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EFFECT OF KARAMUNTING LEAF EXTRACT (*Melastoma malabathricum* L.) ON GLUCOSYLTRANSFERASE ENZYME OF *Streptococcus mutans*

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ABSTRACT

Background: *Karamunting leaf extract has secondary metabolite compounds such as tannin, flavonoid, and phenol that have antibacteria potential to be used as an alternative to mouthwash in addition to Chlorhexidine 0.12% to lower the incidence of tooth caries. Caries is caused by several important virulence factors, including Streptococcus mutans Glucosyltransferase (GTF) enzyme. Karamunting leaf extract can inhibit the work of GTF enzyme by precipitating, denaturing protein and damaging cell walls. Inhibition of Streptococcus bacteria enzyme activity will decrease plaque formation, thus decreasing the potential of dental caries.* ***Objective:*** *The purpose of this study was to determine the effect of Karamunting leaf extract and 0,12% Chlorhexidine on Glucosyltransferase enzyme activity of Streptococcus mutans.* ***Methods:*** *This study used true experimental design with post test only with control group design using 7 groups, including Karamunting leaf extract group with 5%, 10%, 15%, 20%, and 25% concentrations to determine the activity of S. mutans GTF enzyme.* ***Results:*** *The results showed that Karamunting leaf extract with 20% concentration was able to decrease the activity of Glucosyltransferase enzyme in Streptococcus mutans.* ***Conclusion:*** *Karamunting leaf extract affects Streptococcus mutans Glucosyltransferase enzyme activity.*

Keywords*: Chlorhexidine 0,12%, Glucosiltransferase, Melastoma malabathricum L., Streptococcus mutans.*

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INTRODUCTION

Dental caries is an infectious disease and a demineralization process on the hard tissue, which are the enamel, dentin and cementum, caused by certain bacterial activity which ferments carbohydrates such as sucrose and glucose, thus forming acid and decrease pH to < 5, which leads to demineralization process on tooth surface.[1] The main bacteria that causes caries is *Streptococcus mutans*, which is a coccus gram-positive bacterium and a normal flora in the oral cavity.[2] The caries process is begun by the attachment of *Streptococcus mutans* (*S. mutans*) on tooth surface. The infection is caused by the adhesive property of glucan, the result of Glucosyltransferase (GTF) enzyme synthesis owned by *S. mutans*.[2-7] GTF enzyme produces glucan which is not soluble in water and adhesive in nature, thus GTF enzyme become a virulence factor in the pathogenesis of caries.[2-7] The adhesive property of glucan increases bacterial colonization and accumulation on tooth surface and acts in increasing plaque integration, thus lowering oral cavity pH which in turn lead to demineralization process.[2-7] Caries prevention can be performed using 0.12% chlorhexidine, which is a gold standard in caries prevention. However, chlorhexidine can have side effects if used for prolonged period.[8]

Herbal medicine has been through many developments because natural ingredients are thought to be relatively safe with fewer side effect.[9] One of the medicinal plants often used by the people is Karamunting (*Melastoma malabatricum* L.) where it is used empirically in Kalimantan, especially around the upstream region, as a medicine for infection and diarrhea.[9,10] The secondary metabolite substances contained in Karamunting are tannin, flavonoid, and phenol.[11] Phenol is the most dominant secondary metabolite substance in Karamunting, reaching 67.1%.[12] According to previous study, Karamunting

leaf extract with 25%, 50%, 75%, and 100% concentrations can inhibit the growth of *Staphylococcus aureus*.[9] Other study also showed that Karamunting leaf extract with 5%, 10%, 15%, 20% and 25% concentrations can inhibit the growth of *Streptococcus mutans*.[22] According to the statement, a study was conducted to know the effect of Karamunting leaf extract on Glucosyltransferase enzyme of *Streptococcus mutans*.

MATERIALS AND METHODS

This study has received ethical permission with No.091/KEPKG-FKGULM/EC/XII/2018 issued by the Health Research Ethical Committee, Faculty of Dentistry, Lambung Mangkurat University. This study was true experimental with post test only with control group design. The population of this study was Glucosyltransferase enzyme isolated from the colony of *Streptococcus mutans* ATCC® 25175™.

The samples in this study were Karamunting leaf extract (*Melastoma malabathricum* L.) with different concentrations (5%, 10%, 15%, 20%, 25%), positive control using 0.12% chlorhexidine, and negative control using sterile distilled water with four times repeated measurements. The seven treatment was subjected to GTF enzyme activity test using UV-Vis spectrophotometry. Data were analyzed using Saphiro-Wilk normality test, Levene's homogeneity test, One Way ANOVA, and Post Hoc LSD.

