Insisiva Dental Journal: Majalah Kedokteran Gigi Insisiva
Website: http://journal.umy.ac.id/index.php/di/index

Research Article

The Effectiveness of α-Mangostin in Reducing the Streptococcus Mutans Biofilm Thickness

Rahmawati Sri Praptiningsih[1]*, Arlina Nurhapsari[2], Rifqi Wahyu Febrian[3]
[1]Department of Oral Biology, Faculty of Dentistry, Universitas Islam Sultan Agung, Jl Kaligawe Raya No.Km.4 Semarang, Jawa Tengah 50112
[2]Department of Conservative Dentistry, Faculty of Dentistry, Universitas Islam Sultan Agung, Jl Kaligawe Raya No.Km.4 Semarang, Jawa Tengah 50112
[3]Faculty of Dentistry, Universitas Islam Sultan Agung, Jl Kaligawe Raya No.Km.4 Semarang, Jawa Tengah 50112

Received date: August 10th, 2022; revised date: October 20th, 2022; accepted: November 11st, 2022
DOI: 10.18196/di.v11i2.15809

Abstract

Dental caries occurs due to the demineralization of tooth structure caused by microorganisms in colonies called biofilms. One of the microorganisms involved in dental caries is *Streptococcus mutans*. Oral mouthwash, in addition to mechanical cleaning, is known to prevent the growth of oral microorganisms. Mangosteen is known as an anti-cancer ingredient with high anti-bacterial properties. This study aims to identify the effectiveness of mangosteen skin extract (*Garcinia mangostana L.*) in decreasing *Streptococcus mutans* biofilm. The study is experimental research with a post-test control group design. The research sample was divided into five groups; 3 treatment groups and 2 control groups. The biofilm thickness test was carried out with OD (Optical density) with a wavelength of about 620 nm using an ELISA reader. Kruskal Wallis analysis was employed as a non-parametric statistical test analysis. Statistical Kruskal Wallis indicated significant differences in the thickness of 5 test groups of *Streptococcus mutans*. The lowest average yield of biofilm thickness was in the α-mangosteen group at 12.5 g/ml. Conclusion: α-mangosteen in mangosteen skin extract effectively reduced the thickness of *Streptococcus mutans* biofilm.

Keywords: α-mangostin; biofilm thickness; *Streptococcus mutans*

INTRODUCTION

Dental caries is a dental disorder that often occurs in the community. According to Indonesian health research by the ministry of health in 2018, 57.6% of Indonesians experienced dental and oral health problems, with a caries prevalence of 45.3%.[1] Dental caries occur due to the demineralization of tooth structure caused by microorganisms in colonies called biofilms. Microorganisms produce acids forming cavities in the tooth structure on the surface of the enamel, dentin, or cementum.[2] Cariogenic organisms are capable of creating acids from fermentable carbohydrates. These microorganisms can live and reproduce in an acidic environment and stick to the tooth surface.[3] One of the microorganisms involved in dental caries is *Streptococcus mutans*.[2,4] *Streptococcus mutans* is one of the aerobic gram-positive bacteria and belongs to the *Streptococcus viridans* group.[4] It is a normal flora in the oral cavity. *Streptococcus mutans* are used to living in areas rich in sucrose. Bacterial activity will produce acid (pH <5.5) in the oral cavity and impact the demineralization of tooth structure. The inhibited demineralization process will lead to the formation of cavities. The cavity will become an entry point for bacteria to enter the pulp and even the periapical tissue,

* Corresponding author, e-mail: rahmawati@unissula.ac.id

which can cause various diseases, including fatal infections.[2] A biofilm is a structured and attached bacterial cell colony; one example of a biofilm is attached to the tooth surface. One of the bacteria that plays a role in colony formation is *Streptococcus mutans.*[5]

Caries control is an effort to prevent the growth of microorganisms into biofilms mechanically or chemically. Chemical biofilm prevention is carried out using fluoride and mouthwash to help inhibit caries' formation.[6] Chlorhexidine gluconate 0.2% is a mouthwash that contains an antibacterial substance with bactericidal properties that are effectively used for gram-positive and gram-negative bacteria.[7] However, the Maillard reaction is catalyzed by chlorhexidine which causes discoloration of the teeth.[8] In addition, chlorhexidine as a mouthwash is known to have side effects if used for an extended period, such as stained teeth and an unpleasant sensation and taste.[7] Therefore, alternative materials are needed, namely herbal ingredients; one of the herbal ingredients is mangosteen fruit and skin.[9]

