



# The Effectiveness of Herbal Mouthwash with Mangosteen Peel Extract in Inhibiting Dental Plaque Formation

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## Abstract

**Objective** Dental plaque control is important for preventing periodontal tissue diseases. Dental plaque control therapy is enhanced when supported by adjunctive therapy, including the use of mangosteen peel extract mouthwash. Mangosteen peel extract contains  $\alpha$ -mangostin, saponins, alkaloids, tannins, flavonoids, quinones, and triterpenoids, which have antibacterial properties against bacteria that cause dental plaque. This study aims to determine the effectiveness of mangosteen peel extract mouthwash at concentrations of 2, 4, and 6% in inhibiting plaque formation.

**Materials and Methods** The study used a quasi-experimental design with pre- and posttreatment examinations. Samples were taken using purposive sampling on 32 patients of Periodontology Clinic of Padjadjaran University Dental Hospital. The patients underwent prophylactic treatment (scaling), then the dental plaque index was measured using the Q-ray Cam Pro and the Loe and Silness Index before (day 1) and after (day 3) gargling with distilled water or mangosteen peel extract mouthwash at concentrations of 2, 4, and 6% for 2 days without oral hygiene in the maxillary area. The data were analyzed using the Wilcoxon test, analysis of variance (ANOVA), and the Kruskal–Wallis test.

**Results** A phytochemical analysis revealed that the mangosteen peel extract contains antibacterial compounds such as flavonoids, saponins, polyphenols, quinones, and triterpenoids. The mangosteen peel extract mouthwash group exhibited lower mean differences in plaque index compared with the aquades group. The 2% mangosteen peel extract mouthwash shows the smallest mean difference of 0.25 in the Q-ray Cam Pro examination and 0.062 in the Loe and Silness Index examination.

**Conclusion** Mouthwash with 2, 4, and 6% mangosteen peel extract has an effect in inhibiting dental plaque formation, with 2% concentration exhibiting the best inhibitory effect on dental plaque formation.

## Keywords

- ▶ mouthwash with mangosteen peel extract
- ▶ dental plaque
- ▶ Q-ray cam Pro
- ▶ Loe and Silness index

## Introduction

Oral health is one of the most essential aspects influencing human general health. Some studies reported that microbial instability in the oral cavity contributes not only to the pathology of oral diseases but also to the pathology of systemic diseases; hence, it disrupts the individual quality of life.<sup>1-7</sup> Therefore, conducting preventive actions to maintain oral hygiene effectively is substantial, including preventing dental plaque formation. Dental plaque control as a preventive action is essential because plaque accumulation may induce pathological processes in the oral cavity, for example, periodontal tissue diseases.<sup>8</sup> The data from Riskesdas 2018 reported that the prevalence of periodontal tissue diseases among individuals aged  $\geq 15$  years reached 74.1%; this emphasized the importance of preventing the formation of dental plaque.<sup>9</sup> Dental plaque control can be conducted through mechanical and adjuvant chemical therapies.<sup>10</sup> Mechanical therapies involve toothbrushing, dental flossing, toothpick, scaling, and scaling and root planing (SRP).<sup>11-13</sup> Although mechanical therapies provide an excellent result in maintaining oral hygiene, some studies proved that the control result would be better with reinforcement with adjuvant chemical therapy.<sup>11-14</sup> The adjuvant controls involve the usage of antibiotics, mouthwash, etc.<sup>15</sup>

Today, many types of mouthwash are available in the market. The synthetic types are more commonly used. However, there are some reversible side effects of using mouthwash, that is, tawny staining on the teeth, taste alteration, and unusability in allergic conditions.<sup>16,17</sup> Herbal materials have been reported to possess relatively lesser side effects, as stated in a study by Ristianti and Marsono.<sup>18</sup> Moreover, herbal materials are easier to obtain compared with synthetic materials.<sup>13</sup>

