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by Deby Kania Tri Putri

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THE EFFECT OF MAULI BANANA STEM GEL *(MUSA ACUMINATA)* ON BIOFILM MASS OF *STREPTOCOCCUS MUTANS* THE CAUSES OF DENTAL CARRIES

Akhmad Farhansyah Ibrahim[1], Amy Nindia Carabelly[2], Deby Kania Tri Putri[3], Lia Yulia Budiarti[4], Maharani Laillyza Apriasari[5]

[1] Program Study of Dentistry, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, South Borneo, Indonesia
[2] Departement of Oral Pathology and Maxilofacial, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, South Borneo Indonesia
[3] Departement of Biomedic, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, South Borneo Indonesia
[4] Departement of Microbiology, Medical Faculty, Lambung Mangkurat University, Banjarmasin, South Borneo Indonesia
[5] Departement of Oral Medicine, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, South Borneo Indonesia

Correspondence:
Akhmad Farhansyah Ibrahim
Program Study of Dentistry
Faculty of Dentistry Universitas Lambung Mangkurat
Veteran Streets No. 128B, Banjarmasin City, South Borneo
Email: akhmadfarhansyahibrahim@gmail.com, 1711111210004@mhs.ulm.ac.id

Abstract

Background: The prevalence of dental caries in Indonesia reaches 88.8%, and the province of South Kalimantan ranks second with a caries rate of 86.9%. Streptococcus mutans is the main etiologic factor causing dental caries, because it can form biofilms. Streptococcus mutans can form biofilms by producing glucosyltransferase enzymes and matrix extracellular polymeric substances. Mauli banana is a typical plant of South Kalimantan. Mauli banana stems are proven to have antibacterial and antifungal properties, but it is not yet known whether they also have antibiofilm properties. **Objective:** To analyze the antibiofilm potential of Mauli banana stem gel (MBSG) against Streptococcus mutans biofilm. **Methods:** Antibiofilm potential was assessed based on the percentage of the remaining biofilm mass and the percentage reduction in biofilm mass. This study used 10 groups, group 1 was given 0.2% chlorhexidine gel (positive control), group 2 was not given treatment (negative control), group 3 was the media control, and group 4-10 was given MBSG with a concentration of 6.25%, 12 .5%, 25%, 37.5%, 50%, 62.5% and 75% All groups were incubated for 18 hours, then stained with crystal violet and observed using a microplate reader. **Results:** The results of the Mann-Whitney test showed a significant difference in all treatment groups. **Conclusion:** MBSG is an antibiofilm against the biofilm of Streptococcus mutans.

Keywords: Biofilm, Crystal violet, Mauli banana stem gel, Streptococcus mutans.

INTRODUCTION

Dental caries is a condition of demineralization of the hard tissues of the teeth caused by the production of acid produced by carbohydrate fermentation by bacteria.[1] Streptococcus mutans (S. mutans) has been shown to be the main etiologic factor causing dental caries, this is because S. mutans can adhere to solid surfaces and colonize in the oral cavity, and form biofilms. These biofilms are microbial aggregates encased in extracellular polymeric substances (EPS) produced by bacteria. Extracellular polymeric substances (EPS) mediate strong adhesion between bacteria to form biofilms with high cell density that can increase bacterial resistance to antibiotics.[2]

It is very important to prevent caries from an early age and one of the most common ways to prevent dental caries is to use a topical drug, 0.2% chlorhexidine gel. The use of topical 0.2% chlorhexidine gel for a long period of time can cause side effects in the form of tooth discoloration, changes in taste sensation and irritation of the oral mucosa so that an alternative to herbal ingredients is needed.[3] Herbal oral topical drugs are suitable for oral prophylaxis because they can maintain good oral health without causing toxic effects to our bodies.[4] One of the ingredients that can be used as an alternative to prevent caries is Mauli banana stem extract.

Mauli banana stem is a medicinal plant that has many benefits in the community, one of which is as an antibacterial ingredient.[5] Based on preliminary studies, there are several compounds contained in Mauli banana stems, such as cinnamic acid which is a polyphenolic compound and isoleucine amino acid, in addition there are secondary metabolites that are mostly in Mauli banana stems, namely tannins. Cinnamic acid has hydrophobicity and low lipophilic molecules that allow it to interact with cell membranes and damage cell structures and in isoleucine compounds it acts as an antimicrobial peptide (AMP) which can cause membrane lysis.[6,7] These three compounds cause bacterial death which can indicate an inhibition of the biofilm produced and the biofilm coating by EPS can be penetrated and degraded, so it is necessary to measure the mass of the biofilm against biofilm bacteria by looking at the absorbance value of the remaining percentage of biofilm formation.[6,8] This staining was used to evaluate the ability of the extract to inhibit the biofilm.