Preparation of Karamunting Leaf Extract (*Melastoma malabathricum* L.)

The preparation of Karamunting leaf extract was performed using maceration technique. The leaves was derived from Banjarbaru, South Kalimantan. Around 2 kg of Karamunting leaves were rinsed and cut into small pieces, then dried until the water content was less than 10%. The dried simplisia was made to 4/18 powder with a blender, then immersed in 96% ethanol for 1 x 24 hours. After immersion, the leaf extract was filtered using filter paper. The remaining water was inserted into a rotary evaporator to separate the solvent and pure extract at 60^{o}C and test for ethanol content was performed using acetic acid (CH_3COOH) and sulfuric acid (H_2SO_4) with the help of heat. The extract was free from ethanol when no ester smell was found.[9,23]

Culture of *Streptococcus mutans*

Streptococcus mutans was grew on an agar media (Nutrient Agar) and incubated for 2 x 24 hours at 37^{o}C. Afterwards, it was inserted into a bottle of culture media with 10 ml of liquid BHI, then incubated at 37^{o}C for 24 hours.[2,4]

Isolation of *Streptococcus mutans* Glucosyltransferase Enzyme Crude

After cultivation for 24 hours, the culture media was shaken with 150 rpm shaker then centrifuged at 1500 rpm for 30 minutes, thus a supernatant containing GTF enzyme crude was obtained.[2]

Streptococcus mutans Glucosyltransferase Enzyme Activity Measurement

1. Preparation of Test Solution

The treatment and control groups were given with a mixture of 0.9 ml 0.1% sucrose in a pH 7phosphate buffer, added with 0.1 ml GTF enzyme solution and Karamunting leaf extract with each concentration for 0.025 ml (the same volume for positive and negative control), then phosphate buffer with pH 7 was added until a 2 ml total volume was reached. All treatments and control groups were incubated at 37^{o}C for 2 hours, 10% Pb-acetate solution and 5% Na-Oxalate solution were added then filtered using filter paper until all solutions were clear with no white deposits.[2,4]

2. Karamunting Leaf Extract Test on Glucosyltransferase Enzyme Activity

Fructose solution standard curve was made using test tubes with 0 (blank), 20, 40, 60, 80, and 100 mg/L standard fructose solution. Each solution was added with 5 mL anthrone reagent and placed in a water bath at 100^{o}C for 12 minutes then cooled. 2 ml was poured into a cuvette for absorbance reading on maximum wavelength and correlation was made between absorbance and fructose concentration.[24] Afterwards, GTF enzyme activity test was performed using UV-Vis spectrophotometer. One ml of filtered solution from each group was taken and added with 5 ml anthrone reagent and placed in a 100^{o}C water bath for 12 minutes and cooled. 2 ml was poured into a cuvette for absorbance reading on wavelength. The result of the reading was inputted to standard curve equation. One unit of GTF enzyme activity was defined as 1 µmol fructose/ml.[2,4,24]

RESULTS

The result of *S. mutans* GTF enzyme activity showed that Karamunting leaf extract with 5% concentration had average enzyme activity of 164.575 units, 10% concentration had 127.219 units, 15% concentration had 98.939 units, 20% concentration had 72.018 units, and 25% concentration had an average enzyme activity of 228.82 units. Chlorhexidine 0.12% had the average enzyme activity of 100.327 units. Sterile distilled water had the average enzyme activity of 425.177 units. Each concentration of Karamunting leaf extract and control showed effect in decreasing glucosyltransferase

enzyme activity and the most optimal concentration was 20%.

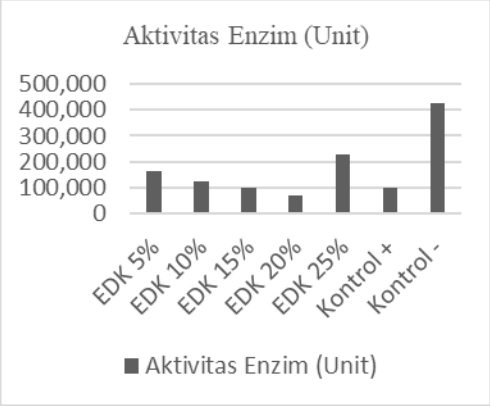

Figure 1. Bar chart of the average value of enzyme activity from each treatment group.