Researchers have proved several ingredients from mangosteen skin and fruit extracts to be beneficial for health. The content of the mangosteen fruit is xanthone active compounds (Mangostin, α-Mangostin, Beta Mangostin, Mangostenol, Mangostin A, Mangostin B), anthocyanins such as Cyanidin-3-sophoroside, yellow latex, pectin, tannins, and resins.[9] Xanthone is an ingredient that is often used as the main ingredient. Xanthones have antioxidant, anti-inflammatory, anti-allergic, antibacterial, antifungal, antitumor, and antiviral activities. The content of xanthones in the mangosteen rind is 27 times more than that of the mangosteen flesh.[3] In addition, α-mangostin in the xanthones of the mangosteen fruit is antibacterial.[10,20]

A Research revealed that the content of mangosteen peel extract, α-mangostin, reduced S*treptococcus mutans.*[10] In addition, Yanura also denoted the inhibitory power of mangosteen peel with concentrations of 3.125, 6.25, and 12.5 g/ml, indicating the presence of an inhibitory zone of α-mangostin against *Mycobacterium tuberculosis.*[11] This study aims to determine whether α-mangostin effectively reduces the biofilm thickness of *Streptococcus mutans*, the most common causative microorganism in dental caries.

MATERIALS AND METHODS

This study is experimental laboratory research with a post-test-only control group design conducted in the Microbiology Laboratory. The optical density results were read at the Biology Laboratory, Faculty of Medicine, Sultan Agung Islamic University. The Ethical clearance in this research is 350/b.1-KEPK/SA-FKG/II/2022. The subject of this study was *Streptococcus mutans ATCC 25175,* obtained commercially, and the independent variable included α-mangosteen from mangosteen skin extract (*Garcinia mangostana L.*) with concentrations of 3.125 g/ml, 6.25 g/ml, 12.5 g/ml, distilled water, and chlorhexidine. α-mangostin is a bioactive compound found in the mangosteen skin. The compound α-mangostin is a yellow substance insoluble in water. The concentration of α-mangostin consisted of 3.125 g/ml, 6.25 g/ml, and 12.5 g/ml. Meanwhile, the dependent variable in this study included the thickness of the *Streptococcus mutans* biofilm. *Streptococcus mutans* biofilms are colonies of *Streptococcus mutans* bacteria that grow in the oral cavity. Furthermore, saliva was collected, and the *Streptococcus mutans* colony was cultured using American Type Culture Cell (ATCC) in TYS20B medium for 48 hours under anaerobic conditions, incubated at 37°C, and then calculated. The thickness of the biofilm was assessed using the five microtiter well plate biofilms and the ELISA Reader by Thermo Scientific at the medical faculty of Sultan Agung Islamic University Integrated Biomedical Laboratory (IBL). This study utilized a

microtiter plate biofilm, brain heart infusion, incubator, test tube, measuring cup, micropipette, ELISA reader, and syringe filter with a diameter of 0.22 μm. The materials of this study included pure *Streptococcus mutans* isolate, α-mangostin isolate, saliva, placebo in the form of distilled water, chlorhexidine 0.2% Minosep, gentian violet crystals 1%, and phosphate buffer saline (PBS). After preparing the materials and tools, the researcher created a research schedule. Tools and materials were prepared and sterilized for research to avoid contamination with compounds or microorganisms affecting research results. The α-mangostin isolates obtained from Tokyo Chemical Industry (TCI) Japan were divided into three groups: 3.125, 6.25, and 12.5 g/ml. Aquadest and chlorhexidine were prepared as a control group. Furthemore, the preparation of a suspension of *Streptococcus mutans* bacteria utilized brain heart infusion (BHI) media. Next, human saliva and pure isolates of *Streptococcus mutans* were pre-cultured in a BHI medium. The growth of *Streptococcus mutans* was adjusted to McFarland's turbidity standard of 0.5, resulting in bacterial suspension of 1.5 x 10^8 CFU /ml. The bacteria were then incubated at 37°C for 24 hours.[5]