Based on a study by Apriasari et al (2013), with methanol extract using Mauli banana stems with concentrations of 6.25%, 12.5%, 25% on the growth of S. mutans, it was found that at all three concentrations, Mauli banana stem extract had the ability to inhibit the growth of S. mutans and at a concentration of 25% are said to be the most effective and have not found a concentration that can kill bacteria as a whole, so researchers want to use concentrations of 6.25%, 12.5%, 25% as a reference and add several concentrations with intervals of 12.5%, namely 37.5%, 50%, 62.5%, 75% which will be compared with the positive control of chlorhexidine gel.[9] Research by Wikan et al (2016) said that the use of topical drug preparations in the form of a gel has advantages over other topical preparations, because it can last a long time and is able to provide high speed in releasing active substances, and can cause a cool sensation and is easy to apply.[10] In the research of Ikono et al (2019), the observation of extract activity in culture to see the percentage of the remaining biofilm mass and the percentage decrease in biofilm mass was at incubation for 18 hours where there was a significant increase in yield, this is because this phase occurred

in the logarithmic phase. where the formation of more and more stable EPS occurs to protect the biofilm from exposure to antibiofilm compounds.[11] Based on this background, it is necessary to conduct research to To analyze the antibiofilm potential of Mauli banana stem gel (MBSG) against Streptococcus mutans biofilm.

MATERIALS AND METHOD

This research is a pure experimental study with a post test only design with a control group design which has been declared ethically feasible by the Health Research Ethics Commission, Faculty of Dentistry, University of Lambung Mangkurat with No. 042/ KEPKG-FKGULM/EC/III/2021.

Preparation of Mauli Banana Stem Extract

Prior to the extraction procedure, a determination test was carried out on the plants to ensure that the plants used were the correct Mauli banana species. Three medium-sized Mauli banana stems were obtained from SMK-PP Banjarbaru. The process of making Mauli banana stem extract was carried out by the maceration method. The banana tree trunk used was taken 10 cm from the root tuber, then washed. The stems have been washed and then cut into smaller pieces. Furthermore, the pieces of banana stems are dried in an oven for 3 days. After drying, the pieces of banana stem were crushed into simplicia using a blender, then soaked in 70% ethanol using a basin for 3x24 hours. The simplicia results obtained are approximately 2.5 kg. After 3 days of immersion, the solution was evaporated using a rotatory evaporator at a temperature of 40°-50°C to obtain a thick extract of Mauli banana stem with a total weight of 116.75 grams. The viscous extract of Mauli banana stem that had been obtained was then tested for ethanol free using acetic acid (CH_3COOH) and sulfuric acid (H_2SO_4), then heated. The extract is declared free of ethanol if the process does not smell ester odor.

Preparation of Mauli Banana Stem Gel

After getting the results of the thick extract of Mauli banana stem from the rotatory evaporator and it was declared free of ethanol, the next step was to process the extract into a gel preparation with concentrations of 6.25%, 12.5%, 25%, 37.5%, 50%, 62,5% and 75%. Mauli banana stem gel is made by mixing Mauli banana stem extract with gel base ingredients in the form of propylene glycol, glycerin, Na-CMC, nipagin, and aqua ad. Each concentration of the Mauli banana stem extract gel that had been formed was then dissolved using dimethyl sulfoxide (DMSO) for the antibiofilm testing process to inhibit the formation of the biofilm mass of Streptococcus mutans bacteria.

Mauli Banana Stem Gel Preparation Test

Mauli banana stem gel that has been made is then tested to see the stability and physical characteristics of the gel with homogeneity test and spreadability test.

Microorganism Culture Process

This study used a strain of Streptococcus mutans ATCC 25175. Streptococcus mutans was cultured separately in triptic soy broth (TSB) plus 1% sucrose for 18 hours at 37°C. Streptococcus mutans was cultured anaerobically (10% CO_2, 80% N_2, 10% H_2).