Data obtained from each treatment were then subjected to normality test using Shapiro-wilk test and Levene's homogeneity test with the required p value > 0.05. Normality test result showed that each treatment group had p value > 0.05, thus the data were normally distributed. Homogeneity test with p value > 0.05 meant that the data were homogenous. Normally distributed and homogenous data were then analyzed using One Way ANOVA parametric test with confidence interval of 95%. The result of One-Way ANOVA showed that there were significant differences between treatment groups. The administration of Karamunting leaf extract at each concentration and 0.12% chlorhexidine gave different effect on *Streptococcus mutans* GTF enzyme activity. Afterwards, Post Hoc Least Significant Difference (LSD) test was performed to determine which group gave significant difference and p value = 0.000 was obtained for each group. Therefore, it could be concluded that there were significant differences ($p < 0.05$) for each treatment group. However, several treatment groups showed no significant difference.

DISCUSSION

The results showed that Karamunting leaf extract with 5%, 10%, 15%, 20%, and 25% concentrations were able to decrease *Streptococcus mutans* glucosyltransferase (GTF) enzyme activity. The value of enzyme activity for treatment group with 5% concentration was 164.575 units, 10% with 127.219 units, 15% with 98.939 units, 20% with 72.018 units, and 25% with 228.82 units. This showed that there was antibacterial property from the secondary metabolites of Karamunting leaf extract, such as tannin, flavonoid, and phenol in decreasing the activity of glucosyltransferase enzyme. These substances include secondary metabolite substances that can inhibit the growth of bacteria by denaturing bacterial protein, thus decreasing enzyme activity. The results showed that the most effective concentration in decreasing GTF enzyme activity was 20%. This was caused by tannin in the extract could precipitate protein with tannin complex bonding.[11,13-17]

Tannin is a growth inhibitor which can inhibit the growth of bacteria. The enzyme released by bacteria is basically protein, and protein is precipitated by tannin, thus the enzyme became inactive. Generally, tannin derived from green plants had complex bond with stronger protein. The bond between tannin and protein is made through three types of bond, i.e. hydrogen bond, ion bond, and covalent bond. Hydrogen bond formed between phenol group from tannin and carboxyl group (aromatic and aliphatic) from protein, then strong bond between tannin and protein causes inactive enzyme.[17] This was supported by Akiyama and Chung in Rahman FA *et al*. (2017) who stated that tannin can precipitate protein through cell membrane damage because of tannin toxicity and the formation of metal ion bond from tannin which acts in tannin toxicity. Bacteria that grow in aerobic condition need iron for various functions, including reduction from precursor ribonucleotide DNA. The bond between tannin and iron will disturb various functions of bacteria. *S. mutans* is an anaerobic facultative bacterium, which still able to live in aerobic condition. Bacteria that living in aerobic condition will be disturbed by tannin. Other than tannin toxicity, this substance was also worked by inhibiting reverse transcriptase enzyme and topoisomerase DNA, so the bacterial cell can not completely form and also causes incomplete formation of bacterial cell wall.[18] Karamunting leaf also has other secondary metabolite, called flavonoid, that also helps in decreasing enzyme activity.[13]

The mechanism of flavonoid in Karamunting leaf extract in decreasing glucosyltransferase enzyme activity is by damaging bacterial cell wall which consists of lipid and amino acid. The lipid and amino acid will react with alcohol group in flavonoid substance, thus damaging cell wall and flavonoid will enter bacterial nucleus. Inside the nucleus, flavonoid will react with DNA and causes damage to DNA lipid structure, so that leads to bacterial lysis. This was caused by difference in polarity between bacterial DNA structure lipid with flavonoid alcohol group. Beside damaging cell walls, flavonoid will also inhibit the function of bacterial cell membrane by forming complex substance from soluble extracellular protein which disturb the integrity of bacterial cell membrane. Any disturbance on this cell membrane permeability will affect proton electrochemical gradient that passes through membrane. This is very important for bacteria

in case of ATP synthesis, membrane transport, and bacterial movement. The presence of flavonoid will lead to disturbances in ATP synthesis, bacterial movement and membrane transport.[13,14]

Phenol substance contained in Karamunting leaf extract can also decrease GTF enzyme activity. The substance will denaturate the bacterial protein. The hydrogen bond that formed between phenol and protein causes damage to protein structure and affected cell wall permeability, thus causing macromolecule imbalance in cell, leading to cell lysis. In low concentration, phenol will destroy cytoplasm membrane and in high concentration, phenol will coagulate with cellular protein.[15,16]

This study used 0.12% chlorhexidine as positive control that has bactericidal and bacteriostatic properties toward gram-positive and gram-negative bacteria in the oral cavity. The mechanism of chlorhexidine in decreasing glucosyltransferase enzyme activity depends on the concentration. Cation charge (positive) in chlorhexidine creates a strong attachment on bacterial cell membrane, because most bacterial molecular charge is anion (negative), thus causing changes in bacterial cell membrane permeability and causes cell cytoplasm and cell components with lower molecular weight to pass through cell membrane, causing cell death.[19]