Mangosteen peels contain components such as xanthone, saponin, alkaloid, tannin, flavonoid, quinone, and triterpenoid groups, which act as antibacterial.<sup>19-21</sup> A study by Kemala et al supported the theory through their study result, which reported the antibacterial potency of mangosteen peel's (*Garcinia mangostana* L.) ethanol extract against *Streptococcus sanguinis* (ATCC 10556) due to antibacterial components of the extract.<sup>21</sup> Other studies concluded that  $\alpha$ -mangostin ( $\alpha$ MG) of xanthones was a potent glycolysis inhibitor.<sup>11</sup> Sivaranjani et al reported that  $\alpha$ MG targeted *Streptococcus* successfully leading to obliteration of the cytoplasm membrane integrity and resulting in rapid bactericidal activity.<sup>22</sup> In another study, Widyarman et al reported that purified  $\alpha$ MG could be used as a resistor of biofilm formation caused by *S. mutans* through the inhibition of the glucosyltransferase enzyme activity.<sup>23</sup> Based on these studies, it can be concluded that the antibacterial components of mangosteen peel can disrupt bacterial activity, eliminate bacteria, and prevent the growth of bacterial plaque; hence, the components of mangosteen peel can inhibit dental plaque formation.

The concentration used in a study regarding the testing of mangosteen peel ethanol extract against *S. sanguinis* (ATCC 10556) as the primary bacterial plaque by Kemala et al

reported that the zone of inhibition was detected in the 0.1 to 20% concentration of ethanol extract from mangosteen peels.<sup>21</sup> A study regarding the testing against *Aggregatibacter actinomycetemcomitans* as the secondary bacterial plaque by Hendiani et al reported that the minimal inhibitory concentration (MIC) score was 6.25% and the minimal bactericidal concentration (MBC) score was 12.5%.<sup>24</sup> The 4% concentration of mangosteen peel extract in a gel dosage form has been evaluated in previous studies, and it was reported to have tolerable flavor and was subjectively and objectively evaluated as biocompatible.<sup>25</sup> After consideration, the concentrations used in this study were 2, 4, and 6% since a clinical trial for these concentrations has not been conducted yet.

Many studies have proven that specific components of the mangosteen peel affect the bacteria causing dental plaque and the formation of dental plaque. The most common type of study is in vitro testing of mangosteen peel extract against bacteria. However, there have not been any studies that discuss how the activity of mangosteen peel extract mouthwash can inhibit dental plaque formation. Hence, the objective of this study was to discover the mangosteen peel extract mouthwash's activity and the optimum concentration of mouthwash in inhibiting dental plaque.

## Materials and Methods

### The Study Design

The present study used the quasi-experimental method with a pre- and poststudy design. We followed a double-blind system using a simple randomized design. This study compared the dental plaque index results of every treatment group. Ethical approval (249/UN6.KEP/EC/2023) for this study has been acquired from the Research Ethical Committee of Padjadjaran University.

### Population

The population of this study was 32 patients of the Periodontology Clinic in the Dental Hospital of Padjadjaran University. Sample size determination was set according to the Federer formula  $(T-1)(N-1) \geq 15$ . Based on the results of the formula calculation, the number of samples for each group was set as at least six persons. However, there is a consideration of the possibility of reducing participants for several reasons outside the criteria, so the determination of the sample size considers a simple 5-and-20 rule of thumb  $N/(1-\text{drop out percentage})$ . In this study, a dropout value of 20% was used to consider the worst-case scenario of reduced participants and increase the success rate of the study. Based on these considerations, this study determined a total sample of 32 people with a sample of 8 people in each group.

The sample of this study was divided into four groups (three treatment groups and one control group). The inclusion criteria were the following: (1) patients who are willing to participate in the study; (2) those with good general health; (3) those with six anterior maxillary teeth; (4) those with no absence of any anterior maxillary teeth; and (5) those who were free of dental caries. The exclusion criteria were the following: (1) patients who do not sign the

informed consent; (2) those with any lesions on oral soft tissue; (3) those who smoke; (4) those who consumed antibiotics in the last 3 months; (5) those who used fixed orthodontics appliances; (6) those who have crowding on anterior maxillary teeth; and (7) those who use other types of mouthwash.

### Date and Location of the Study

The manufacturing of mouthwash was conducted at the Herbal Study Center of the Faculty of Pharmacy Padjadjaran University and the Center for Pharmaceutical Developments of the Faculty of Pharmacy Padjadjaran University. The study was conducted at the Periodontology Clinic of Dental Hospital Padjadjaran University from December 2022 to April 2023.