Saliva Coating

Saliva in this study was obtained from artificial saliva of McDougall's solution by Fusayama Meye method which was dissolved in PBS until the concentration reached 200 ng/mL. Then, 200 L of saliva was taken to be applied to a 96-well plate and incubated at 37°C for 1 hour.[12]

Table. 1 Ingredients for Formulation of Mauli Banana Stem Gel (MBSG)

	Material Name	Utility	Gel preparation (%) (Unit in grams (g))						
			F1	F2	F3	F4	F5	F6	F7
1	Mauli banana stem extract	Active ingredients	6,25% (1,25)	12,5% (2,5)	25% (5)	37,5% (7,5)	50% (10)	62,5% (12,5)	75% (15)
2	Propilen Glikol	Maintain viscosity	5% (1)	5% (1)	5% (1)	5% (1)	5% (1)	5% (1)	5% (1)
3	*Glycerin*	*Emollient* and *humectant*	10% (2)	10% (2)	10% (2)	10% (2)	10% (2)	10% (2)	10% (2)
4	Na-CMC	*Gelling agent*	10% (2)	10% (2)	10% (2)	10% (2)	10% (2)	10% (2)	10% (2)
6	Nipagin	Preservative	0,1% (0,02)	0,1% (0,02)	0,1% (0,02)	0,1% (0,02)	0,1% (0,02)	0,1% (0,02)	0,1% (0,02)
7	Aqua ad	Solvent	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml

Information:

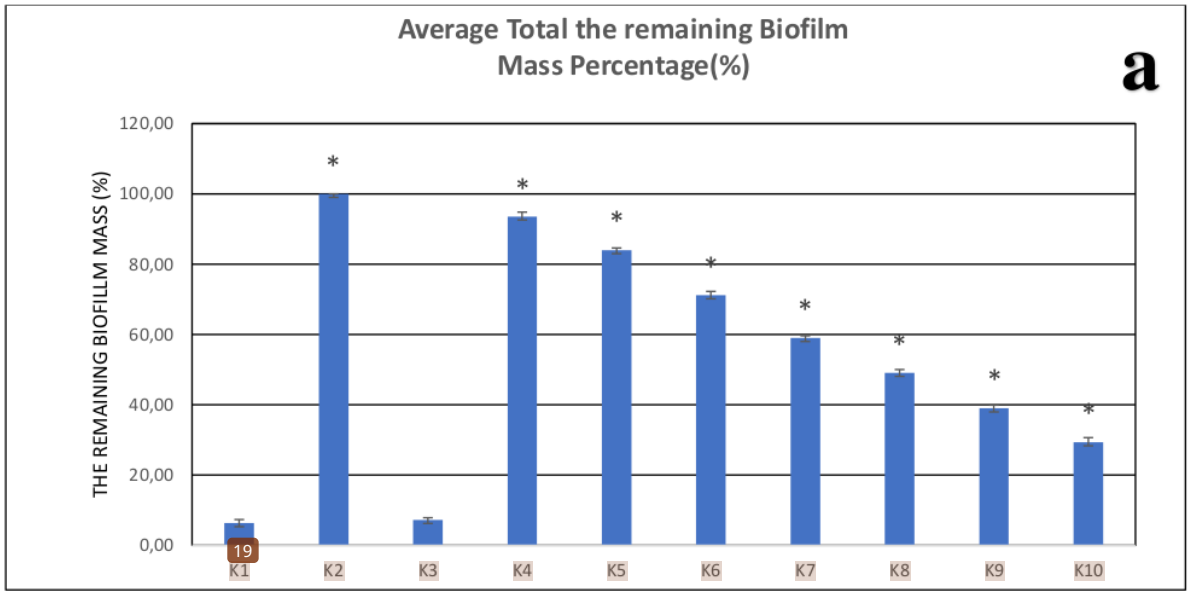
F1: MBSG 6,25% F2: MBSG 12,5% F3: MBSG 25% F4: MBSG 37,5% F5: MBSG 50%
F6: MBSG 62,5% F7: MBSG 75%

Biofilm formation

Streptococcus mutans that had been cultured for 18 hours were harvested by centrifugation (5000 rpm, 10 minutes, 4°C). Each pellet was dissolved using TSB + 1% sucrose, until it reached OD600 = 0.1, then measured using a UV/VIS spectrophotometer. In the Streptococcus mutans biofilm, 200 L of a suspension of Streptococcus mutans microorganisms was inoculated on each well-plate. The well-plate was incubated for 90 minutes under anaerobic conditions (10%CO2, 80% N2, 10% H2). After 90 minutes, the supernatant was removed and replaced with 200 L of different concentrations of Mauli banana stem extract gel, namely: 0% (negative control group), 6.25%, 12.5%, 25%, 37.5%, 50% 62.5%, 75% and 0.2% chlorhexidine gel solution (positive control) were dissolved with DMSO. The well-plate was re-incubated anaerobically (10% CO2.80% N2, 10% H2) at an incubation time of 18 hours, after incubation the supernatant was aspirated and the well-plate was washed with PBS twice.

