence of dental caries in Indonesia is considered high. Based on a 2004 survey held by the Indonesian Household Health Survey, 71.2% of the population aged 15 and above had caries, and 52.3% of those cases were left untreated.^[1] According to the basic health research conducted by the Indonesian government in 2007 and 2013, the decayed missing and filling teeth index was 4.9 and 4.6, respectively.^[2] Other than caries, the main problems in the oral cavity are gingivitis and periodontitis. These diseases are caused mostly by bacteria. Most bacteria can naturally form biofilms as an act of survival.^[3] Bacterial accumulation that forms plaque on the surfaces of teeth is commonly known as oral biofilm.^[4] Oral

biofilm is defined as a diverse community of microorganisms found on the surface of the tooth embedded in a matrix of extracellular polymeric substances (EPS).^[5] *Streptococcus mutans* is the primary bacteria that forms oral biofilms is the main cause of dental caries.^[6]

Oral biofilm is one of the key causes of caries and periodontal diseases, including gingivitis and periodontitis.^[7] If left untreated, gingivitis may lead to periodontitis, which is defined as an inflammatory disease of the tissues supporting the teeth caused by a specific microorganism or group of microorganisms that lead to progressive destruction of periodontal ligament and alveolar bone. This disease can involve periodontal pocket formations, recession, or both, as well as tooth loss.^[8]

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Although many therapeutic agents to treat oral diseases are available, some of these agents may have side effects, including multiple drug resistance.^[9] Therefore, the search for natural bioactive compounds that can treat oral diseases with few to no adverse effects continues. Many indigenous Indonesian cultures often use herbs to treat a variety of diseases in the oral cavity.^[10,11] Native Indonesian plant species such as mangosteen fruit (*Garcinia mangostana* L.) are known to have medicinal potentials.^[12] Mangosteen, especially the peel, contains abundant amounts of xanthenes, a class of polyphenolic compounds that possess many significant biological activities *in vitro*,^[13] and other bioactive compounds including flavonoids, tannins, and anthocyanins.^[14] Several studies show that xanthone has antioxidant, anti-inflammatory, antiallergy, antibacterial, anticancer, and antifungal effects.^[15] The purpose of this study was to analyze the antibiofilm properties of mangosteen peel extracts against *S. mutans* and *Porphyromonas gingivalis* biofilms in the oral cavity.

Materials and Methods

This study is an *in vitro* experimental design which was conducted at the Microbiology Center of Research and Education (MiCORE) Laboratory, Faculty of Dentistry, Trisakti University. In this study, we used crude forms of Indonesian mangosteen peel extract *G. mangostana* L. were collected from Bogor (Java Island, Indonesia). The microorganisms used in the study were standard strains of *S. mutans* ATCC 3198 and *P. gingivalis* ATCC 33277. They were obtained from the microbiology laboratory of Dipa Pharmed Intersains (PT Dipa Healthcare) in Jakarta, Indonesia.

Extraction of *Garcinia mangostana* L. peels

G. mangostana L. peels were dried from 8 a.m. to 3 p.m. for 2 days. Then, dried *G. mangostana* peel was inserted into the tool grinder for 10 min. The results were obtained in the form of powder. Subsequently, the *G. mangostana* peel powder was extracted by the maceration method using 96% ethanol (Merck, Darmstadt, Germany) as a solvent. The mangosteen peel skin powder was diluted with 96% ethanol (1:5 ratio) for 1 day in a dark container, protected from light, and occasionally stirred. After 1 day, the solution was taken with filter paper. The filtered solution was then processed in the evaporator to evaporate the remaining ethanol solution. After the evaporation process, we obtained *G. mangostana* peel extract. The *G. mangostana* peel extract was then diluted with 5% dimethyl sulfoxide (Merck, Darmstadt, Germany) to obtain 100% concentration, and then serial dilution was performed using brain–heart infusion (BHI) broth to form an extract with concentrations of 50%, 25%, 12.5%, and 6.5%.

