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**EFFECTIVENESS OF KARAMUNTING FLOWER EXTRACT (*Melastoma malabathricum* L) ON THE *STREPTOCOCCUS MUTANS* GLUCOSYLTRANSFERASE ENZYME ACTIVITY**

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**ABSTRACT**

**Background:** Caries is a chronic disease of mineral ions continuous loss caused by bacteria in oral cavity. One of the main bacteria is *Streptococcus mutans*. This bacterium is able to synthesize the glucosyltransferase enzyme (GTF), which function is to catalyze polysaccharides in the form of sucrose into sticky glucans. This pathogenic property also has function in helping bacteria to stick and accumulate on the tooth surface, so it can increase the occurrence of the demineralization process and dental caries. Karamunting flower extract, as a natural ingredient, has antibacterial property. This property is caused by the content of phenol, flavonoid and tannin, which can reduce the GTF *S. mutans* enzyme activity. **Objectives:** This study aimed to analyze the effectiveness of karamunting flower extract (*Melastoma malabathricum* L) on the activity of the *Streptococcus mutans* glucosyltransferase enzyme. **Methods:** This study used a post-test only with control group design with karamunting flower extract samples in various concentration, including 40%, 50%, 60%, 70%, measuring enzyme activity with 4 repetitions in each group. **Results:** The data were analyzed using One Way ANOVA, which got  $p = 0,000$  ( $p < 0.05$ ) and followed by Post Hoc LSD test that showed significant difference between each group ( $p < 0.05$ ). Data showed that concentrations of 40%, 50%, 60%, and 70% can reduce GTF *S. mutans* enzyme activity. **Conclusion:** Karamunting flower extract has the effectiveness of reducing the GTF enzyme activity of *S. mutans*.

**Keywords:** Glucosyltransferase enzyme, Karamunting flower extract, Spectrophotometry UV –VIS, *Streptococcus mutans*.

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**INTRODUCTION**

The prevalence of dental and mouth problems in Indonesia show a very large number. Based on the result of Riskesdas (2018), 57.6% of Indonesian suffer from dental and oral diseases. Most tooth and mouth damage occur due to dental caries. Dental caries is a chronic disease which characterized by continuous loss of mineral ions from the surface of the enamel. This damage can initially only be seen microscopically, but progresses over time, the lesion will be seen on the email and irreversibly softening of semen in the roots.<sup>1,3</sup>

The aetiology of dental caries is *Streptococcus mutans* (*S. mutans*) bacterium. *S. mutans* is capable of synthesizing glucosyltransferase enzyme, which is the main virulence factor in causing dental caries. The virulence of this enzyme is able to

catalyze sucrose polysaccharide to synthesize sticky glucan. The pathogenic property of the sticky glucan plays important role in helping the bacteria to stick and accumulate on the tooth surface, thus increase bacterial cariogenicity in producing organic acid, which result in the demineralization process.

Effort to prevent dental caries continues to be carry out, one of which is the use of antibacterial agents. This bacteriostatic property is able to inhibit enzymes and denaturate protein, but this material causes various effects when use continuously, such as hypersensitivity reaction, bad breath, tooth discolorization, and bacterial resistance, so that the use of herbal plant as an alternative antibacterial agent is recommended. Herbal plants are expected to be more effective and not causing harms for long term use, compared to synthetic materials or other materials

Karamunting (*Melastoma malabathricum L*) is one of the Kalimantan forest herbal plants, commonly used by the community for various treatments, such as dyspepsia, dysentery, bacillary, diarrhea, hepatitis, leukorrhea, sprue, malnutrition and ulcers.<sup>5</sup> Karamunting contains several chemical compounds, such as flavonoid, alkaloid, steroid, anthocyanin, saponin and tannin that found in the root, stem, leaf, flower and fruit. According to study by Danladi's, karamunting flower has large amount of phenol and flavonoid and also is a part of karamunting plant that is rarely used, even though that is the most effective as they are rich in antioxidant (IC<sub>50</sub> = 48 µg / ml). It has free radical inhibitory percentage of 86.06%. The antibacterial activity of Karamunting flower has also been shown to inhibit oral bacteria.<sup>6,7</sup>

Based on this description, the purpose of this study is to analyze the benefit of Karamunting flower extract (*Melastoma malabathricum L*) on the activity of the *Streptococcus mutans* glucosyltransferase enzyme.