### Mangosteen Peel Extract Mouthwash

Mangosteen peel powder was obtained from the Special Region of Yogyakarta and was analytic-certified by Lansida Herbal Technology. The mangosteen peel extract was produced using the maceration method with 70% ethanol, and then it was evaporated until a thick extract was obtained. The manufacturing process was conducted at the Pharmaceutical Technology Laboratory of the Center for Pharmaceutical Developments Faculty of Pharmacy Padjadjaran University. The formulation was based on a previous study by Nurhadi and was modified according to the Handbook of Pharmaceutical Excipients.<sup>26,27</sup> The manufacturing of mouthwash started with preparing and measuring each material. Tween 80 was dissolved into aquades in a beaker glass using a magnetic stirrer until homogenous v/v (solution 1). Sodium saccharin and sodium benzoate were added gradually into solution 1 and mixed until homogenous w/v (solution 2). Peppermint oil was dissolved into the thick mangosteen peel extract v/v (solution 3). Solutions 2 and 3 were mixed using a magnetic stirrer until it became homogenous. The homogenous final solution was distilled and packed into opaque bottles to preserve the quality of the solution (► **Table 1**).

### Study Instruments

The instruments utilized in the study were mouth mirrors, probes, Q-ray Cam Pro, examination forms, and ultrasonic scalers. The dental plaque assessment utilized the Q-ray Cam Pro (AIOBIO Co., Seoul, Republic of Korea) on the buccal

surfaces of 11, 12, 13, 21, 22, and 23 and the Loe and Silness index on the buccal and palatal surfaces of 11, 12, 13, 21, 22, and 23. The materials used in the study were 2, 4, and 6% mangosteen peel extract mouthwashes and aquades.

One of the measurements used is the Loe and Silness plaque index. This index is used to measure plaque thickness based on its location and amount near the gingival margin, so only plaque in the cervical third of the teeth without regard to plaque that extends into the middle or incisal third. This index is measured by air drying the teeth first and then checking the plaque thickness on each tooth measured using a sonde or explorer and mouth glass. The teeth examined include the buccal and palatal surfaces and then these were scored on the Loe and Silness index.<sup>28</sup>

The Q-ray Cam Pro (AIOBIO Co.) is a high-resolution, lightweight, handheld camera equipped with autofocus. It is designed for capturing dental images, particularly of anterior teeth, following the quantitative light-induced fluorescence (QLF) digital photography protocol. To use the Q-ray Cam Pro, the camera should be positioned as close as possible to the patient's teeth, with ambient light levels adjusted to minimize excessive lighting. The camera includes various settings such as full high-definition resolution, automatic shutter speed, automatic aperture, and a 2.3-MP image sensor for object distance detection. Fluorescence images of the vestibular surface of the anterior teeth are automatically saved as bitmap files by default using QLF software. The software analyzes these images to calculate the  $\Delta R$  value for each tooth surface pixel, which measures the increase in red fluorescence relative to the surface impression. Higher  $\Delta R$  values indicate areas with more active bacterial metabolism in the dental plaque, signifying greater dental plaque maturation.<sup>29</sup>

### Randomization Procedure

Randomization was done using a simple randomized method. The subject randomization was determined using the Web site randomizer.org. The mouthwash bottles were labeled with particular codes by Researcher B; the codes symbolized the concentration of each bottle. Then, the mouthwash bottles were distributed to the patients according to the randomization result. Only Researcher 2 knew the concentration value of each code. The study participants and Researcher 1, as the operator of the dental plaque

**Table 1** The formulation of mangosteen peel extract mouthwash

Materials	Formulation (%)			Function
	F1	F2	F3	
Mangosteen peel extract	2	4	6	Active component
Peppermint oil	0.2	0.2	0.2	Flavoring
Sodium benzoate	0.3	0.3	0.3	Perseverative
Sodium saccharin	0.2	0.2	0.2	Sweetener
Tween 80	1	1	1	Emulator
Aquades	350	350	350	Solvent

assessment, did not know the concentration of the mouthwash of each participant.

