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Research Report

The inhibition of *Streptococcus mutans* glucosyltransferase enzyme activity by mangosteen pericarp extract

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ABSTRACT

Background: *Streptococcus mutans (S. mutans) is a bacterium that plays an important role in the pathogenesis of dental caries. Streptococcus mutans produces the glucosyltransferase enzyme which is capable of catalyzing glucan synthesis in the progression of dental caries. Certain treatments involving traditional plant use have been developed to eradicate Streptococcus mutans as a means of preventing the formation of dental caries. One of these is mangosteen pericarp extract containing a number of polyphenols that have the capacity to act as antibacterial agents, namely; tannin, mangostin, and flavonoid.* ***Purpose:*** *The study aimed to investigate the inhibitory power of mangosteen pericarp extract against Streptococcus mutans producing the glucosyltransferase enzyme.* ***Methods:*** *The research used mangosteen pericarp extract at concentrations of 0.39% and 0.78% as the treatments, while 0.12% chlorhexidine gluconate was used as a positive control, and distilled water as a negative control. Each group consisted of six samples. Mangosteen peels extracted with 96% ethanol (maceration method) and mangosteen extract constituted 5% of the total weight of the mangosteen pericarp. Supernatant containing Gtf enzyme produced from a culture medium and centrifuged at 1500 rpm for 10 minutes at 4° C. Glucosyltransferase enzyme activity was measured by analyzing the extensive fructose area by means of High Performance Liquid Chromatography (HPLC). The extensive fructose area was determined according to time retention in each group.* ***Results:*** *Mangosteen peel extract at concentrations of 0.39% and 0.78% demonstrated greater ability than the negative control group (sterile aquades) and similar ability to the positive group (chlorhexidine 0.12%) to inhibit the activity of the Gtf enzyme or S. mutans bacteria.* ***Conclusion:*** *Mangosteen pericarp extract has the ability to inhibit the activity of Streptococcus mutans in producing glucosyltransferase enzyme.*

Keywords: *glucosyltransferase enzyme; inhibitory power; mangosteen pericarp extract; Streptococcus mutans.*

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INTRODUCTION

According to the Basic Health Research (RISKESDAS) project of 2013, the DMF-T index of the Indonesian population was 4.6, categorized as 'high' by the World Health Organization (WHO). The high prevalence of dental caries cannot, in fact, be separated from the virulent properties of cariogenic bacteria in the oral cavity.[1] Cariogenic bacteria, together with fermentable carbohydrates and saliva, contributes to the demineralization cycle and dental remineralization. The main cariogenic bacterium responsible for dental caries is *Streptococcus mutans* (*S. mutans*), also considered to be the dominant bacterium in the oral cavity.[2]

S. mutans produces the glucosyltransferase (Gtf) enzyme, a virulent factor in dental caries pathogens. The Gtf enzyme can catalyze the formation of soluble and insoluble glucan derived from sucrose, while also playing a significant role in the polysaccharide matrix composition of dental plaque. This is because glucan can support both the attachment to and accumulation on tooth surfaces of cariogenic *S. mutans* bacteria. As a result, the activity of the Gtf enzyme generated by *S. mutans* bacteria should be inhibited in order to prevent dental caries.[3]

In the field of dentistry, certain antiseptics and other substances, including chlorhexidine, triclosan, and sanguinarine, are often used as dental plaque control

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agents. One antibacterial material considered to be the gold standard in the field of dentistry is chlorhexidine. Nevertheless, it still manifests several deficiencies leading to tooth discoloration and discomfort. Consequently, further research should focus on the development of a new specific agent capable of inhibiting Gtf enzyme activity as a means of controlling the formation of dental plaque with minimal side effects on the oral cavity.[4]

New agents the medical benefits of which have been investigated are naturally occurring materials enjoying wide availability and limited side effects. One frequently used natural material whose benefits are particularly well-known throughout Southeast Asia is mangosteen (*Garcinia mangostana L.*). This fruit or, more specifically, its peel provides many benefits contains several compounds producing a range of pharmacological benefits, including; anti-aging, anti-bacterial, anti-viral and anti-hypertension.[5] The results of laboratory tests confirm that mangosteen peel extract contains a range of elements, including; xhantone (10.70%), saponin (3.82%), tannins (5.92%), αα-mangostin (2.82%), β mangostin (7.88%), flavonoids (1.88%), and mangostanin (11.88%).[6]

S. mutans produce three Gtf enzymes: GtfB synthesizes the insoluble glucan alpha (1-3) polymer, GtfC synthesizes a mixture of insoluble glucan alpha (1-3) and non-alpha soluble (1-6), whereas GtfD synthesizes soluble glucan alpha (1-6). The content of flavonoids and alpha αα-mangostin in mangosteen pericarp extract is effective in inhibiting GtfB and GtfC enzymes restricting their activity by up to 70%. However, the main one is GtfB because it can catalyze the formation of insoluble, alfa-linked glucan.[7] Inhibition of enzyme activity is also known to be caused by tannin which has an inhibitory power of 31.39%.[8] In addition, flavonoids also actively limit the activity of the Gtf enzyme as well as promoting antibacterial activity.

