Majalah Kedokteran Gigi Indonesia
Vol 8 No 3 – December 2022
ISSN 2460-0164 (print), ISSN 2442-2576 (online)
Available online at https://jurnal.ugm.ac.id/mkgi
DOI: http://doi.org/10.22146/majkedgiind.80636

RESEARCH ARTICLES

The concentration effect of kulim leaf (*scorodocarpus borneensis)* extract on *Streptococcus mutans* ATCC 25175 bacterial hydrophobicity and adhesion

Trianna Wahyu Utami*, Adhaninggar Ratna Hapsari, Dhea Rifdania Hanalda**, Asikin Nur*, Heribertus Dedy Kusuma Yulianto*, Nunuk Purwanti*✉**

*Department of Biomedical Dental Science, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
**Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
*Jl Denta No 1 Sekip Utara, Yogyakarta, Indonesia; ✉ correspondence: n_purwanti@mail.ugm.ac.id

Submitted: 3rd December 2022; Revised: 7th December 2022; Accepted: 12nd December 2022

ABSTRACT

With the ability to reduce hydrophobicity and inhibit the adhesion of S. mutans ATCC 25175 bacteria, Kulim leaf extract can be used as an alternative to chlorhexidine mouthwash in caries prevention. The objectives of this study are to determine the effect of Kulim leaf extract on hydrophobicity and adherence of the cariogenic bacteria *S. mutans* ATCC 25175. The test groups were divided into negative control (DMSO 2%), the treatment group (Kulim leaf extract concentrations of 1.25%, 2.5%, 5%), and positive control (0.1% chlorhexidine). All of groups were received three replicated tests for hydrophobicity and adherence inhibition of *S. mutans* ATCC 25175 bacteria. The hydrophobicity test was conducted by providing 3 ml of bacterial suspension of *S. mutans* ATCC 25175 which had been adjusted to the McFarland 0.5 standard for each group. Furthermore, each test group was vortexed for one minute and left to stand for 15 minutes. Each treatment was tested with a wavelength spectrophotometer of 550 nm before and after the provision of 200 µl of n-hexadecane. The absorbance value on the spectrophotometer was then included in the hydrophobicity formula to determine the hydrophobicity percentage of *S. mutans* ATCC 25175 against n-hexadecane. To test the bacterial adhesion, the 96 wells microplate was inserted with the kulim leaf extract of each concentration, BHI-B, bacteria according to the McFarland 0.5 standard, and for the positive control and negative control. Afterwards, they were incubated at 37 °C for 24 hours before they were rinsed with distilled water, and stained with 0.1% crystal violet. Then, an optical density reading was performed using a microplate reader with a wavelength of 540 nm. The absorbance value was then included in the formula for percentage of bacterial adhesion inhibition. Post-Hoc LSD test showed a significant difference in mean difference between the negative control group and the other treatment groups ($p<0.05$). In addition, it was revealed that there was no significant mean difference between treatment groups, and there was no significant difference between positive control and treatment groups of 2.5% and 5% in the hydrophobicity test. However, there was a significant difference between the positive control and the treatment group of 1.25% in the hydrophobicity test and the treatment group of 1.25%, 2.5%, and 5% in the adherence test. This study concluded that Kulim leaf extract concentration affected hydrophobicity and attachment of *S. mutans* ATCC 25175 with an effective concentration of 2.5%.

Keywords: adhesion; biofilm; hydrophobicity; Kulim (*S. borneensis*) leaf extract; *S. mutans* ATCC 25175

INTRODUCTION

The main oral health problem in Indonesia is dental cavities or caries. The Basic Health Research data (Riskesdas) in 2013 and 2018 indicated that the DMF-T index for ages 35-44 years increased from 5.4 to 7.02.[1,2] Without any intervention and prevention, the existing problem of cavities if allowed will definitely affect a person's quality of life, because it causes pain, discomfort, and can lead to acute and chronic infections.[3]

Caries is a process of demineralization or loss of minerals in the hard tissue of the tooth that involves the interaction of several factors, namely the resistance of enamel, microorganisms, carbohydrates, time and saliva[4] *Streptococcus mutans* is one of the normal oral bacteria known as caries-causing (cariogenic) bacteria which has the ability to metabolize carbohydrates to acid (acidogenic), and the ability to thrive in an acidic environment (aciduric).[5] The bacteria *S. mutans* ATCC 25175 are frequently found in dentinal caries.