### Study Procedure

The study began when the patients arrived without brushing their teeth and then an oral prophylaxis procedure was performed, that is, SRP to remove dental plaque. There were no specific instructions given to the patients prior to the initial examination or baseline examination. After the dental plaque was removed to a minimum, a baseline dental plaque examination was performed directly at the same time on the anterior maxillary teeth (11, 12, 13, 21, 22, and 23) using the Q-ray Cam Pro and the Loe and Silness index measurement. The dental plaque assessment using the Q-ray Cam Pro was conducted by taking images of the buccal teeth surface, and then the images were automatically assessed by the Q-ray software. The dental plaque assessment using the Loe and Silness index assessed the plaque on the cervical one-third of the buccal and palatal tooth surfaces. The patients were instructed to gargle 15 to 20 mL of mouthwash for 30 seconds. This was done twice a day (after breakfast and before sleeping) for 2 days without performing any other oral hygiene procedures on the teeth in the upper jaw, neither toothbrushing nor flossing. After using mouthwash, it is advisable to wait approximately 30 minutes before eating or drinking. This gives time for the active ingredients in the mouthwash to work effectively in the mouth and teeth without being disturbed by food or drink that may rinse it away. The research was executed in a short time (2 days) to minimize the patients' boredom, but the duration was still adequate to acquire the study's objective for dental plaque formation. After 2 days of using the mouthwash, the patients were reexamined for dental plaque assessment using the Q-ray Cam Pro and the Loe and Silness index.

### Data Analysis

Data analysis uses parametric tests that are more accurate on normally distributed data and nonparametric tests on non-normally distributed data. The mean difference of the dental plaque index score before and after using the mouthwash of each group was analyzed using the Wilcoxon test. A comparison of the mean difference of the dental plaque index score

before and after using the mouthwash between the groups was done using the Kruskal–Wallis test because the data were not normally distributed, except for data after gargling with mouthwash measured using the Loe and Silness index because the data were normally distributed analyzed using the analysis of variance (ANOVA) test. The comparison of the mean derivation of the dental plaque index score between the groups was performed using the Kruskal–Wallis test.

## Results

### The Phytochemical Screening Results

The phytochemical screening of mangosteen peel extract (*Garcinia mangostanae pericarpium extractum*).

The objective of the screening was to determine the antibacterial components embodied in the mangosteen peel extract used in this study. The phytochemical screening was conducted at the Herbal Study Center of the Faculty of Pharmacy Padjadjaran University. The screening results showed that the mangosteen peel extract contained antibacterial components, that is, flavonoid, polyphenol, quinone, saponin, and triterpenoid. The screening results are provided in **Table 2**.

### Intervention Results

Screening of the study patients resulted in 32 patients of the Periodontology Clinic in the Dental Hospital of Universitas Padjadjaran who were fulfilled the criteria of this study. During the research, only 30 of 32 patients attended the whole sequence of the dental plaque assessment because the patients could not be present for the dental plaque assessment after the treatment research (the third day). As seen in **Table 3**, the patients were characterized according to sex and age. In this study, the number of female patients was higher than male patients—20 females and 10 males. According to the age grouping of the health department, all the patients were classified as young adults (17–25 years).<sup>30</sup>

**Table 4** compares the mean score of the dental plaque index assessed using the Q-ray Cam Pro before (day 1) and after (day 3) using the mouthwash in each group. The data showed that the mean score of each group was various, but the mean difference before (day 1) and after (day 3)

**Table 2** The phytochemical screening test of the mangosteen peel extract

Component	Result	
Alkaloid	No deposition formed following the addition of dragendorff reagent	–
Flavonoid	Yellow color formed on the amyl layer of alcohol	+
polyphenols	Color change to blackish blue following the addition of FeCl <sub>3</sub>	+
Tannin	No deposition formed following the addition of gelatin	–
Quinone	Color change to red following the addition of KOH	+
Saponin	Persistent bubbles formed following the shaking and the addition of HCl	+
Triterpenoid	Purple/blueish green color formed following the addition of Liebermann–Burchard reagent	+

Note: +: Component detected.

–: Component not detected.