Analysis of Streptococcus mutans Biofilm Mass Inhibition with Crystal Violet (CV) Solution

The well-plate that has been aspirated and has dried will be given 0.1% crystal violet dye into the well, so that the biomass in the biofilm can be stained which will then be incubated for 2 minutes at room temperature. The contents of the well-plate were rinsed using sterile PBS solution so that cells stained on the biofilm but not attached to the well-plate could be removed. Then the well-plate was allowed to dry again at room temperature, then 200μL of 96% ethanol solution was added into the well and incubated for 20 minutes at room temperature. 96 well-plates were observed using a microplate reader with a wavelength of 600 nm. The results of the calculation using the microplater reader will get the value of optical density (OD). The result of inhibition of biofilm mass is expressed as a percentage value divided by the percentage of remaining biofilm mass and the percentage reduction in biofilm mass obtained by entering the OD value into the following formula:



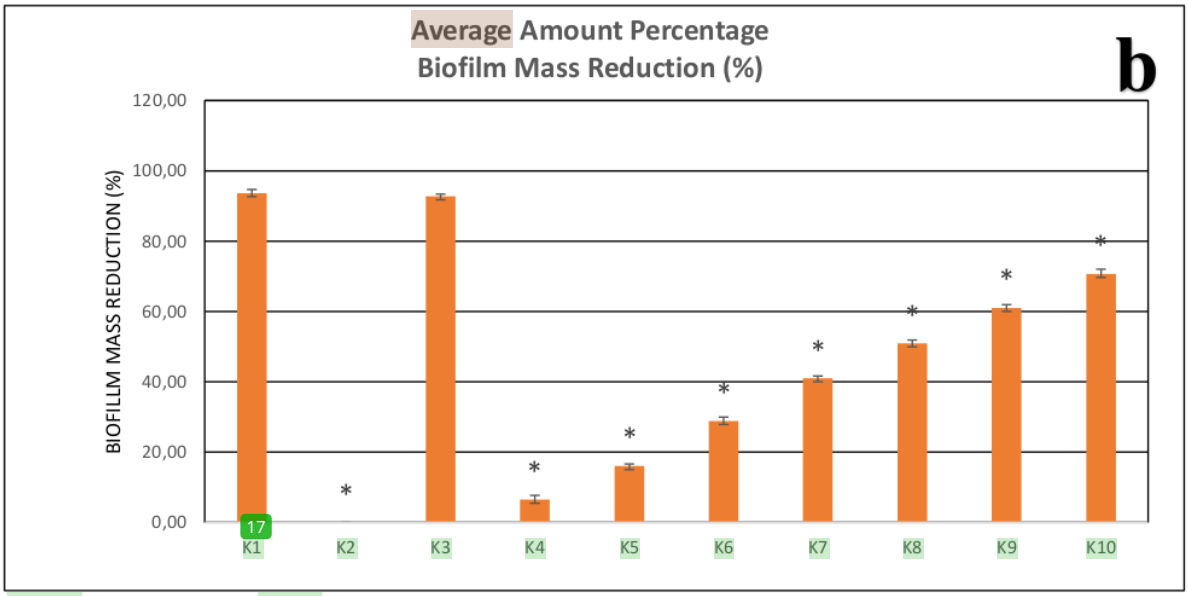


Figure 1. Observation of the antibiofilm test, (a) Average Total the Remaining Biofilm Mass Percentage, and (b) Average Amount Percentage Biofilm Mass Reduction.

Information

K1/ K(+)	: Positive Control (*Chlorhexidine gluconate 0,2% gel)*
K2/ K (-)	: Negative control (without treatment)
K3/ KM	: Media Control (*Tryptic Soy Broad)*
K4	: Treatment group with concentration of 6,25% MBSG
K5	: Treatment group with concentration of 12,5% MBSG
K6	: Treatment group with concentration of 25% MBSG
K7	: Treatment group with concentration of 32,5% MBSG
K8	: Treatment group with concentration of 50% MBSG
K9	: Treatment group with concentration of 62,5% MBSG
K10	: Treatment group with concentration of 75% MBSG
MBSG	: Mauli Banan Stem Gel