The American Type Culture Collection (ATCC) that serves as a national repository and distribution center for cultures of microorganisms classifies these bacteria for laboratory research. These bacteria are used as the gold standard in research because they have passed very meticulous laboratory procedures that set them as the quality control. The *S. mutans* ATCC 25175 bacteria have several genes with the same function as the function of genes in the oral cavity *S. mutans*, known as the spaP gene. This gene plays a crucial role in the adhesion of bacteria to teeth. In addition, *S. mutans* ATCC 25175 also expresses brpA and relA genes, which is crucial in the process of biofilm formation. The ability of bacteria to adhere to the host is a major factor at the start of the bacterial infection process.[6] This ability depends on the hydrophobic interaction between bacteria and the tooth surface. Hydrophobic interactions are determined by the hydrophobicity nature of bacteria.[7]

To inhibit the formation of plaque, antibacterial chemicals can be applied to surfaces that may serve as plaque growth sites.[6] One of the physical agents commonly used to prevent plaque formation is chlorhexidine. However, its use has several side effects, such as staining of the teeth, oral dryness and a burning sensation.[8] This side effect of chlorhexidine encourages the development of herbal ingredients as antibacterial agents. One of the potential plants is kulim (*Scorodocarpus borneensis*). This plant is commonly found in the forests of East Kalimantan.[9] Based on the research of Lim, Kulim (*S. borneensis*) has several benefits, including as an antiplatelet, anticancer, and antimicrobial agent.[10] The purpose of this study was to determine the effect of Kulim leaf extract concentration on hydrophobicity and adherence of the cariogenic bacteria *S. mutans* ATCC 25175. An increase of Kullim leaf extract concentration will decrease hydrophobicity and adherence of the cariogenic bacteria *S mutans* ATCC 25175.

MATERIALS AND METHODS

The extract was made using the maceration method at Mulawarman University. The collected Kulim leaf samples were washed under running water and drained before being dried in the oven. The dried leaves were then mashed using a blender to form a powder. Subsequently, Kulim leaf powder was put into the Erlenmeyer and soaked using n-hexane for a certain time with occasional stirring. Afterwards, the soaking results were filtered to obtain the filtrate, which was put into a vacuum rotary evaporator with a certain temperature and time until 100% viscous extract was obtained.

The dilution of kulim leaf extract was carried out at the Unit IV Laboratory of the Faculty of Pharmacy, Universitas Gadjah Mada. A total of 4 g of 100% kulim leaf extract was added with 80 ml of 2% DMSO. The extract was then dissolved using an ultrasonic device until it was homogeneous. The following step was filtering with filter paper and by using 0.45 mm millipore, resulting in a kulim leaf extract with concentration of 5%. Afterwards, 30 ml of 5% concentration of kulim leaf extract was taken and transferred to a new conical tube and then added with 30 ml of 2% DMSO, before it was vortexed to produce a 2.5% concentration of kulim leaf extract. Following this, 20 ml of kulim leaf extract with a concentration of 2.5% was taken, transferred to a new conical tube, and added with 20 ml of 2% DMSO to produce a kulim leaf extract with concentration of 1.25%.

The preparation of *S. mutans* ATCC 25175 bacterial suspension was carried out at the Integrated Research Laboratory of the Faculty of Dentistry, Universitas Gadjah Mada with number ethical clearance 00350/KKEP/FKG-UGM/EC/2020. The *S. mutans* bacterial suspension was prepared by taking bacterial colonies using sterile ose then transferred to a conical tube containing BHI broth and incubated for 24 hours. The conical tube was centrifuged for 15 minutes at a speed of 3000 rpm. Bacterial colonies were taken using sterile ose and transferred to test tubes containing sterile distilled water until turbidity was obtained according to the standard of 0.5 McFarland 1.5 x 108 CFU / ml.

The hydrophobicity test was carried out at the Integrated Research Laboratory of the

Majalah Kedokteran Gigi Indonesia. December 2022; 8(3): 200-209
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

Faculty of Dentistry, Universitas Gadjah Mada. The hydrophobicity test was carried out with five sterile test tubes. Five test tubes were labeled 1, 2, 3, 4, and 5. The first tube to the fifth tube was filled with 3 ml of bacterial suspension. The second to fourth tubes were filled with Kulim leaf extract at the concentration to be used in the study. The concentration was determined based on the MIC test result, namely 2.5%. The MIC test was conducted at Mulawarman University by Kuspradini et al.[11] The concentrations used for this study were 1.25%, 2.5%, and 5%. The first tube as a negative control was 3 ml DMSO 2%. The second to fourth tubes were filled with 3 ml of extract, each with a concentration of 1.25%, 2.5%, and 5%. The fifth tube as a positive control was filled with 3 ml of 0.1% chlorhexidine. The solution in the five tubes was homogenized with a vortex mixer at 3150 rpm for one minute. The tube was left to stand for 15 minutes. Furthermore, 2.5 ml of solution per tube was inserted into the cuvette for absorbance measurement with a 550 nm wavelength spectrophotometer. The results of observations was used to determine the value of At, namely the optical absorbance of bacteria before the addition of n-hexadecane.[7]