**Table 3** The study participants' characteristics

Variable	Placebo group (aquades)	2% concentration group	4% concentration group	6% concentration group	Number of variables
Male	2	2	2	4	10
Female	6	6	5	3	20
Age (mean)	20	21	22	22	21

**Table 4** The mean dental plaque index score using the Q-ray Cam Pro on buccal surfaces before (day 1) and after (day 3) treatment research (Wilcoxon test)

Group	n	Time of assessment	Mean score	SD	Mean difference	p-value
Placebo (aquades)	8	Before (day 1)	0.25	0.71	1	0.063
		After (day 3)	1.25	1.49		
2% concentration	8	Before (day 1)	0.5	1.41	0.25	0.157
		After (day 3)	0.75	1.75		
4% concentration	7	Before (day 1)	0.29	0.49	0.28	0.48
		After (day 3)	0.57	0.79		
6% concentration	7	Before (day 1)	0.29	0.49	0.42	0.083
		After (day 3)	0.71	0.76		

Abbreviations: SD, standard deviation; n, number of participants.

Note: Wilcoxon test.

p-value < 0.05 significant.

**Table 5** The mean dental plaque index score using the Loe and Silness index on buccal and palatal surfaces before (day 1) and after (day 3) treatment research (Wilcoxon test)

Group	n	Time of assessment	Mean score	SD	Mean difference	p-value
Placebo (aquades)	8	Before (day 1)	0.01	0.29	0.29	0.012 <sup>a</sup>
		After (day 3)	0.30	0.17		
2% concentration	8	Before (day 1)	0.00	0.00	0.062	0.034 <sup>a</sup>
		After (day 3)	0.062	0.06		
4% concentration	7	Before (day 1)	0.02	0.04	0.1	0.071
		After (day 3)	0.12	0.09		
6% concentration	7	Before (day 1)	0.02	0.04	0.1	0.063
		After (day 3)	0.12	0.76		

Abbreviations: SD, standard deviation; n, number of participants.

Note: Wilcoxon test.

<sup>a</sup>p-value < 0.05: significant.

treatment in all the groups was not statistically significant ( $p > 0.05$ ), indicating that the plaque score before and after using mouthwash was similar, and there was no dental plaque formation in all the groups.

► **Table 5** compares the mean score of the dental plaque index assessed using the Loe and Silness index before (day 1) and after (day 3) using mouthwash in each group. The data showed a significant difference in the mean scores of the dental plaque index in the aquades and 2% mangosteen peel extract mouthwash before (day 1) and after (day 3) the treatment research; the significance values were 0.012 and 0.034, respectively; there was formation of dental plaque.

The mean score of the dental plaque index in the 4% mangosteen peel extract before (day 1) and after (day 3) treatment did not differ significantly ( $p > 0.05$ ), indicating that the plaque score remained similar; no dental plaque was formed.

The comparison of the mean dental plaque score before (day 1) and after (day 3) using the mouthwash between the groups is provided in ► **Table 6**. The data showed that the Q-ray Cam Pro assessment conducted before (day 1) the treatment research resulted in insignificant scores between the groups ( $p > 0.05$ ); therefore, the data before the treatment research were considered homogenous. The data from

**Table 6** The comparison of mean dental plaque index score before (day 1) and after (day 3) the treatment research (placebo, 2%, 4%, and 6% mangosteen peel extract mouthwash)

Parameter	Surface	Time of assessment	Score (mean ± SD) mouthwash				p-value
			Aquades	2%	4%	6%	
Q-ray Cam Pro	Buccal	Before (day 1) <sup>a</sup>	0.25 ± 0.71	0.5 ± 1.41	0.29 ± 0.49	0.29 ± 0.49	0.874
		After (day 3) <sup>a</sup>	1.25 ± 1.49	0.75 ± 1.75	0.57 ± 0.79	0.71 ± 0.76	0.689
Loe and Silness index	Buccal and palatal	Before (day 1) <sup>a</sup>	0.01 ± 0.29	0.00 ± 0.00	0.02 ± 0.04	0.02 ± 0.04	0.388
		After (day 3) <sup>b</sup>	0.30 ± 0.17	0.062 ± 0.06	0.12 ± 0.09	0.12 ± 0.076	0.003 <sup>c</sup>

Abbreviation: SD, deviation standard.

<sup>a</sup>Kruskal–Wallis test.

<sup>b</sup>ANOVA test.