Inhibition of Biofilm Thickness Formation: Sterilization was conducted to avoid contamination by other compounds or microorganisms. To make a pellicle on five well plates, 200 μl of saliva was added and filtered using a 0.22 μl minecart syringe filter in each well plate. It was then incubated for 15 minutes at 37°C, removed using a micropipette, and then rinsed with PBS 200 μl. The *Streptococcus mutans* solution of human saliva was divided into five put on the well plate media, and 200 μl of the *Streptococcus mutans* solution was added to each well.[5] Each solution added to the well plate media was tested with α-mangosteen compound with concentrations of 3.125, 6.25, 12.5 g/ml, 0.2% Chlorhexidine, and an aquadest. The sample was incubated for 24 hours at 37°C. The remaining planktonic cells were removed, rinsed with sterile PBS 3 times, and dried at room temperature. The well plate media was dripped with 150 μl of 1% gentian violet crystals for 20 minutes, rinsed with PBS solution three times, and then dried for 5 minutes.[12,13] Finally, 200 μl of 98% alcohol was added to the well plate media and left for 15 minutes. The result was measured and read utilizing optical density (OD) with a wavelength of 620 nm and an ELISA-reader by Thermo Scientific at the Biology Laboratory, Faculty of Medicine, Sultan Agung Islamic University.[14] Furthermore, Shapiro-Wilk and homogeneity tests were carried out with Levene's statistical test. In the case of this study, the data from the normality test and the homogeneity of the data were not normal and not homogeneous. These results can be identified with the Kruskal Wallis non-parametric test. The data were then processed using the SPSS program.

RESULT

The *Streptococcus mutans* thickness test results were carried out using an ELISA reader. The study revealed the average value of *Streptococcus mutans* thickness in 3 treatment groups with mangosteen with a concentration of 3.125 g/ml, 6.25 g/ml, 12.5 g/ml, and 2 control treatment groups with 0.2% chlorhexidine administration, and aquadest. It can be seen in table 1.

Table 1. Mean and standard deviation of *Streptococcus mutans* biofilm thickness

No	Group	Mean±SD
1	α-mangostin 3,125	0.641±0.418
2	α-mangostin 6,25	0.364±0.014
3	α-mangostin 12,5	0.356±0.157
4	*Chlorhexidine* 0,2%	0.614±0.253
5	*Aquadest*	1.029±0.278

The average thickness of the *Streptococcus mutans* biofilm with the addition of α-mangostin 12.5 g/ml was the

lowest compared to the other groups. On the other hand, the thickness of *Streptococcus mutans* (0.356) with the addition of aquadest was the highest compared to the other groups (1.029). Therefore, the Shapiro-Wilk test was utilized to determine normality. The results can be seen in table 2.

Table 2. The Normality Result using the Shapiro-Wilk test

Group	Sig.
α-mangostin 3.125 µg/ml	0.157
α-mangostin 6.25 µg/ml	0.501
α-mangostin 12.5 µg/ml	0.013
*Chlorhexidin*e 0.2%	0.589
Aquadest	0.803

According to table 2, 1 group showed abnormal data, and 4 other groups showed normal distribution. Therefore, it can be concluded that the data were not normally distributed. The data were then analyzed for homogeneity using the Levene Statistical test.

Table 3. The result of homogeneity using the Levene Statistical test

	Sig.
Streptococcus mutans biofilm thickness	0,006

The significance of 0.006 ($p<0.05$) indicates that the *Streptococcus mutans* thickness is homogeneous. Furthermore, a non-parametric Kruskal Wallis test was employed to identify the differences in the thickness of *Streptococcus mutans* in each group. Kruskal Wallis test results can be seen in the following table:

Table 4. Kruskal Wallis hypothesis test

	Sig.
Between groups	0.02

The significance number in the table is 0.02 ($p<0.05$), indicating significant differences in the five groups of the *Streptococcus mutans* thickness test. Therefore, the Mann-Whitney test was conducted to determine which group had the essential difference. The test results can be seen in the following table:

Table 5. Mann-Whitney test

The group comparison		Sig.	Difference
α-mangostin 3.125	α-mangostin 6.25 µg/ml	0.251	Not significant
	α-mangostin 12.5 µg/ml	0.117	Not significant
	*Chlorhexidin*e 0.2%	0.917	Not significant
	Aquadest	0.175	Not significant
α-mangostin 6.25	α-mangostin 12,5 µg/ml	0.117	Not significant
	*Chlorhexidin*e 0,2%	0.117	Not significant
	Aquadest	0.009*	Significant
α-mangostin 12.5	*Chlorhexidin*e 0,2%	0.251	Not significant
	Aquadest	0.009*	Significant
*Chlorhexidin*e 0.2%	*Aquadest*	0.047*	Significant

The Mann-Whitney test displayed a significant difference in the thickness value of *Streptococcus mutans* ($p<0.05$) in the distilled water group. The different thickness values were demonstrated in the α-mangostin group 6.25 g/ml, 12.5 g/ml, and 0.2% chlorhexidine.

DISCUSSION

The study results revealed an effect of adding α-mangostin 3.125 g/ml, 6.25 g/ml, 12.5 g/ml, 0.2% chlorhexidine, and aquadest on the average optical density of *Streptococcus mutans* biofilm. The average optical density value with a 12.5 g/ml concentration showed the lowest compared to aquadest. Statistical analysis denoted that there was a significant difference between each group. The lower the optical density value is, the lower the thickness of the *Streptococcus mutans* biofilm will be. In the Mann-Whitney follow-up test, there

was a difference between the groups of α-mangostin 6.25, 12.5 g/ml, Chlorhexidine 0.2%, and aquadest.

One of the bacteria in the biofilm is *Streptococcus mutans*, which can produce acid. The acid will damage tooth minerals (calcium hydroxyapatite), forming dental caries.[14] Biofilm growth can be inhibited with α-mangostin compounds as the antibiofilm properties against planktonic *Streptococcus mutan*s.[15] α-mangostin has potential and fast bactericidal activity targeting the inner bacterial cell membrane. This activity causes a decrease in bacterial mutations as α-mangostin can induce rapid removal of membrane potentials to avoid the occurrence of resistance.[11]

Alpha-mangostin in this study effectively reduced the biofilm thickness of *Streptococcus mutans* due to its antibiofilm properties against planktonic *Streptococcus mutans* through several activities. First, this activity reduces acid production by damaging the membranes of these organisms.[15] Afterward, α-mangostin targets the inner membrane of bacterial cells. The isoprenyl group of α-mangostin increases the hydrophobicity of α-mangostin, leading to an increase in bacterial membrane permeability and leakage of intracellular components, resulting in cell death.[11] Antibiofilm activity of α-mangostin is bactericidal with the mechanism of action of damaging the cytoplasmic membrane of bacteria. Damage to the cytoplasmic membrane causes intracellular components in bacteria leading to death in bacteria.[16]

The mangosteen activity inhibited *Staphylococcus aureus* biofilms only in the formation of young biofilms (early stage) and had no activity in mature biofilms (late stage). This activity was also demonstrated in a previous study on *Streptococcus mutans* biofilms. The bactericidal activity of mangostin on biofilms is highly dependent on the stage of biofilm formation, and mangostin may be more efficient when combined with other antimicrobial compounds to maximize its activity.[17] The minimum level of inhibition was obtained from research conducted, which displayed that mangosteen was less effective in killing gram-negative but more effective against gram-positive bacteria. Uropathogenic *Escherichia coli* is a gram-negative bacterium with an outer cell membrane composed of lipopolysaccharide. Meanwhile, one of the gram-positive bacteria that is effectively inhibited by mangostin is *Streptococcus mutans.*[16]

The observations from this study showed the results of the average optical density with the concentration of mangosteen 3.125 g/ml, 6.25 g/ml, and 12.5 g/ml. At 12.5 g/ml, the average optical density of *Streptococcus mutans* biofilm thickness was the lowest one. The results of these data concluded that the higher the concentration of mangosteen is, the lower the optical density value will be. It indicates that the administration of mangostin can inhibit the growth of *Streptococcus mutans* biofilm. There was a decrease in the thickness of the *Streptococcus mutans* biofilm with mangostin accompanied by mechanical treatment. The decrease in the thickness of the *Streptococcus mutans* biofilm in this study was with concentrations of mangostin 100, 150, and 200 mM.