The next step was the addition of 200 µl n-hexadecane into each tube. The solution in the tube was homogenized with a vortex mixer with a speed of 3150 rpm for one minute, then left for 15 minutes so that there was a separation between the water phases at the bottom of the solution. The water phase was taken with a 2.5 ml micropipette and put into a cuvette to measure the absorbance using a 550 nm spectrophotometer. The results of the observations were to determine the Au value, namely the optical absorbance of bacteria after the addition of n-hexadecane. All measurements were repeated three time by taking into account the average and standard deviation of the observed data. The absorbance percentage of bacterial cells against n-hexadecane was calculated by the following formula:

$$Ab = \frac{(At - Au)}{At} \times 100\%$$

At: Optical absorbance of total bacterial suspension before addition of n-hexadecane

Au: Optical absorbance of total bacterial suspension after addition of n-hexadecane

Ab: The percentage of hydrophobicity adsorption of bacterial cells against n-hexadecane[7]

The bacterial adhesion test was carried out using microplate flexible U-bottom PVC 96 wells filled with 20 µl of Kulim leaf extract with a concentration of 5%; 2.5%; and 1.25%. Afterwards, each well was added with 170 µl of BHI broth containing 2% sucrose and 10 µl of *S. mutans* bacterial suspension. The positive control group was added with 0.1% chlorhexidine gluconate. The negative control group was added with DMSO 2%. The subjects were then incubated at 37 °C for 24 hours. After that, the well was emptied by removing the media and bacteria that were not attached to the wall of the well. Subsequently, the microplate was washed with distilled water to remove bacteria that did not adhere to the walls of the well before the microplate was dried and stained with 200 µl of 0.1% crystal violet and allowed to stand for 15 minutes. The remaining staining was cleaned twice using distilled water. The microplate was drained again, and the remaining dye adhering to the cells adhering to the tube walls was cleaned using 200 µl of 99% ethanol. After that, 150 µl of the contents of each well was transferred to the microplate flat bottom PVC 96 wells and absorbance measurements were carried out using a wavelength of 540 nm.

According to Dewi et al the percentage of bacterial adherence inhibition was calculated using the formula:

$$\% \text{ inhibition} = \left(1 - \frac{\text{DO test sample} - \text{DO blank sample}}{\text{DO test solvent} - \text{DO Solvent blank}}\right) \times 100\%$$

Note:
DO test sample: optical density measurement results of the test group
DO blank sample: optical density measurement results extract + BHI + sterile distilled water
DO test solvent: measurement results of DMSO optical density + bacterial suspension
DO solvent blank: optical density measurement results DMSO + BHI + sterile distilled water[11]

The hydrophobicity data of bacteria were obtained from the absorbance measurement using

a 550 nm spectrophotometer. Meanwhile, the bacterial adhesion inhibition data were obtained from the absorbance measurement results with a 540 nm microplate reader. Then, each data was entered into the hydrophobicity formula and the percentage formula for bacterial adherence inhibition to be calculated and statistically analyzed using the SPSS Statistics 26 program with a statistical analysis confidence level of 95% (α = 0.05). The significance of the effect of the concentration of kulim leaf extract on the adhesion of S. mutans bacteria was assessed using a one-way ANOVA test. To further assess the significance of the mean difference between treatment groups, the Post Hoc Least Significant Difference (LSD) test was used.

RESULTS

The hydrophobicity test was carried out in the Integrated Research Laboratory of the Faculty of Dentistry, Universitas Gadjah Mada. The data obtained were presented in Table 1. Table 1 shows that the highest average hydrophobicity was found in the negative control group, namely aquades, while the lowest hydrophobicity average was found in the positive control group. The highest hydrophobicity means that there are a lot of bacteria attached to hexadecane, while the low hydrophobicity indicates a decrease in the ability of bacteria to adhere to hexadecane. The description of the mean hydrophobicity of each treatment group is presented in the graph in Figure 1. The graph shows that the percentage of hydrophobicity from high to low was found in the negative control of Kulim leaf extract with concentration of 1.25%, 2.5%, 5%, and in the positive control, respectively. The percentage of hydrophobicity of *S. mutans* ATCC 25175 bacteria decreased along with the increase in extract concentration.