<sup>c</sup>p-value <0.05: significant

**Table 7** Follow-up test of mean dental plaque score after (day 3) the treatment research (placebo, 2%, 4%, and 6% mangosteen peel extract mouthwash) measured with Loe and Silness Index

Comparison of treatment research	p-value	Critical region	Conclusion	Mean differences	Comparison
Aquades vs. 2%	0.002*	H <sub>0</sub> rejected	A significant difference	0.23887	Aquades > 2%
Aquades vs. 4%	0.029*	H <sub>0</sub> rejected	A significant difference	0.18241	Aquades > 4%
Aquades vs. 6%	0.029*	H <sub>0</sub> rejected	A significant difference	0.18270	Aquades > 6%
2% vs. 4%	0.793	H <sub>0</sub> accepted	A nonsignificant difference	-0.05646	2% < 4%
2% vs. 6%	0.795	H <sub>0</sub> accepted	A nonsignificant difference	-0.05618	2% < 6%
4% vs. 6%	1	H <sub>0</sub> accepted	A nonsignificant difference	0.00029	4% > 6%

<sup>a</sup>p-value <0.05: significant.

the Q-ray Cam Pro assessment conducted after (day 3) the treatment research were also insignificant between the groups ( $p > 0.05$ ). ► **Table 6** also compares the mean dental plaque score using the Loe and Silness index. The mean dental plaque score before (day 1) the treatment research resulted in insignificant scores between the groups ( $p > 0.05$ ); therefore, the data before the treatment research were considered homogenous. The data from the Loe and Silness index assessment conducted after (day 3) the treatment research were significant between the groups ( $p < 0.05$ ), indicating that there were groups with significant dental plaque index compared with other groups (► **Table 7**).

The following test was conducted to determine the significance of the group comparison data. The following test results of the mean dental plaque score after the treatment result using the Loe and Silness index showed that the group with the highest mean difference was the aquades group, indicating that aquades had the least activity of inhib-

iting dental plaques. The mean differences of 2, 4, and 6% concentration were insignificant, indicating that the groups had a relatively similar activity of dental plaque inhibition.

► **Table 8** provides data on the comparison of the mean deviation of dental plaque index score using the Q-ray Cam Pro between the groups. The test resulted in a p-value of 0.0628 ( $p > 0.05$ ); therefore, the mean deviation between all the groups was nonsignificant, indicating that the groups had a similar activity of dental plaque inhibition. ► **Table 8** also provides the data of the Loe and Silness index. The test resulted in a p-value of 0.01 ( $p < 0.05$ ); therefore, significant differences in the mean derivation of all the groups were discovered.

The Mann–Whitney U test results above indicate that the Loe and Silness index assessment resulted in a significant mean derivation of dental plaque index ( $p < 0.05$ ) between the aquades group and the mouthwash group. ► **Table 9** shows that the highest dental plaque formation occurred in the aquades group, indicating a deficient activity in dental

**Table 8** The comparison of mean deviation of the dental plaque index score in the treatment research

Assessment	Mouthwash				p-value
	Aquades	2%	4%	6%	
Q-ray Cam pro	1.00 ± 1.31	0.25 ± 0.46	0.29 ± 1.11	0.43 ± 0.53	0.628
Loe and Silness index	0.9 ± 0.172 <sup>a</sup>	0.062 ± 0.06 <sup>b</sup>	0.095 ± 0.11 <sup>b</sup>	0.095 ± 0.11 <sup>b</sup>	0.01 <sup>c</sup>

<sup>a,b</sup>Nonsignificant.

<sup>c</sup>p-value <0.05: significant.

**Table 9** The Mann–Whitney *U* test for comparison of the mean deviation of the dental plaque index score on buccal and palatal surfaces after the treatment research using the Loe and Silness Index

Comparison of treatment research	<i>p</i> -value	Critical region	Conclusion	Comparison
Aquades vs. 2%	0.002 <sup>a</sup>	$H_0$ rejected	A significant difference	Aquades > 2%
Aquades vs. 4%	0.019 <sup>a</sup>	$H_0$ rejected	A significant difference	Aquades > 4%
Aquades vs. 6%	0.025 <sup>a</sup>	$H_0$ rejected	A significant difference	Aquades > 6%
2 vs. 4%	0.431	$H_0$ accepted	A nonsignificant difference	2% < 4%
2 vs. 6%	0.757	$H_0$ accepted	A nonsignificant difference	2% < 6%
4 vs. 6%	0.895	$H_0$ accepted	A nonsignificant difference	4% > 6%

<sup>a</sup>*p*-value <0.05: significant.

plaque inhibition. The comparison data in the 2, 4, and 6% concentrations showed nonsignificant mean derivations ( $p > 0.05$ ), indicating that the groups had similar activity in dental plaque inhibition, with the lowest mean derivation occurring in the 2% concentration.