The percentage of the remaining mass of biofilm is calculated by the following formula:

$$\frac{\text{OD Kelompok Perlakuan (KP)}}{\text{OD Kontrol Negatif (KN)}} x\ 100\%$$

While the percentage reduction in the mass of biofilm is calculated by the following formula:

$$\frac{\text{OD Kelompok Perlakuan (KP)} - \text{OD(KN)}}{\text{OD Kontrol Negatif (KN)}} x\ 100\%$$

RESULTS

Evaluation of Mauli Banana Ste Extract Gel Antibiofilm against Streptococus mutans Biofilm

The effect of administration of mauli banana stem extract gel on biofilm growth was observed by observing the value of changes in biofilm mass. Figure 1 shows the biofilm inhibition ability of the Mauli banana stem extract gel confirmed by observing changes in the mass of S. mutans biofilm. Figure 1a shows the effect on the remaining biofilm mass. The results showed the average percentage of the remaining biofilm mass against the Streptococcus mutans biofilm for 18 hours given the Mauli banana stem gel solution. The highest average percentage was found in the untreated group/K(-) because it only contained untreated Streptococcus mutans biofilm media (100%) and the lowest average was found in the positive control group/K(+) which contained Streptococcus mutans biofilm. with 0.2% (6.31%) chlorhexidine gel treatment. In the treatment group, the Mauli banana stem gel showed an effect on the remaining biofilm mass with a decrease in the mass of the remaining biofilm.

The ability to inhibit the biofilm mass was added with other observations to increase the accuracy of the data, so the effect of the Mauli banana stem extract gel on the decrease in biofilm mass was also carried out. Figure 1b shows the results of decreasing the percentage of biofilm mass. These results indicate the average percentage reduction in biofilm mass against Streptococcus mutans biofilm for 18 hours given the Mauli banana stem gel solution. The lowest average percentage was found in the untreated group/ K(-) because it only contained untreated Streptococcus mutans biofilm media (0%) and the highest average was found in the positive control group/ CHX gel 0,2% which contained Streptococcus mutans biofilm. with 0.2% (93.69%). In the result of treatment group, the Mauli banana stem gel showed an effect on the remaining biofilm mass with an increase in the percentage decrease in the remaining biofilm mass. The increase in the concentration of the Mauli banana stem gel in the two observations of changes in the mass of the biofilm shows that the more the concentration, the value of the wasted biofilm mass (lysis) will increase and the mass formed will be less.

Data analysis

The results of the analysis on the inhibition of biofilm mass showed that in the normality test, a significance value ($p<0.05$) was obtained for all groups, except for the no-treatment group (-) and the treatment group with 37.5%. These results indicate that in all groups are not normally distributed. The results of the Levene Test show a significance value of $p = 0.000$ ($p<5$), so the data is continued with a non-parametric test with Kruskal-Wallis followed by the Mann-Whitney test. The results of the Kruskal Wallis test showed a significance value of $p = 0.000$ ($p <0.05$) which stated that there was a significant difference and could be continued with the Mann-Whitney test. The results showed that there was a significant difference between the treatment group given the Mauli banana stem extract gel and all groups. Results that showed significant differences were also found between the group given chlorhexidine 0.2% (positive control) and all groups, except the media group.

DISCUSSION

The development of biofilms in the oral cavity, especially those attached to the tooth surface, will play a role in the initiation and progression of caries, so preventing the formation of biofilms can reduce the growth of biofilms and prevent caries. Biofilm mass is one way to detect the presence of biofilm growth to prevent caries by looking at the OD value. This can be done using the microtiterplate method which is the gold standard for biofilm detection. Based on the results of this study, it was shown that the administration of a gel solution of Mauli banana stem extract with concentrations of 6.25%, 12.5%, 25%, 37.5%, 50%, 62.5%, 75% on the Streptococcus mutans biofilm could be said to be an antibiofilm. because it can show a decrease in the percentage of the remaining biofilm mass and show an increase in the percentage decrease in biofilm mass in the Streptococcus mutans biofilm that causes dental caries.[5]

The treatment group with the administration of Mauli banana stem extract gel with a concentration of 75% showed the highest decrease in the percentage of remaining biofilm mass and the highest increase in the decrease in the percentage of biofilm mass. Each increase in the concentration of the Mauli banana stem extract gel will cause a decrease in the percentage of remaining biofilm mass and an increase in the percentage decrease in biofilm mass in Streptococcus mutans biofilm. The antibiofilm ability of the Mauli banana stem gel is due to the active ingredients in the banana stem, namely the main phytochemical compounds in the form of isoleucine and cinnamic acid (CA), and can also be caused by several other secondary metabolites possessed by the Mauli banana stem such as alkaloids, phenols, tannins, saponins, and flavonoids in Mauli banana stem gel.[5,17,18]