## MATERIAL AND METHODS

This research began with submission of research permits and ethical clearance issued by the Faculty of Dentistry, Lambung Mangkurat University No. 092 / KEPKG-FKGULM / EC / XII / 2018. This study was a pure experimental study (true experimental) with a post-test only with control group design. The sample used in this study was Karamunting flower extract (*Melastoma malabathricum L*) with different concentrations of 40%, 50%, 60%, 70%, with positive control using chlorhexidine gluconate 0.12% and distilled water as a negative control with the number of 4 times repetitions for each group.

### Extraction of karamunting flower

Karamunting flower that used were native plants originating from South Kalimantan. The extraction technique was maceration technique. Karamunting flowers were washed and dried at 40-50 ° C for 72 hours using the oven. The dried karamunting flower was mashed into powder using a blender. The powder was removed to glass beaker and added 100% methanol, mixed and allowed to stand for 72 hours. The sample mixture of karamunting and 100% methanol was then filtered with filter paper. The pulp was removed and the filtrate obtained was evaporated with vacuum rotary evaporator and waterbath heater at 40°C, thus karamunting flower extract is obtained. The extract was tested for methanol free and diluted with sterile distilled water to obtain concentrations of 40%, 50%, 60%, and 70%.<sup>7,19</sup>

### Isolation of crude *Streptococcus mutans* glucosyltransferase enzymes

*Streptococcus mutans* was inoculated into a culture media tube containing 10 ml of liquid BHI, then incubated at 37 ° C for 24 hours. After 24 hours of cultivation, the culture media was vibrated with a 150 rpm shaker and then centrifuged 1500 rpm for 30 minutes and a supernatant containing the glucosyltransferase enzyme will be obtained.<sup>3,4</sup>

### Measurement of *Streptococcus mutans* glucosyltransferase enzyme activity

#### 1. Preparation of the test solution

The test tube was added with mixture of 0.9 ml 0,1% sucrose and 0.1 ml GTF enzyme solution, added 0.025 ml for each treatment, which were karamunting extract concentration of 40%, 50%, 60%, 70%, chlorhexidine gluconate 0.12%, sterile distilled water on each tube and phosphate buffer of pH 7, added to the total volume in the 2 ml tube. All groups were incubated at 37°C for 2 hours and added 10% Pb-acetate solution and 5% Na-Oxalate, then carried out repeatedly and filtered with filter paper until all solutions were clear, clean and no white precipitate was formed.<sup>4</sup>

#### 2. Karamunting flower extract test on the activity of the GTF enzyme using UV-Vis Spectrophotometer

##### 1) Making a standard curve of fructose solution

The standard curve was made by preparing fructose solution of 0, 20, 40, 60, 80, and 100 mg/L concentration. Each solution was then added with 5 mL of anthrone reagent, heated in 100°C waterbath for 12 minutes and cooled with flowing water. After that, absorbance was read (760 nm) and relationship between absorbance and fructose concentration was determined.<sup>20,21</sup>

##### 2) Testing the test solution

The solution of each group that was filtered was taken 1 ml and added with 5 mL of anthrone reagent, heated in 100°C waterbath for 12 minutes and cooled with running water. Absorbance reading (760 nm) is obtained using a UV-Vis spectrophotometer. The results of the reading were entered into the fructose standard curve equation. One unit of GTF enzyme activity was defined as 1µmol fructose / ml.<sup>3,4,20</sup>

## RESULTS

Based on the results of the study, the data were obtained in the form of absorbance value (760 nm) of the effectiveness of Karamunting flower extract to the activity of *S. mutans* glucosyltransferase enzymes

**Table 1.** Fructose absorbance values in each treatment group

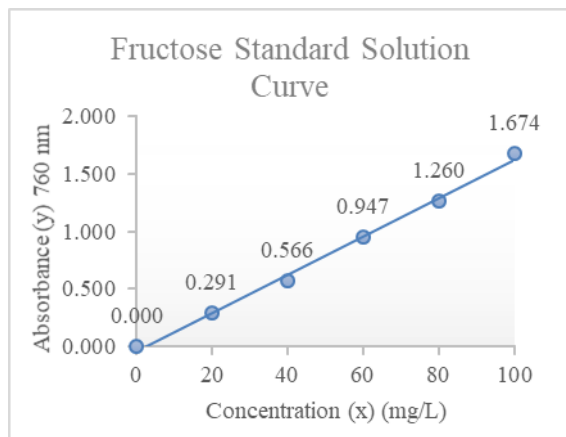
Treatment Group	Absorbance (y)			
	P1	P2	P3	P4
EBK 40%	0,998	1,022	1,030	1,025
EBK 50%	0,896	0,799	0,927	0,852
EBK 60%	0,750	0,755	0,797	0,706
EBK 70%	0,576	0,546	0,540	0,575
Clorhexidine 0,12%	0,238	0,245	0,328	0,226
Distilled water	1,260	1,256	1,228	1,201