## Discussion

The phytochemical test results showed that the mangosteen peel extract comprised antibacterial components, that is, flavonoid, saponin, polyphenol, quinone, and triterpenoid. The extract was also assumed to contain  $\alpha$ MG since previous studies reported that the xanthone derivation is the most commonly found component in the mangosteen peel.<sup>31</sup> Xanthone in the mangosteen peel is a class of polyphenols, one of the components evidenced in this study.<sup>32</sup> The screening test of the mangosteen peel ethanol extract showed that tannin was not present in the extract. The antibacterial components provided by the phytochemical test depicted that the mangosteen peel ethanol extract in this study had an antibacterial property.

Despite the benefits of mangosteen peel, minimal side effects might arise due to inappropriate use. Minimal side effects affecting the gastrointestinal, skin, and respiratory and general conditions were reported from a study on the consumption of mangosteen peel extract in a capsule dosage form. The most common side effects involved the gastrointestinal system, which was assumed to relate to the antibacterial property of mangosteen peel, thereby causing disturbances in intestinal microbes.<sup>33</sup> After the first rinsing, only 30% of the mouthwash remains in the saliva for 5 hours and in the oral mucosa for 12 hours and no concentration was found in the plasma. It is due to the poor absorption of mouthwash in the gastrointestinal system.<sup>34</sup> Therefore, oral administration using mouthwash is considered safer than systemic administration. The safety of  $\alpha$ MG as an antibacterial component was verified by a study by Wahyuni et al, who evaluated the in vitro cytotoxicity toward the leukocyte cell culture and the antioxidant activity test. The study indicated that the tested concentrations are safe to consume, that is, 3.125, 6.25, 12.5, 25, 50, and 100  $\mu$ g/mL.<sup>35</sup>

This present study showed that mangosteen peel extracts generate excellent dental plaque inhibition. **Tables 4 and 5** provide information that after the equalization treatment

(prophylaxis therapy) and the dental plaque assessments before and after the treatment research; the test groups (2, 4, and 6% mangosteen peel extract mouthwash) resulted in less dental plaque formation compared with the control group that used aquades. These findings can be seen in the mean deviation of the dental plaque index score, indicating how much plaque was formed. The highest dental plaque formation occurred in the control group, with mean deviations of 1 and 0.29 from the Q-ray Cam Pro and the Loe and Silness index assessment results, respectively. The lowest dental plaque formation occurred in the 2% concentration group, with the mean derivation of 0.025 and 0.062 from the Q-ray Cam Pro and the Loe and Silness index assessment results, respectively.

**Table 8** provides data on the comparison of the mean deviation of dental plaque index score using the Loe and Silness index between the groups; based on the data, the *p*-value was 0.01, indicating differences in dental plaque formation between the groups. Based on the following test in **Table 9**, the aquades group yielded a different outcome from all groups ( $p < 0.05$ ) and the 2% concentration group resulted in the least dental plaque formation. These findings show that the 2, 4, and 6% mangosteen peel extract mouthwash inhibited dental plaque formation. This is consistent with previous in vitro research studies about the mangosteen peel extract testing against *S. mutans* that reported that the mangosteen peel extract inhibited dental plaque formation.<sup>11,19–23</sup>

The mean differences in dental plaque formation in the 2, 4, and 6% mangosteen peel extract mouthwash were not very different. In the 2% mangosteen peel extract mouthwash group, there were no male patients, while in the 4 and 6% groups male patients had a relatively higher dental plaque formation than female patients during the oral prophylaxis process, probably because men are considered to be less concerned with hygiene and aesthetics than women. Besides that, several factors can affect the results, including patient compliance, the condition of the patient's teeth and mouth, the food they eat, and other. Therefore, the habits of the patients in this group may affect the results of the study. These factors cannot be controlled by researchers, unless the patients are quarantined. This does not mean that the 4 and 6% mangosteen peel extract mouthwash concentrations are ineffective. The 2, 4, and 6% mangosteen peel extract mouthwash concentrations can inhibit the formation of dental

plaque. However, from the results of this study it can be concluded that only a 2% concentration is sufficient to help inhibit the formation of dental plaque.