The results of the Post-Hoc LSD test (Table 2) showed that there were significant mean differences between the negative control group and the other treatment groups and between the 2.5% concentration of Kulim leaf extract treatment group and the positive control group. This is indicated by a significance value of less than 0.05. Hence, the mean value of the negative control group's hydrophobicity was the highest compared to the other groups. The positive control group compared to the Kulim leaf extract group with concentrations of 2.5% and 5% had a significance value greater than 0.05. This shows that there was no significant mean difference between the positive control group and the Kulim leaf extract

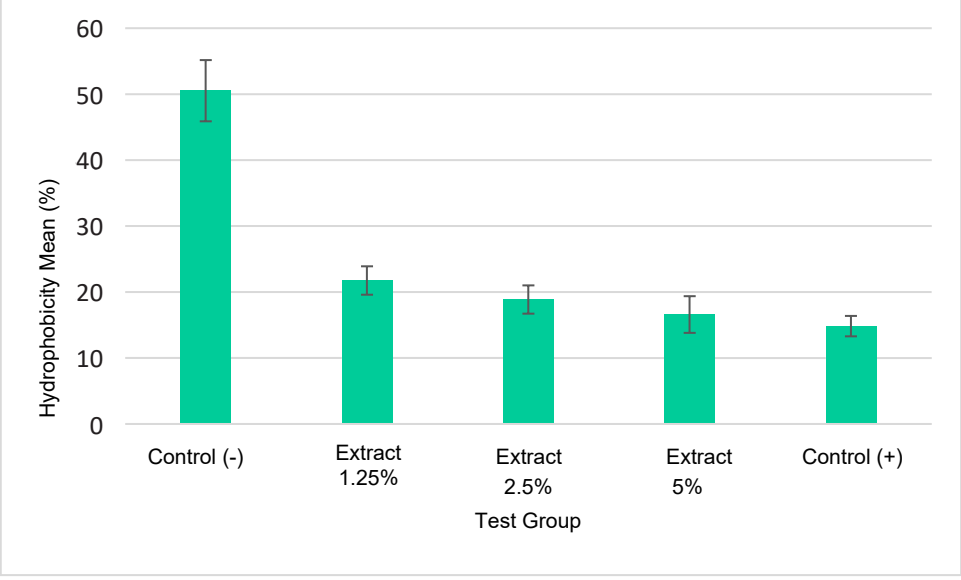

Figure 1. Graph of the mean hyfrophobicity of S. mutans

Majalah Kedokteran Gigi Indonesia. December 2022; 8(3): 200-209
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

Table 1. Percentage of hydrophobicity of *S. mutans* bacteria

Treatment	Hydrophobicity (%)			Mean	Standard Deviation
	Test 1	Test 2	Test 3		
Control (-)	45.45	51.61	54.54	50.35	4.64
Concentration 1.25%	19.51	21.95	23.80	21.75	2.15
2.5% concentration	16.66	19.04	20.93	18.87	2.14
5% concentration	13.79	16.66	19.35	16.60	2.78
Control (+)	14.30	16.60	13.63	14.84	1.55

Table 2. Post-Hoc LSD Result

	Control -	Extract 1.25%	Extract 2.5%	Extract 5%	Control +
Control -	-	0.000*	0.000*	0.000*	0.000*
Extract 1.25%	-	-	0.246	0.052	0.014*
Extract 2.5%	-	-	-	0.353	0.115
Extract 5%	-	-	-		0.469
Control +	-	-	-	-	-

*significant $p < 0.05$

Table 3. Percentage of adherence inhibition of *S. mutans* ATCC 25175 bacteria

Treatment	% Adherence Inhibition			Mean	Standard deviation
	Test 1	Test 2	Test 3		
Positive control	97.88%	96.88%	96.33%	97.03%	0.78581%
Extract 5%	86.69%	72.91%	76.64%	78.7467%	7.12746%
Extract 2.5%	80.14%	79.16%	77.25%	78.85%	1.46973%
Extract 1.25%	77.60%	77.08%	75.40%	76.693%	1.14984%
Negative control	1.86%	1.68%	1.64%	1.7267%	0.11719%