Phytochemical analysis assay

Phytochemical analysis assay is a series of rapid and simple qualitative methods to determine the presence of

bioactive compounds in the plant extracts. In this study, each phytochemical analysis assay was performed in the Indonesian Medicinal and Aromatic Crops Research Institute (local acronym BALITTRO) to detect the presence of saponins, tannins, alkaloids, phenolic compounds, flavonoids, triterpenoids, glycosides, and plant sterols. Saponins detection method: extracts were diluted in a tube containing the distilled water. The tube was shaken continuously in a shaker for 15 min. The formation of persistent foam layer at the top of the tube indicates the presence of saponins. Tannins detection method: 1% gelatin-sodium chloride solution was added into the tube containing the extract. The presence of tannins was indicated by the formation of white precipitate at the bottom of the tube. Alkaloids detection method: extracts were treated with hydrochloric acid (HCl) and filtered. A few drops of potassium mercuric iodide solution (Mayer's reagent) were added into the filtrate. The presence of alkaloid compounds was indicated by the formation of white creamy-colored precipitate. Phenol detection method: the ethanolic extract was spotted on a piece of filter paper, added with a drop of phosphomolybdic acid reagent and exposed to ammonia vapors. The presence of phenol was indicated by the formation of blue coloration of the spot. Triterpenoids detection method: detection was carried out using Liebermann–Burchard test. Mangosteen peel extract (100 mg) was shaken with chloroform in a tube. A few drops of acetic anhydride was added to the solution. The tube was boiled in a water bath and cooled into iced-water, followed by the addition of concentrated H₂SO₄ (2 mL) into the tube. The presence of triterpenoids was indicated by the formation of deep red color in the solution. Flavonoids detection method: a piece of magnesium ribbon was added into 2–3 mL of mangosteen peel ethanolic extract, followed by the addition of 1 mL of concentrated HCl. The presence of flavonoids was indicated by reddish-pink or red coloration in the solution. Glycosides detection method: HCl was added into the extract as a pretreatment to induce hydrolysis. Then, the extract was treated with ferric chloride solution and immersed in boiling water for 5 min. After cooling down, an equal volume of benzene was added into the extract. The benzene layer was separated and added with ammonia solution. The presence of plant glycosides was indicated by the formation of rose-pink color in the solution.^[16-18]

Preparation and treatment of the biofilms

S. mutans ATCC 25175 and *P. gingivalis* ATCC 33277 were used in this study. *S. mutans* was inoculated into BHI broth (Thermo Scientific, Waltham, MA, USA) and then incubated at 37°C for 24 h in anaerobic conditions (CO₂, H₂, and N₂). *P. gingivalis* was cultured in BHI broth and incubated in a GasPak Jar System (Becton Dickinson, Franklin Lakes, NJ, USA). The cultures that were incubated were then homogenized with a vortexer, and then their optical density (OD) was measured with a

microplate reader on a wavelength of 600 nm. A volume of 200 μL bacterial suspension (1×10^7 colony-forming unit/mL) were distributed into the 96-well plates and incubated at 37°C for 24 h in anaerobic conditions to form biofilm. After 24 h, each well plate was rinsed with phosphate buffer solution (PBS) twice. The *G. mangostana* peel extracts with different concentrations (100%, 50%, 25%, 12.5%, 6.25%) were distributed into wells that were rinsed with PBS. As positive control of 0.2% chlorhexidine was distributed into the wells. Observations were made after 1 h, 3 h, 6 h, and 24 h of incubation time at 37°C in anaerobic conditions. The extracts were then removed, and the well plate was rinsed twice using PBS (AMRESCO, Solon, OH, USA), crystal violet (Merck, Darmstadt, Germany) (0.05%) were distributed into well plates and incubated for 15 min. The extraction of crystal violet in each well plate was calculated by adding 96 μL of 96% ethanol, while the amount of biofilm by absorbance was calculated at 600 nm with a microplate reader (AccuReader, Metertech, Taipei, Taiwan).^[19] Chlorhexidine at 0.2% was used as a positive control. Biofilms without *G. mangostana* peel extracts were used as negative controls. All treatments were done in triplicate.