Factors affecting the research results include patient compliance in implementing the instructions (using the mouthwash without performing toothbrushing) during the research. Many factors impact patient compliance, that is, negligence while using mouthwash, fear of side effects, worrying about the drug quality, and long-term research.<sup>36</sup> Some preventive actions were taken in this study to overcome these factors. During the research, the researchers reminded the patients about the correct steps of using the mouthwash and not brushing their anterior maxillary teeth. At the beginning of the study, the patients signed the informed consent, and were informed about the whole research sequence beforehand to minimize patients' solicitudes regarding the side effects and quality of the drugs. Other external factors beyond the researchers' control were the type of foods consumed by the patients during the research. Dental plaque accumulation would increase if the patient ate plenty of soft and glutinous foods, particularly high-sucrose foods.<sup>37</sup> Water or aquades gargle can also help cleanse food residue between the teeth. While gargling, water flows in between the spaces of the teeth and removes the impacted residual foods; therefore, it helps reduce the residual food accumulation that can be a source of nutrition for bacteria.<sup>38</sup> This is one of the excuses that aquades gargle statistically can inhibit dental plaque formation, ► **Table 4**.

Aside from patient compliance and type of foods, Newman et al stated that oral hygiene, diet, medical condition, smoking, and genetics were also predisposing factors of dental plaque formation. Smoking can reduce salivary flow; saliva contributes to dental remineralization with its components, for example, calcium, and phosphate, which contribute to strengthening the tooth enamel layer.<sup>15</sup> Newmann et al also mentioned that the tar component in the cigarette could stick to the tooth surface and settle on the gingival tissue. The presence of tar increases bacteria and food residue retention in the teeth, thereby causing dental plaque accumulation.<sup>39</sup> A study by Al-Jobair et al reported that teeth with uneven surfaces were prone to dental plaque accumulation, as it would be hard to remove the plaque from such surfaces effectively. Therefore, the risk of dental plaque accumulation increases. Moreover, a narrow or wide gap between the teeth influences dental plaque formation. In a narrow gap, it is hard to remove the plaque thoroughly.<sup>40</sup> The same can be observed in crowding teeth; it is hard for a toothbrush and dental floss to reach the gaps in crowding teeth, resulting in a high risk of dental plaque formation.<sup>41</sup> These factors are difficult to control in a study and cause different outcomes in every individual.

The difference in significance in measurements using the Q-ray Cam Pro and the Loe and Silness index observed in ► **Tables 4** and **5** can be influenced by the number of tooth surfaces examined. In the measurement using Q-ray Cam Pro, the tooth surface examined was only the buccal surface due to the researchers' limitation to take pictures on the palatal surface, while in the measurement using the Loe and

Silness index, the tooth surface examined was the buccal and palatal surfaces so that the values of the two examinations can be different. However, both examination methods have their own advantages. The Q-ray Cam Pro examination is considered more objective because the assessment uses a tool, while the Loe and Silness index does not have limitations to assess the palatal surface of the teeth so that the data are assessed well because more tooth surfaces are assessed. The advantages of both methods suggest that both methods can be used.

Furthermore, although the mangosteen peel extract can be applied as an additional effort to get rid of dental plaques, mechanical dental plaque control is still considered the primary priority because dental plaque cannot be fully removed using mouthwash. No previous studies have concluded that using mouthwash can be a substitute for mechanical dental plaque control. Nevertheless, mouthwash is worth applying as a different approach to cleaning dental plaque.

## Conclusion

Based on the study results, the 2, 4, and 6% mangosteen peel extracts possessed the potency to inhibit dental plaque formation. The 2% mangosteen peel extract was discovered to have the most prominent dental plaque inhibition potency.

### Suggestion

Further studies with a larger sample size are recommended. In addition, it is essential to control for factors that could influence the study result to increase its validity.

### Authors' Contributions

I.H., T.D.F., B.C.H., and C.A.B. participated designing the study and collection of data. I.M.S.P. participated in data collection. I.H., T.D.F., and B.C.H. participated in analysis and writing the manuscript. All the authors read and approved the final version of the manuscript.

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### Conflict of Interest

None declared.

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