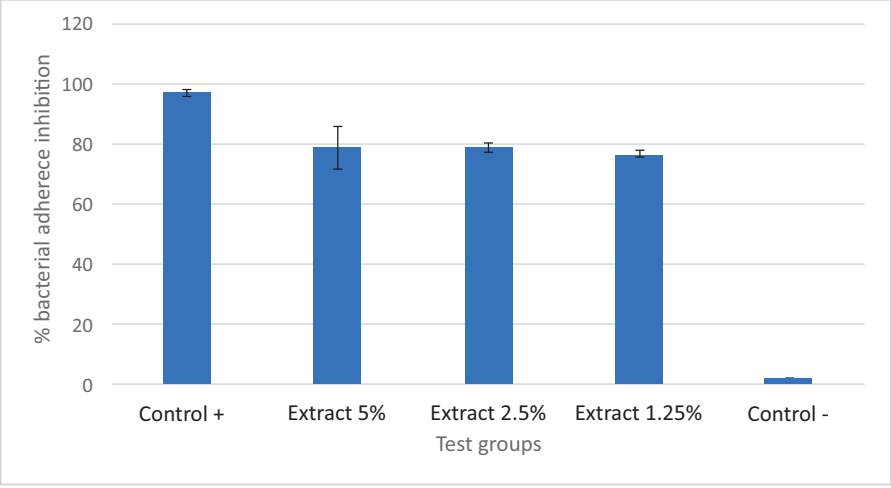

Figure 2. Graph of mean percentage adherence inhibition of S. mutans ATCC 25175

Table 4. Summary of Post-Hoc LSD results

	Control +	Extract 5%	Extract 2.5%	Extract 1.25%	Control -
Control +		0.000 *	0.000 *	0.000 *	0.000 *
Extract 5%			0.970	0.465	0.000 *
Extract 2.5%				0.444	0.000 *
Extract 1.25%					0.000 *
Control -					

*significant $p < 0.05$

group with a concentration of 2.5%, and 5%. In addition, there was no significant mean difference between the three concentrations of Kulim leaf extract used.

The data for calculating the mean percentage of bacterial adherence inhibition in the treatment of kulim leaf extract with a concentration of 5%, 2.5%, 1.25%, negative control and positive control are shown in Table 3. An overview of the mean percentage of bacterial adherence can be seen in the graph in Figure 2. It is conclusive that the negative control group (DMSO 2%) showed the lowest percentage of bacterial adherence inhibition compared to the other treatment groups. Meanwhile, the highest percentage of bacterial inhibition was owned by positive controls (chlorhexidine gluconate 0.1%). The percentage of bacterial attachment inhibition of the kulim leaf extract concentration group from a concentration of 5% to a concentration of 2.5% resulted in an increase, while a concentration of 2.5% to 1.25% obtained a decrease. However, the analysis of the mean difference between the treatment groups in Table 3 indicated that the 5% extract had insignificant differences ($p > 0.05$) compared to the 2.5% and 1.25% extracts. This means that the 5% concentration of kulim leaf extract had almost the same effectiveness as the 2.5% and 1.25% extract in inhibiting the adherence of *S. mutans* ATCC 25175 bacteria. The concentration of 5%, 2.5%, and 1.25% extracts had significant differences ($p < 0.05$) compared with positive and negative controls, which means that the Kulim leaf extract concentrations of 5%, 2.5%, and 1.25% had different effectiveness in inhibiting the adherence of *S. mutans* ATCC 25175 bacteria compared to positive control and negative control.

DISCUSSION

Caries can be defined as the localized deterioration of the hard tissues of the teeth by bacterial fermentation of carbohydrate-containing foods. Caries can occur preceded by the formation of a biofilm. The formation of biofilm/dental plaque includes three stages, namely the formation of a thin layer on the surface of the tooth or pellicle, adhesion or attachment of bacteria, and maturation of the biofilm. Bacterial adhesionis the most important stage and can be compromised using the right approach. *Streptococcus mutans* is a cariogenic bacterium that has hydrophobicity properties.[12] Hydrophobicity is a factor that influences the attachment of *S. mutans* bacteria to the tooth surface.[13]

The results of the hydrophobicity test showed that the hydrophobicity character of *S. mutans* ATCC 25175 decreased along with the increase in the concentration of Kulim leaf extract. Adherence of bacteria to the teeth was influenced by the nature of hydrophobicity, [14] while the hydrophobicity nature of bacteria was determined by the surface of the bacterial cell.[7] The decreased hydrophobicity of bacteria after being exposed to the Kulim leaf extract indicated that the content contained in the Kulim leaf extract could affect the surface of bacterial cells.