Statistical analysis

The Shapiro–Wilk test was used to test for normality, and Levene’s test was used to test for homogeneity of variance. The normal data ($P > 0.05$) continued to be analyzed by a one-way analysis of variance test, and $P < 0.05$ was considered the level of significance. The significant difference between groups was analyzed with Tukey’s *post hoc* least significant difference test. Statistical calculations were performed with IBM SPSS Statistics of Windows, Version 20.0. Armonk, NY: IBM Corp.

Results

Phytochemical analysis result

The result of phytochemical test shown in Table 1. These assays confirmed the presence of saponins, tannin, alkaloids, phenolic compounds, flavonoids, triterpenoids, glycosides, and plant sterols in Indonesian mangosteen peel crude extract.

Garciana mangostana L. peel extract reduced the growth of *Streptococcus mutans* biofilm

The results were achieved with 100% concentration of *G. mangostana* L. peel extract, which inhibited the growth of the *S. mutans* biofilm during an incubation period of 6 h. The statistical test results showed significant reduction of *S. mutans* biofilms after the treatment with *G. mangostana* L. peel extract in all concentrations at the 1 h incubation period compared to the negative control. All concentrations at the incubation period of 6 h and 24 h showed a significant reduction of *S. mutans* biofilms compared to the negative control ($P = 0.000$) [Figure 1].

Table 1: Qualitative phytochemical analysis of ethanolic mangosteen pericarp crude extract

Plant metabolites	Result
Saponins	+
Tannins	+
Alkaloids	+
Phenolics	+
Flavonoids	+
Triterpenoids	+
Steroids	–
Glycosides	+

+: Metabolites were found in the mangosteen pericarp crude extract

–: Metabolites were not found in the mangosteen pericarp crude extract

Garciana mangostana L. peel extract reduced the growth of *Porphyromonas gingivalis* biofilm

The results showed that the most effective concentration of *G. mangostana* L. peel extract for inhibiting the growth of the *P. gingivalis* biofilm was 100% over an incubation period of 24 h. The statistical analysis results showed a significant decrease in *P. gingivalis* biofilms after the *G. mangostana* L. extract treatment in all concentrations over a 1 h incubation period compared to the negative control ($P = 0.000$) [Figure 2].

Discussion

In this study, *G. mangostana* peel extract inhibited biofilm formation in *S. mutans* and *P. gingivalis* in a dose-dependent manner, with the best concentration being 100% over an incubation period of 6 h. The antimicrobial activity of mangosteen peel extracts was expressed better in *S. mutans* than in *P. gingivalis*. This difference may have been caused by the presence of one main active component in the mangosteen peel extracts, the α -mangostin, which possesses antimicrobial properties against Gram-positive oral streptococci that activate through many mechanisms, including the disruption of peptidoglycan that lead to membrane breakage and cell lysis.^[20] Since *P. gingivalis* is a Gram-negative bacteria that contains a thinner layer of peptidoglycans,^[21] this mechanism may not show the same effect as that of the Gram-positive bacteria.

Based on the results of this study, the authors also observed that achieving maximum antimicrobial activity from mangosteen peel extracts required longer incubation periods and higher concentrations. This pre-conclusional perspective was in agreement with a previous study of Janardhanan *et al.*^[22] who stated that mangosteen peel crude extracts gradually showed better bactericidal effects as the extracts’ concentration increased. Mangosteen peel extracts have much potential to damage and kill bacteria, but bacteria in the biofilm states can be more resistant to antimicrobial agents than to suspension cells.^[23]

Mangosteen peel extract has antioxidant, antibacterial, anticancer, antiviral, and antifungal properties.^[15] Ethanol solvents were reported to be better than methanol solvents