Furthermore, this study demonstrated a decrease in the thickness of the *Streptococcus mutans* biofilm in mangostin 3.125 g/ml, 6.25 g/ml, 12.5 g/ml, and 0.2% chlorhexidine treatment. The results of the average optical density, mangostin 6.25 g/ml and 12.5 g/ml, were more effective than the antibacterial properties of the positive control, 0.2% chlorhexidine, due to its several activities in inhibiting the growth of *Streptococcus mutans* biofilms compared to 0.2% chlorhexidine.[15] Chlorhexidine 0.2% is one type of mouthwash considered a gold standard.[18] Chlorhexidine's mechanism of action effectively inhibits growth or kills gram-positive and gram-negative bacteria. The chlorhexidine molecule has a positive

charge (cation), and the most bacterial molecular charge is negative (anion). It causes the strong attachment of Chlorhexidine to the bacterial cell membrane. Therefore, chlorhexidine will cause changes in the permeability of the bacterial cell membrane. It causes the release of the cell cytoplasm and low molecular weight cell components through the cell membrane, leading to bacterial death.[19] α-mangostin is effective against multiple microbes, including ones with antibiotic resistance. Additionally, α-mangostin, though plant-based, produced similar antimicrobial activity in comparison with commercially available antibiotics.[20] Based on the result, it is concluded that the greater the content of mangostin is, the more inhibited the *Streptococcus mutans* biofilm will be.

CONCLUSION

Alpha-mangostin at a dose of 3.125 g/ml, 6.25 g/ml, and 12.5 g/ml was effective in reducing the biofilm thickness of *Streptococcus mutans*. *Streptococcus mutans* biofilm thickness with the α-mangostin concentration of 12.5 g/ml had the lowest mean biofilm thickness compared to the α-mangostin group of 3.125 g/ml, 6.25 g/ml and the aquadest as the negative control group.

ACKNOWLEDGMENT

The author would like to thank the Dental Faculty of Universitas Islam Sultan Agung, Semarang, Indonesia.

REFERENCE

1. RISKESDAS. Riset Kesehatan Dasar Tahun 2018. Badan Penelitian dan Pengembangan Kesehatan. 2018. p. 198. http://labdata.litbang.kemkes.go.id/images/download/laporan/RKD/2018/Laporan_Nasional_RKD2018_FINAL.pdf
2. Sibarani M. Dental Caries: Etiology, Clinical Characteristics, and Management. Maj Kedokt UKI. 2014;30(1):14–22.
3. Komansilan JG, Mintjelungan CN, Waworuntu O. Daya Hambat Ekstrak Kulit Manggis (Garcinia Mangostana L.) Terhadap Streptococcus mutans. e-GIGI. 2015;3(2). https://doi.org/10.35790/eg.3.2.2015.8824
4. Gunawan PN, Supit A, Manado SR. Uji Efektif Anti Bakteri Esktrak Bunga Cengkeh. 2014;2.
5. Astuti SI, Arso SP, Wigati PA. Aktivitas Pembentukan Biofilm Streptococcus Mutans dan Candida Albicans Setelah Dipapar Dengan Cigarette Smoke Condensate dan Minuman Probiotik. Anal Standar Pelayanan Minimal Pada Instal Rawat Jalan di RSUD Kota Semarang. 2015;3:103–11.
6. Hamsar A, Ramadhan ES. Penggunaan Chlorhexidine Kumur dalam Perbaikan Indeks Kebersihan Gigi Pegawai Poltekkes Kemenkes RI Medan. J Kesehat Gigi. 2019;6(2):99–103. https://doi.org/10.31983/jkg.v6i2.5482
7. Dwipriastuti D, Putranto RR, Anggarani W. Perbedaan Efektivitas Chlorhexidine Glukonat 0,2% Dengan Teh Hijau (Camellia Sinensis) Terhadap Jumlah Porphyromonas Gingivalis. ODONTO Dent J. 2017;4:50–4. https://doi.org/10.30659/odj.4.1.50-54
8. Al-Eraky MM, Mohamed N, Kamel F, Al-Qahtani A, Madini MA, Hussin A, et al. Chlorhexidine – An Insight. Nazam. Int J Adv Res. 2016;4(6):625–34.
9. Darmawansyih. Khasiat Buah Manggis untuk Kehidupan. J Al Hikmah. 2014;XV:60–8.
10. Idawati S, Hakim A, Andayani Y. Isola α - Mangostin dari Kulit Buah Manggis (Garcinia mangostana L.) dan Uji Aktivitas Antibakteri terhadap Bacilus cereus. 2018;118–