Nevertheless, such compounds have been shown to be effective in inhibiting the activity of Gtf enzyme. Unfortunately, no research has yet been conducted into the inhibitory power of a natural material containing all three compounds with regard to the activity of Gtf enzymes. Thus, mangosteen peel, containing flavonoids, α-mangostin, and tannins, is thought to be effective in inhibiting the activity of Gtf enzymes. Mangosteen pericarp extract is also known to be capable of inhibiting and killing *S. mutans* bacteria. The minimum inhibitory concentration (MIC) of the mangosteen pericarp extract on *S. mutans* bacteria is 0.39%, while its minimum bactericidal concentration (MBC) is 0.78%.[6]

Further investigations would be most appropriately focused on the MIC and MBC of the mangosteen pericarp extract since it is expected to be an alternative ingredient of mouthwash within its MIC range. Moreover, this research was also expected to analyze the mangosteen pericarp extract within the concentration range of MIC and MBC. Therefore, the activity of the Gtf enzyme can still continue even though *S.mutans* bacteria have died within the concentration range of MBC.[9]

MATERIALS AND METHODS

The research reported here was an experimental laboratory-based study with randomized control group post test-only design. Research samples consisted of *S. mutans* bacteria obtained from the Laboratory of Microbiology, Faculty of Dental Medicine, Universitas Airlangga, Indonesia. The number of samples totaled six in accordance with Federer formula (1963). Independent variables consisted of the mangosteen pericarp extract at concentrations of 0.39% and 0.78%, while the dependent variable was the activity of Gtf enzyme generated by *S. mutans* bacteria.

Mangosteen pericarp extract was prepared at Materia Medika, Batu, East Java, Indonesia. Meanwhile, the first preparation of *S. mutans* bacteria was conducted in the Laboratory of Microbiology, Faculty of Dental Medicine, Universitas Airlangga, Indonesia. Subsequently, the second preparation of *S. mutans* bacteria was performed at Institute of Tropical Disease, Universitas Airlangga, Indonesia. Thereafter, Gtf enzyme was extracted from *S. mutans* bacteria and tested at Central Testing Services, Faculty of Pharmacy, Universitas Airlangga, Indonesia.

Mangosteen pericarp extract was prepared by treating dried mangosteen pericarp with 96% ethanol solvent and water using a maceration method. Consequently, all less polar, semi polar and polar chemicals could be extracted as far as possible. The ratio of 96% ethanol solvent to mangosteen peel powder used was 1:2. The maceration method is a filtration process of simplicia derived from solvent by agitating the latter several times and then stirring it with a Gerhardt Thermoshaker, Germany (2 x 24 hours) at room temperature. The simplica was then filtered, the resulting clear red filtrates being evaporated with a vacuum evaporator (at 60° C) until the ethanol separated out. Brown mangosteen pericarp extract accounting for 5% of the total weight of the mangosteen pericarp was then produced.

The *S. mutans* bacteria used in this research were drawn from *S. mutans* stock and then injected into BHIB (Brain Heart Infusion Broth) media before being incubated for 24 hours at 37° C. After cultivation for 24 hours, a sterile tube containing *S. mutans* bacteria was prepared in 7 mL of BHIB, incubated at 37° C for 24 hours and, finally, vibrated at 150 rpm with a Gerhardt Thermoshaker, Germany.[10] Next, the culture media were centrifuged at 1500 rpm using an Ultra Sentrifugator Hermle Z36HK for 10 min at 4° C, generating supernatants containing the Gtf enzyme.[11]

Twenty-four test tubes were subsequently utilised during this investigation, six of which were used for each research group, namely the positive control group, the negative control group, the treatment group using the mangosteen pericarp extract at a concentration of 0.39%, and the treatment group using the extract at a concentration 0.78%. The positive control tubes contained 0.875 ml of 0.25 M sucrose in 0.2 M phosphate buffer with pH of 7, then supplemented with 0.1 ml of Gtf enzyme solution and 0.025 ml of 0.12% chlorhexidine. The negative control tubes, on