Antibacterial activity owned by each extract increases along with the concentration. Concentration of an antibacterial substance is one of the determining factors of the ability of the substance in inhibiting tested bacterial growth.[20]

The results of glucosyltransferase enzyme activity do not always turn to activity decrease comparable to increase concentration. In 20% concentration, there was 72.018 units of enzyme activity, while 25% concentration showed 228.824 units of increase enzyme activity. This was thought to be caused by the difference in diffusion speed of antibacterial substance. This change is caused by difference in composition of agar media used, difference in media composition can change the property of the media, thus changes diffusion distance. Agar media affects bacterial activity, diffusion speed of antibacterial, and bacterial growth. Beside the difference in diffusion speed, other suspected cause of increased enzyme activity in 25% concentration was due to increased plant maturity (the older the plant, the higher the tannin content). Increased enzyme activity from 72.018 units in 20% concentration to 228.824 units in 25% concentration is highly suspected to be caused by degraded tannin or tannin can no longer precipitate protein. The loss of ability is caused by polymerization or depolymerization which causes lack of tannin reactivity and also the oxidation which causes the substance to be unable to precipitate protein.[17,20,21]

Karamunting leaf extract has the potential to be used as herbal natural alternative medicine due to antibacterial substance which can inhibit the activity of *Streptococcus mutans* GTF enzyme, that is the bacteria that cause tooth caries. In this study, Karamunting leaf extract was investigated as an alternative of synthetic mouthwash, i.e. 0.12% chlorhexidine as control in caries prevention, which is expected to decrease the side effect of prolonged use of 0.12% chlorhexidine. In conclusion, there was an effect of Karamunting leaf extract (*Melastoma malabathricum* L.) on *S. mutans* glucosyltransferase enzyme activity.

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REFERENCES

1. Adhani R, Rachmadi P, Nurdiyana T, Widodo. *Karies Gigi di Masyarakat Lahan Basah*. Ed 1st. Banjarmasin: MNC Publishing; 2018. p. 23-30.
2. Adindaputri ZU, Purwanti N, Wahyudi IA. Pengaruh Ekstrak Kulit Jeruk Nipis (*Citrus Aurantifolia Swingle)* Konsentrasi 10% Terhadap Aktivitas Enzim Glukosiltransferase *Streptococcus mutans*. Maj Ked Gi. 2013; 20 (2): 126-131.
3. Bidarisugma B, Timur SP, Purnamasari R. Antibodi Monoklonal *Streptococcus mutans* 1 (c) 67 kDa sebagai Imunisasi Pasif dalam Alternatif Pencegahan Karies secara Topikal. BIMKGI. 2012; 1 (1): 1-11.
4. Isnarianti R, Wahyudi IA, Puspita RM. Ekstrak Daun *Mutingia calabura L* dalam Menghambat Aktivitas Glukosiltransferase *Streptococcus mutans.* Journal of Dentistry Indonesia. 2013; 30(3): 59-63.
5. Syahrial AA, Rahmadi P, Putri DKT. Perbedaan Kekerasan Permukaan Gigi Akibat Lama Perendaman dengan Jus Jeruk *(Citrus sinensis. Osb)* Secara In Vitro. Dentino Jurnal Kedokteran Gigi. 2016; 1(1): 1-5.
6. Usha C, Sathyanarayana R. Dental Caries – A Complete changeover Part I. J Conserv Dent. 2009; 12(2): 46-54.
7. Wardani PKM, Supartinah A, Titien IS, Rantinah SBS, Lukito E, Utomo RB, *dkk*. Faktor Resiko Terjadinya Karies Baru dengan Pendekatan Kariogram pada Pasien Anak di Klinik Kedokteran Anak RSGMP Prof. Soedomo Yogyakarta. Maj. Ked Gi. 2012; 19 (2): 107-109.