22.
11. Yanura A. Potensi α-Mangostin dari Ekstrak Perikarp Manggis (Garcinia mangostana L.) dalam Menghambat SekresiProtein Culture FiltrateProtein (CFP)-10 pada Mycobacterium tuberculosis H37Rv. Pharm J Indones. 2016;2(1):12–7. https://doi.org/10.21776/ub.pji.2016.002.01.3
12. Syaravina CB, Amalina R, Hadianto E. Pengaruh Ekstrak Daun Beluntas (Pluchea indica (L.) Less) 25% Terhadap Biofilm Streptococcus Mutans - in vitro. ODONTO Dent J. 2018;5(1):28. https://doi.org/10.30659/odj.5.1.28-33
13. Wangi RPL, Suswati E, Wisudanti DD. Aktivitas Ekstrak Metanol Bawang Putih (Allium sativum) sebagai Penghambat Pembentukan Biofilm pada Pseudomonas aeruginosa (The Activity of Methanolic Extract of Garlic (Allium sativum) in Inhibiting Growth of Biofilm in Pseudomonas aeruginosa). J Pustaka Kesehat. 2017;5(3):537–43.
14. Maghfirah F, Saputri D, Basri. Aktivitas pembentukan biofilm Streptococcus mutans dan Candida albicans setelah dipapar dengan cigarette smoke condensate dan minuman probiotik. J Caninus Dent. 2017;2(1):12–9.
15. Nguyen PTM, Falsetta ML, Hwang G, Gonzalez-Begne M, Koo H. A-Mangostin Disrupts the Development of Streptococcus Mutans Biofilms and Facilitates Its Mechanical Removal. PLoS One. 2014;9(10). https://doi.org/10.1371/journal.pone.0111312
16. Rakhmawatie MD, Rohmani A, Ahyar FW. In Vitro Effect of Alfa Mangostin on Multiresistant Uropathogenic Escherichia Coli. Sains Med J Kedokt dan Kesehat.2017;8(1):20–7. https://doi.org/10.26532/sainsmed.v8i1.1002
17. Phuong NTM, Van Quang N, Mai TT, Anh NV, Kuhakarn C, Reutrakul V, et al. Antibiofilm activity of α-mangostin extracted from Garcinia mangostana L. against Staphylococcus aureus. Asian Pac J Trop Med. 2017;10(12):1154–60. https://doi.org/10.1016/j.apjtm.2017.10.022
18. Tamura H. A Novel Herbal Formulation versus Chlorhexidine Mouthwash in Efficacy against Oral Microflora. 2018;8(831):34–7.
19. Sinaredi BR, Pradopo S, Wibowo TB. Daya Antibakteri Obat Kumur Chlorhexidine, Povidone Iodine, Fluoride Suplementasi Zinc Terhadap Streptococcus mutans dan Porphyromonas gingivalis. Maj Kedokt Gigi. 2014;47(4):211. https://doi.org/10.20473/j.djmkg.v47.i4.p211-214
20. Sultan OS, Kantilal HK, Khoo SP, Davamani AF, Eusufzai SZ, Rashid F, et al. The Potential of α-Mangostin from Garcinia mangostana as an Effective Antimicrobial Agent—A Systematic Review and Meta-Analysis. Antibiotics. 2022;11(6). https://doi.org/10.3390/antibiotics11060717