The results of the research on the adhesive test *S. mutans* ATCC 25175 showed that the three extract concentrations had the effect of inhibiting bacterial attachment, respectively with 78.75%,

78.85% and 76.69%. Although not as effective as the positive control (chlorhexidine) which reached bacteria attachment bridging of 97.03%, kulim leaf extract had the ability in inhibiting the adherence of bacteria.

Kulim leaf extract could reduce hydrophobicity and inhibit the adherence of *S. mutans* bacteria due to the content of active substances in it. The process of making the extract was carried out by the maceration method using n-hexane (C6H14) solution. The maceration method is able to isolate several active substances, such as saponins, flavonoids, and tannins.[15] Extracts made using n-hexane contain several active substances, such as saponins, alkaloids, flavonoids and tannins.[16]

Flavonoids are synthesized by plants and have an antimicrobial response. The activity of flavonoids in inhibiting bacterial infection is related to their ability to form bonds with proteins in the bacterial cell wall. This binding will induce disruption of the bacterial cell membrane.[17] Disruption due to the bond between flavonoids and extracellular proteins can affect the bacterial cell wall.[18] This is related to a decrease in the hydrophobicity of bacteria, which will interfere with the attachment of bacteria to the tooth surface.[7] In addition, flavonoids are also known to have anti-glucosyltransferase activity, which works by binding to the amine group on glucosyltransferase using the C atomic double bond on the flavonoids. This will damage the glucosyltransferase enzyme so that it inhibits bacterial adhesion.[19,20]

Tannins can affect the attachment of bacteria by inactivating the adhesin molecules used by bacteria to adhere to the tooth surface.[21] Adhesin is a protein on the surface of bacterial cells.[14] One of the proteins that functions as adhesin is antigen I / II (PAc).[22] Tannins can modify the main protein on the surface of *S. mutans* bacteria, which is called the I / II antigen. I / II antigen is a medium for attaching bacteria to the tooth surface. Interference with I / II antigen on the surface of *S. mutans* can reduce hydrophobicity and interfere with adhesion *S. mutans* on the tooth surface.[14]

The effect of Kulim leaf extract with a concentration of 1.25%, 2.5%, and 5% on the decrease in hydrophobicity of *S. mutans* ATCC 25175 can be seen from the results of the Post-Hoc Least Significance Different (LSD) test, which showed a significant difference between the Kulim leaf extract treatment groups and the negative control. In addition, the effect of Kulim leaf extract with concentrations of 1.25%, 2.5%, and 5% can be seen from the mean hydrophobicity value which was lower than the mean of the negative control group. The ability of the extract to affect the hydrophobicity of bacteria can be seen from the results of the average hydrophobicity value of the extract group with a concentration of 1.25%, 2.5%, and 5%, which were then compared with the mean hydrophobicity value of the positive control group. Kulim leaf extract with concentrations of 1.25%, 2.5%, and 5% had a higher average hydrophobicity value than the positive control. This result indicates that the Kulim leaf extract concentrations of 1.25%, 2.5%, and 5% were not more influential in reducing the hydrophobicity of *S. mutans* ATCC 25175 bacteria compared to the positive control. Based on the results of the Post-Hoc LSD test, there was a significant difference between the 1.25% concentration of the Kulim leaf extract treatment group and the positive control. This result pinpointed that the ability of Kulim leaf extract concentration of 1.25% was significantly different from the positive control. However, the mean percentage of the hydrophobicity value of the Kulim leaf extract treatment group with a concentration of 2.5% and 5% was not significantly different from the positive control as indicated by the results of the Post-Hoc LSD test. Thus, it is clear that the effect of Kulim leaf extract concentration of 2.5% and 5% does not lead to any significant difference between the mean and positive control.

There was no statistically significant difference between the ability of 5% and 2.5% concentration of kulim leaf extract in inhibiting bacterial adherence. However, previous researches revealed that the ability of 5% concentration of kulim leaf extract in inhibiting bacterial adherence is lower than that of 2.5% concentration of extract. This can occur because of the plateau phase which is shown by

the absence of an increase in the ability of the extract to inhibit bacterial adhesion.[23] According to Braga et al., sub-MIC concentrations or lower concentrations may also have the ability to interfere with bacterial cell function in terms of adhesion, hydrophobicity and motility.[24] This study proved that the smallest concentration of 1.25% still had an inhibitory power equivalent to a concentration of 2 levels above it (2.5% and 5%). The results of the LSD Post-Hoc test found that the ability of the kulim leaf extract in inhibiting the adherence of *S. mutans* ATCC 25175 bacteria between concentrations did not have any significant difference. This may be due to the selection of an adjacent test concentration range.