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the other hand, contained 0.875 ml of 0.25 M sucrose in 0.2 M phosphate buffer with pH of 7, then supplemented with 0.1 ml of Gtf enzyme solution and 0.025 ml of sterile aquadest. In addition, tubes in the first treatment group contained 0.875 ml of 0.25 M sucrose in a 0.2 M phosphate buffer with pH 7, then supplemented with 0.1 ml of the Gtf enzyme solution and 0.025 ml of the mangosteen peel extract at a concentration of 0.39%. Meanwhile, tubes in the second treatment group contained 0.875 ml of 0.25 M sucrose in a 0.2 M phosphate buffer with pH 7, then supplemented with 0.1 ml of the Gtf enzyme solution and 0.025 ml of the mangosteen peel extract at a concentration 0.78%. All such treatment and control materials were then incubated at 37^0 C for two hours.

After incubation and filtering with 0.45 µm, filter papers, the fructose concentration was tested by means of a high performance liquid chromatography (HPLC Agilent, USA), i.e. by injecting 20 µl of the treatment solution or control solution to reveal their retention time. Fructose levels were then calculated by reading the area of fructose in the standard solution as follows:[12]

$$\text{Concentration (\%)} = \frac{\{(AC/AS) \times (VIS/VIC) \times FP\} \times 100\%}{KS}$$

Note:
AC = sample area
AS = standard area
VIC = volume of sample injection
VIS = volume of standard injection
KS = standard concentration
FP = dilution factor

The results of the fructose standard solution test on HPLC in this research showed that the retention time for fructose was approximately 2.8 minutes. The area of fructose generated in each sample can be indicated by the retention time shown on the chromatogram.

The normality test is performed to calculate normally distributed data, with Levene's test being subsequently performed to calculate the homogeneity of the data. The next calculation uses the Kruskal Walles test to calculate the differences between groups. The value of significance between groups is established through application of the Mann-Witney test.

RESULTS

The results of the reading and measuring of fructose levels with HPLC are divided into the control groups and the treatment groups using the mangosteen pericarp extract (Figure 1).

The results of the normality test revealed the significance value to be greater than 0.05. This suggests that the data for all research groups was normally distributed. Meanwhile, the Levene's test results showed a significance value of 0.000 ($p<0.05$) indicating that the data was not homogeneous. The results of the Kruskal-Wallis test indicated a significance value of 0.001 ($p<0.05$), indicating that there were significant differences between the research groups.

To reveal the significance value in each group, a Mann-Whitney test was performed the results of which can be seen in Table 1. The negative control group (sterile aquades)

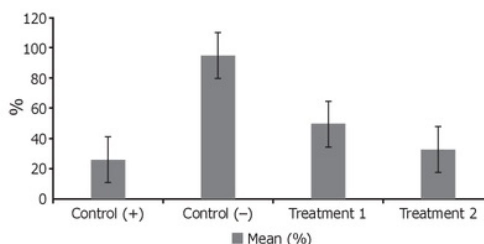

Figure 1. The mean and SD level of fructose in the control and treatment groups.

Table 1. Results of the Mann-Whitney test

	Negative Control	Positive Control	Treatment Group 1	Treatment Group 2
Negative control (aquades sterile)	–	p value= 0,002*	p value= 0.002*	p value= 0,002*
Positive control (chlorhexidine 0,12%)	–	–	p value= 0,065	p value= 0,180
Treatment Group 1 (0,39%)	–	–	–	p value= 0,180
Treatment Group 2 (0,78%)	–	–	–	-

Note:
(+) = The positive control group using 0.12% *chlorhexidine*
(-) = The negative control group using sterile aquadest
I = The treatment Group I using mangosteen pericarp extract at the concentrations of 0.39%
II = The treatment Group I using mangosteen pericarp extract at the concentrations of 0.78%

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ability's to inhibit Gtf *S. mutans* enzyme (p=0.002) was significantly lower than that of the positive control group (chlorhexidine 0.12%), and the 0.78% mangosteen pericarp extract group. The negative control group's (sterile aquades) ability to inhibit Gtf *S.mutans* enzyme (p=0.002) was significantly lower than that of the treatment group of 0.39% mangosteen pericarp extract. The mangosteen pericarp extract concentration group of 0.39% and 0.78% manifested the same ability as the positive control group (chlorhexidine 0.12%) as a substance which could inhibit the bacterial Gtf *S. mutans.*

DISCUSSION

The research results showed that the concentration of fructose in the negative control group had a higher value (94.5%) than the other treatment groups. The elevated concentration of fructose indicated the level of activity of *S. mutans* Gtf enzyme in catalyzing the breakdown of sucrose. This was due to the solution in the reaction tube of the negative control containing sucrose, Gtf enzyme, and aquadest. Since aquadest was neutral, the enzyme broke the sucrose down into fructose and glucose without difficulty.