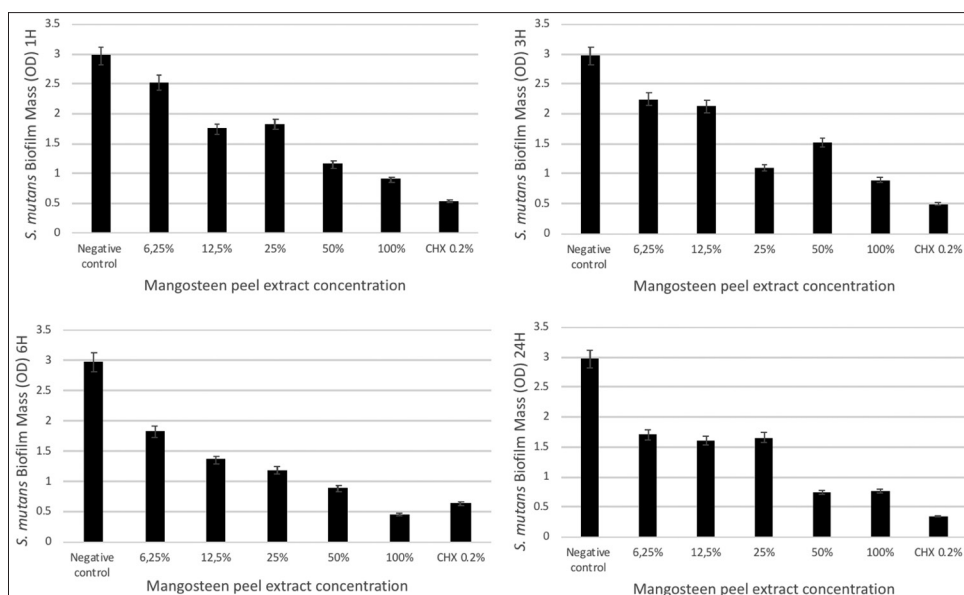


Figure 1: Concentration-response curves for treatment with mangosteen peel extract over different incubation times (1, 3, 6, and 24 h). The vertical axis indicates the *Streptococcus mutans* biofilm mass in optical density. The horizontal axis indicates the concentration of mangosteen peel extract (100%, 50%, 25%, 12.5%, and 6.25%). Chlorhexidine 0.2% was used as positive control and biofilm well without treatment was used as negative control