Cinnamic acid is one of the phenolic compounds in the Mauli banana stem which has a role as an antibacterial agent.[6] This compound has hydrophobicity and low lipophilic molecule. These properties allow it to interact with cell membranes by inhibiting the plasma membrane enzyme H+-ATPase through a diffusion process which then damages the cell wall structure, so that the cell membrane structure will be degraded. This cell degradation causes the cytoplasm to acidify and causes protein denaturation in these microorganisms and disruption of cell integrity in the formation of biofilms.[6,119] Another antibiofilm ability of cinnamic acid is to inhibit the activity of GtfB and GtfC which play a role in the formation of biofilms at the initial attachment stage.[20]

Isoleucine is another major compound that plays a role in Mauli banana which is included in free amino acids.[21] Isoleucine is an amino acid that acts as an antimicrobial peptide (AMP) which has potential as an antimicrobial agent. Antimicrobial peptides can cause a bond that can cause membrane lysis due to the strong electrostatic attraction of AMP to bacterial cells, causing functional disturbances in the bound cell organelles and can damage the permeability of cell membranes.[22] AMP has other antibiofilm abilities by interfering with biofilm formation through cell signaling by interfering with quorom sensing processes in biofilm formation.[22,23]

The antibiofilm ability possessed by the Mauli banana stem can also be obtained from several secondary metabolites which are mostly in the Mauli banana stem, namely tannins (67.59%), saponins (14.49%), and flavonoids (0.25%) by influencing the regulatory process. that bacteria have.[5] The mechanism of action of tannin is by inhibiting the formation of reverse transcriptase and DNA topoisomerase enzymes which cause structural

changes that result in denaturation of the EPS biofilm, so that the biofilm formed will be degraded. Tannins can act as anti-adherents by inhibiting glycosyltransferase thereby inhibiting the synthesis of glucans which play a role in the bacterial attachment process in the early stages of biofilm formation, so that bacteria cannot adhere to surfaces.[24]

Saponins are semipolar compounds that are soluble in lipids and water, which can cause biofilm inhibition by disrupting cell receptors so that there is a disruption in the attachment process in biofilms. Flavonoids have antibiofilm abilities by reducing cellular adhesion and modifying the structure of biofilm formation through the process of inhibiting gtfC enzymes. This inhibition prevents the synthesis of insoluble glucans for the formation of the extracellular matrix in the irreversible biofilm attachment process.[20]

In this study, the treatment group given Mauli banana stem extract gel still did not have results that were comparable to or could exceed the ability of 0.2% chlorhexidine antibiofilm gel in reducing the percentage of remaining biofilm mass and increasing the percentage reduction in biofilm mass percentage. Chlorhexidine 0.2% has antibacterial properties as a cationic molecule by binding to the cell wall of negatively charged bacteria and has a bacteriostatic action, at low concentrations by changing the osmotic balance of bacterial cells, by promoting the release of low-weight molecules. Chlorhexidine causes cell death through cytolysis by increasing the permeability of the bacterial cell membrane resulting in the release of major intracellular components, including potassium, thereby changing the structure of cell proteins and causing precipitation/coagulation of cytoplasmic proteins.[25] Limitation of the results of this study is that the antibiofilm effectiveness value of the Mauli banana stem gel that can match the 0.2% chlorhexidine gel ability, so it is necessary to add other plant compositions that can increase the antibiofilm ability of the Mauli banana stem gel.

CONCLUSION

Mauli banana stem has the ability as an antibiofilm by showing an effect on the mass of Streptococcus mutans biofilm with a concentration of 6.25%, 12.5%, 25%, 37.5%, 50%, 62.5%, 75% after incubation, with concentration of 75% shows the most optimal results. However, the antibiofilm ability of the Mauli banana stem still cannot match the antibiofilm ability of Chlorhexidine gluconate 0.2%

SUGGESTION

In future researchers, the composition of other plant extracts can be added which can increase the effectiveness of the Mauli banana stem gel so that it can match the antibiofilm ability of 0.2% Chlorhexidine gel. In addition, further researchers need to understand and pay more attention to the composition of the gel base material so as not to affect the physical preparation of the gel which can interfere with the antibiofilm ability of the gel.

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