Polyphenol compounds in mangosteen pericarp extract are, moreover, reported as inhibiting the activity of the Gtf enzyme. Polyphenol compounds consist of flavonoids, tannins, and α mangostin and can inhibit the activity of the Gtf enzyme by destroying other enzymes and microbial proteins. Therefore, this enzyme which is mostly composed of proteins can be denatured by the polyphenol compounds generated by the mangosteen pericarp extract.[13] Flavonoids playing a role in the inhibition of the Gtf enzyme are flavones and flavonols since both compounds have double bonds between C-2 and C-3 atoms in their chemical structure chains. The existence of this double bond provides a space for nucleophilic addition (a tendency to donate electrons or react with fewer electron sites, such as protons). The side chain of the Gtf enzyme in the form of aspartic acid (CH_2COOH) then possibly acts as a nucleophile and reacts with flavones and flavonols, thereby causing the Gtf enzyme to be inhibited.[3]

Another polyphenolic compound is tannin, which features a hydroxyl group in its structure, which potentially leads to a redoxive reaction with the Gtf enzyme. This reaction, in turn, triggers the inhibition of Gtf enzyme activity. The redoxive reaction is an oxidation-reduction reaction involving the exchange of electrons between two chemical structures. Tannins can inhibit Gtf enzyme activity by 31.93%.[8] Meanwhile, the third polyphenol compound in the mangosteen pericarp extract, α mangostin, inhibiting the activity of the Gtf enzyme was investigated by means of docking studies. Such studies predict the affinity and conformity of a molecule against a target protein. The molecule studied was α mangostin, while the target protein was the Gtf enzyme. In the results of that research, amino acids in the chain of the Gtf enzyme were found to be strongly and stably bound with α mangostin, indicating the effects of α mangostin on the Gtf enzyme.[7]

Based on the results of the analysis of those treatment groups using mangosteen pericarp extract, it could be seen that this extract at a concentration of 0.78% had greater inhibitory power against the activity of the Gtf enzyme (with a fructose level of 32.63%) than the mangosteen pericarp extract at a concentration of 0.39% (with a fructose level of 49.77%). Nevertheless, the results of the Mann-Whitney test showed there to be no significant difference between the two treatment groups. As a result, it could be concluded that the mangosteen pericarp extract at both concentrations had almost the same inhibitory power.

Meanwhile, the positive control group used 0.12% chlorhexidine since this material is the gold standard of material used in mouthwash. Chlorhexidine is an antibacterial active ingredient that is relatively effective compared to other antibacterial agents. In addition, chlorhexidine also can inhibit the activity of the Gtf enzyme. Chlorhexidine at bacteriostatic concentrations can even inhibit membrane enzymes and disrupt the interaction between lipids and proteins in the membrane. Consequently, chlorhexidine can inhibit the *S. mutans* glucosyltransferase enzyme and denature the enzyme protein.[14] Similarly, in this research, the fructose level was significantly lower than in the negative control group, indicating that 0.12% chlorhexidine was effective in inhibiting the activity of the Gtf enzyme. The fructose level in the positive control group was 25.87%, lower than that in the treatment groups using the mangosteen pericarp extract. This suggests that chlorhexidine had an inhibitory effect against Gtf enzymes superior to that of the mangosteen pericarp extract.

In addition, the results of the Mann-Whitney test showed that the significance value between the positive control group and treatment Group I was 0.065 (p>0.05), while that between the positive control group and treatment Group II was 0.180 (p>0.05). This means that there was no significant difference between the positive control group and those two treatment groups using the mangosteen pericarp extract at respective concentrations of 0.39% of 0.78%. In other words, the ability of mangosteen pericarp extract at these concentrations was equivalent to that of chlorhexidine at the concentration of 0.12% used as the positive control.

In conclusion, mangosteen pericarp extract (at concentrations of 0.39% and 0.78%) demonstrated inhibitory power against the activity of *S. mutans* producing Gtf enzyme. However, it proved less effective in this regard than 0.12% chlorhexidine.

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