8. Nurafifah D. Hubungan Perilaku Pencegahan Karies Gigi dan Kejadian Karies Gigi pada Anak di Dusun Sumberpanggang Desa Lopang Kecamatan Kembangbahu Kabupaten Lamongan. 2013; 1(14): 51-57.
9. Niah R, Baharsyah RN. Potensi Ekstrak Daun Tanaman Karamunting *(Melastoma malabathricum L.)* di Daerah Kalimantan Sebagai Antibakteri *Staphylococcus Aureus.* Jurnal Ilmiah Manuntung. 2018; 4(1): 36-40.
10. Niah R. Uji Aktivitas Ekstrak Etanol 96% Daun Karamunting *(Melastoma malabathricum L.)* terhadap *Salmonella Typh.* Jurnal Insan Farmasi Indonesia. 2018; 1(1): 113-121.
11. Ramadana F, Boleng DT, Jailani. Pengaruh Ekstrak Daun Karamunting *(Melastoma malabathricum L.)* terhadap Pertumbuhan Bakteri *Propionibacterium acnes.* Pendidikan Biologi. 2016; 1(1): 1-8.
12. Dharmaraj S, Danladi S, Azemin AW, Sani YN, Mohd KS, Rao MUS, *dkk.* Phytochemical Screening, Antioxidant Potential and Cytotoxic Activity of *Melastoma malabathricum Linn.* From Different Locations. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 7 (7): 408-413.
13. Ernawati, Sari DK. Kandungan Senyawa Kimia dan Aktivitas Antibakteri Ekstrak Kulit Buah Alpukat *(Persea americana P.Mill)* Terhadap Bakteri *Vibrio alginolyticus.* Jurnal Kajian Veteriner. 2015. 3(2): 203-211.
14. Rahman FA, Haniastuti T, Utami TW. Skrining Fitokimia dan Aktivitas Antibakteri Ekstrak Etanol Daun Sirsak (*Annona muricata L.)* pada *Streptococcus mutans* ATCC 35668. Majalah Kedokteran Gigi. 2017. 3 (1): 1-7.
15. Bontjura S, Waworuntu OA, Siagian KV. Uji Efek Antibakteri Ekstrak Daun Leilem (*Clerodendrum minahassae l.)* terhadap Bakteri *Streptococcus mutans*. Jurnal Ilmiah Farmasi. 2015; 4 (4): 96-101.
16. Sukandar D, Hermanto S, Amelia ER, Zaenudin M. Aktivitas Antibakteri Ekstrak Biji Kapulaga *(Amomum compactum* Sol. Ex Maton). JKTI. 2015; 17 (2): 119-129.
17. Sofiani E, Mareta DA. Perbedaan Daya Antibakteri antara *Klorheksidin Diglukonat 2%* dan Ekstrak Daun Jambu Biji (*Psidium Guajava Linn*) Berbagai Konsentrasi (Tinjauan Terhadap *Enterococcus Faecalis*). IDJ. 2014; 3 (1): 119-129.
18. Ngajow M, Abidjulu J, Kamu VS. Pengaruh Antibakteri Esktrak Kulit Batang Matoa *(Pometia pinnata)* terhadap Bakteri *Staphylococcus aureus* Secara In Vitro. Jurnal MIPA UNSRAT. 2013; 2 (2): 128-132.
19. Sinaredi BR, Pradopo S, Wibowo TB. Daya Antibakteri Obat Kumur *Chlorhexidine, povidone iodine, fluoride* Suplementasi *Zinc* terhadap *Streptococcus mutans* dan *Porphyromonas gingivalis.* Dental Journal Majalah kedokteran Gigi. 2014; 47 (4): 211-214.
20. Utomo SB, Fujiyanti M, Lestari WP, Mulyani S. Uji Aktivitas Antibakteri Senyawa C-4 Metoksifenilkaliks [4] Resorsinarena Termodifikasi Hexadecyltrimethylammonium-Bromide Terhadap Bakteri *Staphylococcus aureus* Dan *Escherichia coli*. Jurnal Kimia dan Pendidikan Kimia. 2018; 3 (3):109-209.
21. Septiani, Dewi EN, Wijayanti I. Aktivitas Antibakteri Ekstrak Lamun *(Cymodocea rotundata)* Terhadap Bakteri *Staphylococcus aureus* Dan *Escherichia coli*. Saintek Perikanan. 2017; 13 (1): 1-6.
22. Marsepriani, Fifendy M, Hidayat Y. Daya Hambat Ekstrak Daun Senduduk (*Melastoma malabathricum L.)* Terhadap Pertumbuhan Bakteri *Streptococcus mutans.* Program Studi Pendidikan Biologi. 2017; 1(1) : 1-4.
23. Kurniawati E, Daya Antibakteri Ekstrak Etanol Tunas Bambu Apus Terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus* Secara In Vitro. Jurnal Wiyata. 2015; 1 (1): 1-7.
24. Al-kayyis HK, Susanti H. Perbandingan Metode Somogyi-Nelson dan Anthrone-Sulfat Pada Penetapan Kadar Gula Pereduksi Dalam Umbi Cilembu (*Ipomea batatas L.)*. Jurnal Farmasi Sains dan Komunitas. 2016; 1 (1): 81-89.