This study used 0.1% chlorhexidine as a positive control and Dimethyl sulfoxide (DMSO) 2% as negative control. Chlorhexidine 0.1% was chosen as the positive control because chlorhexidine is the gold standard anti-plaque mouthwash that can work on both gram-positive and gram-negative bacteria. Besides, chlorhexidine at low concentrations is known to affect the integrity of the bacterial cell wall and inhibit the attachment of bacteria to a surface to prevent the formation of biofilms.[25] DMSO 2% solution was used as a negative control because it was used as a solvent for the extract, which was then diluted to obtain concentrations of 1.25%, 2.5%, and 5%. DMSO was used as a solvent because DMSO can dissolve both polar and non-polar compounds. In addition, DMSO 2% has no effect on bacteria, and thus the results of the reduction in hydrophobicity are not influenced by solvents but because of the activity of compounds contained in Kulim leaf extract.[26]

Streptococcus mutans ATCC 25175, which has been exposed to the active substance contained in Kulim leaf extract is proven to have decreased hydrophobicity and inhibition of adherence. This indicates that the Kulim leaf extract concentrations of 1.25%, 2.5%, and 5% are capable of affecting the hydrophobicity and adhesion of bacteria. The results of the Post-Hoc Least Significance Different (LSD) test showed that the difference in the effect of the three concentrations on the decrease in hydrophobicity and the inhibition of the adherence of *S. mutans* ATCC 25175 was not significant. However, the Kulim leaf concentration of 1.25% had a significant difference in mean with positive control. This demonstrates that the effective concentration that can be used to reduce hydrophobicity and inhibit *S. mutans* attachment is a concentration of 2.5%. The decrease in hydrophobicity causes the bacteria to be unable to adhere to the tooth surface.[14] Bacteria that are not attached to the surface of the teeth will remain in the oral cavity because these bacteria are natural bacteria in the oral cavity or are called microflora. This causes re-infection in the human oral cavity unavoidable. However, these bacteria do not have the same activity as when they are in the environment that forms plaque. Therefore, prevention of bacterial adhesion through decreased hydrophobicity and inhibition of adhesion needs to be done as a way to inhibit bacterial growth before forming plaque and to reduce the risk of caries.[14,27] Further researches on Kullim leaf as an antibacterial agent are still needed to get an effective concentration to reduce hydrophobicity and bacteria adherence. It is necessary to coculture it with other microbes in oral cavity.

CONCLUSION

The results showed that the concentration of Kulim leaf extract had a considerable effect on hydrophobicity and adherence of *S. mutans* ATCC 25175 bacteria with an effective concentration in reducing hydrophobicity and adherence inhibition of *S. mutans* ATCC 25175 by 2.5%.

ACKNOWLEDGMENT

This study was submitted to Faculty of Dentistry Universitas Gadjah Mada as a part of undergraduate thesis for the degree of Sarjana Kedokteran Gigi of Adhaninggar Ratna Hapsari and Dhe Rifdania Hanalda. The authors declare no conflict of interest with the data contained in the manuscript. The authors would like to express gratitude to laboratory staffs of Unit IV Laboratory of the Faculty of Pharmacy and Integrated Research

Laboratory of the Faculty of Dentistry, Universitas Gadjah Mada for their support in this reseach.