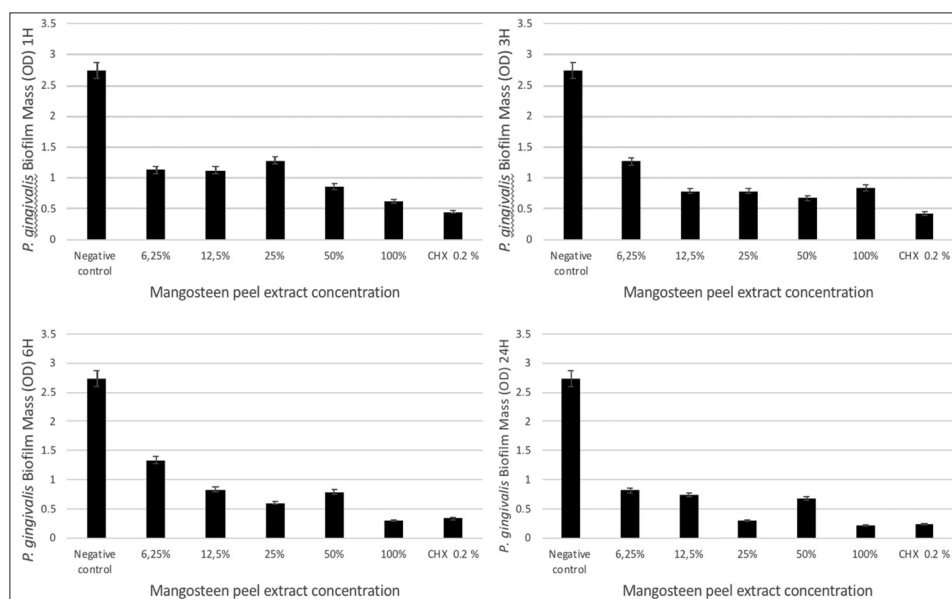


Figure 2: Concentration-response curves for treatment with mangosteen peel extract over different incubation times (1, 3, 6, and 24 h). The vertical axis indicates the *Porphyromonas gingivalis* biofilm mass in optical density. The horizontal axis indicates the concentration of mangosteen peel extract (100%, 50%, 25%, 12.5%, and 6.25%). Chlorhexidine 0.2% was used as positive control and biofilm well without treatment was used as negative control

at extracting mangosteen peels in terms of the antioxidant activity content.^[24] In the previous study, the toxicity of α -mangostin (4000 $\mu\text{g}/\text{mL}$) extracted using hexane and ethyl acetate was tested against gingival fibroblast cells, and no significant cytotoxicity effect was observed for 480 min.^[20] Therefore, the α -mangostin was considered safe for the cells.

Previous studies showed that xanthone-containing mangosteen peel extract significantly inhibits the *Candida albicans* and *S. mutans* growth *in vitro*.^[20,25] Oral biofilms are formed through several successive stages, starting with the formation of an acquired pellicle in the form of

a thin layer of salivary glycoproteins attached to the tooth surface. Then, the primary bacteria begin to form colonies that can change the surrounding conditions to be more suitable for the growth of obligate anaerobic bacteria.^[5] The bacterial colonies that have formed then synthesize EPS such as glucans, which lead to the formation of a biofilm matrix to help the bacteria remain attached to the acquired pellicle.^[26] The bacteria inside the biofilm surroundings communicate to survive the environment. One form of communication is quorum sensing: the bacterial process that requires the production of a signal molecule to control

bacterial attachment, production of extracellular matrices, and the virulence factor.^[27] The biofilm structure reduces the effectiveness of the antimicrobial substance when compared to planktonic cells; the presence of enzyme modification and the efflux pump is associated with the higher resistance factor of biofilms.^[28]

The presence of uncleaned oral biofilm can lead to chronic gingivitis and may even progress to periodontitis. Research shows that in the case of periodontitis, Gram-negative bacteria such as *P. gingivalis* are more common than Gram-positive bacteria.^[29] The lypopolysaccharides components present in *P. gingivalis* will lead to inflammatory processes if interacting with host cell antibodies, which can lead to inflammation and recession of periodontal tissue.^[4,30] Oral biofilm cleansing is one of the key treatments for gingivitis and periodontitis.^[29]

A previous study showed that xanthenes can damage the mechanical stability of *S. mutans* biofilm and inhibit glucosyltransferase enzyme activity, which results in the inhibition of biofilm growth.^[31] The xanthone-derived compounds contained in the mangosteen peel include α -mangostin, gartanin, and γ -mangostin, garcinone B, garcinone E, and mangostin.^[32,33] α -mangostin has antifungal properties, and studies show that α -mangostin is more effective at killing *C. albicans* than the same concentrations of clotrimazole and nystatin.^[34] Further research proves that α -mangostin can also inhibit the growth of *Mycobacterium tuberculosis* with a minimal inhibitory concentration value of 6.25 $\mu\text{g/mL}$.^[35] Flavonoids tend to bind proteins, thus interfering with bacterial metabolism.^[36] Tannins are antibacterial because they destroy the permeability of cell walls, resulting in the inhibition of bacterial cell activity.^[37] Saponins are also antibacterial because they increase membrane permeability, resulting in cell hemolysis.^[38]

Conclusion

Mangosteen peel extract is effective at inhibiting the growth of *S. mutans* and *P. gingivalis* in biofilms. This study shows antibiofilm effect can be an alternative therapy in preventing caries and periodontal disease. However, future studies are needed to explore this effect with other oral pathogens.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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