After getting the absorbance value in the treatment group, the next step was to find the fructose concentration in the treatment group by making a fructose standard curve to determine the formula equation. This fructose standard curve was obtained by adding the standard fructose absorbance values of various kind of concentrations. The fructose absorbance values were as follow: The results of Shapiro-Wilk normality test showed the result obtained in the form  $p \text{ value} > 0.05$  which meant all data was normally distributed. Levene's Test result showed that the value of  $p = 0.981$  ( $p > 0.05$ ) which meant homogeneous data.

**Table 2.** Fructose Solution Standard Absorbance

No	Fructose concentration (mg/L)	Absorbance
1	0	0,000
2	20	0,291
3	40	0,566
4	60	0,947
5	80	1,260
6	100	1,674

From the the result data of table 2, standard curve was determined as follows:



**Figure 1.** Standard Curve Graph for Fructose Solution Concentrations of 0, 20, 40, 60, 80 and 100 mg / L

Based on the fructose standard curve above, to determine the amount of fructose concentration in

the treatment group, the absorbance value was added into the following equation:

$$y = 0,0167x - 0,043$$

$$x = y + \frac{0,043}{0,0167}$$

Annotation

x = fructose concentration (mg / L)  
y = value of absorbance of the treated enzyme

After added into the formula, the average value of *S. mutans* glucosyltransferase enzyme activity was obtained in each treatment which was presented in table form as follow:

**Table 3.** Mean values of Glucosyltransferase *S.Mutans* Enzyme Activity at Each Treatment

Group	Mean± Standard Deviation (Unit)
EBK 40%	353,00 ± 2,46
EBK 50 %	302,92 ± 18,51
EBK 60 %	264,35 ± 12,26
EBK 70 %	200,22 ± 6,33
Clorhexidine gluconate 0,12%	100,35 ± 15,44
Distilled water	425,15 ± 9,08

Table 3 shows that there was a difference in the average enzyme activity of each treatment. The karamunting flower extract with concentrations of 40%, 50%, 60%, 70%, 0.12% chlorhexidine gluconate and distilled water resulted in average activity of *S. mutans* glucosyltransferase enzyme of 353.00 units, 302.92 units, 264.35 units, 200.22 units, respectively. While the group was given by 0.12% chlorhexidine gluconate and distilled water, it was produced the average activity of GTF *S. mutans* enzyme of 100.35 units and 425.15 units.

The value of the effectiveness of each treatment group in the study was illustrated by the lower value of the enzyme activity that was produced, the more effective the material in reducing the activity of *S. mutans* glucosyltransferase enzymes. The result of this study indicated that the positive control group of 0.12% chlorhexidine gluconate had has the greatest ability to reduce the enzyme activity of GTF *S. mutans* and in the negative control group, distilled water, had has the smallest ability to reduce the activity of the enzyme GTF *S. mutans*, compared to the other groups. This showed that 0.12% chlorhexidine gluconate used in this study provided optimal result in reducing *S. mutans* glucosyltransferase enzyme activity.

The collected data was tabulated, then a normality test using Saphiro-Wilk and homogeneity test using Leven's test were conducted. Based on the result of these tests, it was

indicated that each group was normally distributed ( $p > 0.05$ ) and homogeneous  $p = 0.197$  ( $p > 0.05$ ). Normal and homogeneous data can be analyzed using One Way ANOVA, which was got  $p = 0,000$  ( $p < 0.05$ ) and followed by Post Hoc LSD test which showed significant difference between each treatment group  $p = 0,000$  ( $p < 0.05$ ).

## DISCUSSION

Based on the result of this study, karamunting flower extract (*Melastoma malabathricum L*) had has the ability to reduce GTF enzyme activity in each concentration group. The results proved that in the karamunting flower extract treatment group, the concentration of 70% had the most effective ability to reduce the activity of the *S mutans* GTF enzyme, compared to concentrations of 40%, 50% and 60%. The increased concentration of the extract was equivalent to its effectiveness in reducing the activity of the *S mutans* GTF enzyme. The higher the extract concentration, the more active substances dissolved. At a concentration of 70%, karamunting flower extract had has more effective activity in reducing the activity of the GTF *S mutans* enzyme. This was due to the content of active phenol compounds flavonoid and tannin, which were higher than other concentrations. These compounds had have their own ways of reducing the activity of the GTF enzyme.<sup>8,9</sup>

Phenol compound is the most active compound which contained in karamunting flower extract. The type of phenol compound is gallic acid, which has hydroxyl group and 2 hydrogen ions. The hydroxyl group results in the hydroxylation process with the GTF enzyme and will be oxidized, while the hydrogen bond will bind to the GTF enzyme group, so that the enzyme becomes unstable and malfunction. This process will result in denaturation and inactivation of the GTF enzyme and decrease in the activity of the GTF enzyme.<sup>10,11,12</sup>

Flavonoid have the ability to inactivate the GTF enzyme, which is located in the cell membrane of *S. Mutans*, thus disrupting the process of accumulation and adhesion of this bacterium.<sup>13</sup> This ability is due to double bond between C number 2 atom and C number 3 nucleophile of the compound that will react with the amine group of the GTF enzyme aspartic acid ( $\text{CH}_2\text{COOH}$ ), so that it will bind the group and damage the GTF enzyme which mostly composed of protein.<sup>3,14</sup>

Tannin also have the ability to inhibit the GTF enzyme due to take over the required substrate.<sup>4</sup> The hydroxyl group structure able to produce reduce reaction (reduction-oxidative), so that the enzyme will not be active and no enzymatic process occurs.<sup>14</sup>

The results of this study also showed that 0.12% chlorhexidine has the effectiveness of reducing

enzyme activity more effectively than karamunting flower extract with various concentrations, with an average value of 100.35 units. 0.12% chlorhexidine is the most effective antimicrobial gold standard agent in reducing the number of colonies of *Streptococcus mutans*.<sup>15</sup> Chlorhexidine 0.12% is also a broad-spectrum bactericidal and bacteriostatic antimicrobial agent that is very effective against gram-positive and gram-negative bacteria.<sup>16</sup> This is caused by positive ion charge on this material. At the initial stage of contact, the positive ion will interact strongly with the phosphorus acid teikoate group on the bacterial cell wall. The teikoat acid (lipoteikoat acid) is bound to the plasma membrane, decreasing its permeability. This decrease interferes with the interaction between lipid and protein, which is an essential factor for the survival of bacteria, resulting in cell membranes leakage and lysis of the bacteria.<sup>3,17</sup>

In this study, the ineffectiveness of karamunting flower extract was caused by many possible factors that influence the results of the study, so that the karamunting flower extract worked less optimal. This condition may be influenced by the rate of reaction of each ingredient. Herbal ingredients take longer than chemicals. The short reaction that occurred in this study with 2 hours incubation time, when antibacterial agents mixed with the GTF enzyme, affect the antibacterial agent made by herbs less optimal. The process of enzyme isolation also affected the results of the study. The isolation process, which only got the crude enzyme, caused the possibility that there were enzymes or other components that were also influential, so that the extract did not work at one enzyme.<sup>18</sup>

Although karamunting flower extract shows less optimal effect than of 0.12% chlorhexidine in reducing enzyme activity or as an antibacterial, karamunting flower has other effects. Based on the research of Danladi et al. karamunting flower was part of the karamunting plant which has the highest antioxidant effect ( $\text{IC}_{50} = 48 \mu\text{g} / \text{ml}$ ) and has the highest percentage of free radical inhibition of 86.06%.<sup>7</sup> According to Jun, et al. in Rosidah and Tjitraresmi,  $\text{IC}_{50}$  values ( $< 50 \mu\text{g} / \text{ml}$ ) classified in a very high categories. This shows that although it is not optimal as an antibacterial, karamunting flower extract can be used as an antioxidant.<sup>22</sup> Based on these descriptions, it can be concluded that there is effectiveness of karamunting flower extract (*Melastoma malabathricum L*) on the activity of the *Streptococcus mutans* glucosyltransferase enzyme.

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