REFERENCES

1. Ministry of Health Republic Indonesia. Basic Health Survey. Jakarta: Ministry of Health Republic Indonesia; 2014. Available online at http://labdata.litbang.kemkes.go.id/images/download/laporan/RKD/2013/Laporan_riskesdas_2013_final.pdf
2. Ministry of Health Republic Indonesia. Basic Health Survey. Jakarta: Ministry of Health Republic Indonesia; 2014. Available online at http://repository.bkpk.kemkes.go.id/3514/1/Laporan%20Riskesdas%202018%20Nasional.pdf
3. Ligali TO, Orenuga OO, Oredugba FA. Caries impact on quality of life among visually impaired adolescents: A cross-sectional study. Spec Care Dentist. 2020; 40(2): 184-191. doi: 10.1111/scd.12447
4. Rashkova M, Peneva M, Doychinova L. Study of the risk factors for the development of dental caries and creation of a system for assessment the risk of caries in children in Bulgaria. OHDMBSC. 2008; 7(2): 3–11.
5. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, Abaranches J, Bady LJ. The biology of Streptococcus mutans. Microbiol Spectr. 2019; 7(1): 1-26. doi: 10.1128/microbiolspec.GPP3-0051-2018
6. Tortora GJ, Funke BR, Case CL. Microbiology: An Introduction Elevent Edition. Boston: Pearson; 2013. 160-161, 432, 713.
7. Haniastuti T. Penurunan hidrofobisitas permukaan sel bakteri plak gigi setelah dipapar rebusan daun sirih merah konsentrasi 10%. Dentika Dental Journal. 2016; 19(1): 38-41.
8. Attamimi FA, Ruslami R, Maskoen AM. Uji aktivitas antibakteri ekstrak kasar umbi sarang semut (Myrmecodia pedens) dibanding dengan klorheksidin terhadap Streptococcus sanguinis. MKB. 2017; 49(2): 94-101. doi: 10.15395/mkb.v49n2.1053
9. Simanjuntak P. Studi kimia tumbuhan obat tradisional asal Kalimantan Timur “Bawang Hutan”, Scorodocarpus borneensis Becc”: Suatu Tinjauan Pustaka. Prosiding Seminar Nasional Kimia. 2017; 1-5.
10. Lim TK. Scorodocarpus borneensis. Edible Medicinal and Non-Medicinal Plants; 2016. 10, 77-81.
11. Dewi ZY, Nur A, Hertriani T. Efek antibakteri dan penghambatan biofilm ekstrak sereh (Cymbopogon nardus L.) terhadap bakteri Streptococcus mutans. Majalah Kedokteran Gigi Indonesia. 2015; 1(2): 136-141. doi: 10.22146/majkedgiind.9120
12. Mieher JL, Larson MR, Schormann N, Purushotham S, Wu R, Rajashankar KR, Wu H, Deivanayagam C. Glucan binding protein C of Streptococcus mutans mediates both sucrose-independent and sucrose-dependent adherence. Infect Immun. 2018; 86(7): e00146-00118. doi: 10.1128/IAI.00146-18
13. Hasan S, Danishuddin M, Adil M, Singh K, Verma PK, Khan AU. Efficacy of E. officinalis on the Cariogenic properties of Streptococcus mutans: a novel and alternative approach to suppress quorum-sensing mechanism. PLoS ONE. 2012; 7(7): 1-12. doi: 10.1371/journal.pone.0040319
14. Sungkar S, Agustina D, Supartinah A, Haniastuti T. The effect of jamblang (Syzygium cumini (L) Skeels) leaves ethanolic extract on the adhesion of Streptococcus mutans to hydroxyapatite. International Dental Conference of Sumatera Utara 2017 (IDCSU 2017). 2018; 8: 294-297. doi: 10.2991/idcsu-17.2018.74
15. Pangow ME, Bodhi W, Queljoe E. Skrining fitokimia dan uji toksisitas dari ekstrak etanol daun manggis (Garcinia mangostana L.) dengan metode Brine Shrimp Lethality Test (BSLT). Pharmacon. 2018; 7(3): 97- 209.
16. Musa YM. Isolation and purification of flavonoids from leaves of mitracarpushirtus plant. IOSR Journal of Applied Chemistry. 2015; 8(4): 1-3. doi: 10.9790/5736-08420103

17. Godstime OC, Felix EO, Augustina J, Christopher EO. Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens-a review. J Pharm Chem Biol Sci. 2014; 2(2): 77-85.
18. Arsyada IF, Rianti D, Munadziroh E. Antibacterial activity of mixed pineapple peel (Ananas comosus) extract and calcium hydroxide paste against Enterococcus faecalis. Dental Journal. 2018; 51(1): 20-24. doi: 10.20473/j.djmkg.v51.i1.p20-24
19. Vasconcelos LCS, Sampaio FC, Sampaio MCC, Pereira MV, Higino JS, Peixoto MHP. Minimum inhibitory concentration of adherence of Punica granatum Linn (pomegranate) Gel Against S. mutans, S. mitis, and C. albicans. Braz Dent J. 2006; 17(3): 223-227. doi: 10.1590/s0103-64402006000300009
20. Jeffrey J, Satari MH, Kurnia D. Antibacterial effect of Lime (Citrus aurantifolia) peel extract in preventing biofilm formation. Journal of Medicine and Health. 2019; 2(4): 1020-1029. doi: 10.28932/jmh.v2i4.1841
21. Evendi, A., Uji fitokimia dan antibakteri ekstrak daun salam (Syzygium polyanthum) terhadap bakteri salmonella typhi dan eschericia coli secara in vitro. Mahakam Medical Laboratory Technology Journal. 2017; 2(1): 1-9.
22. Brady LJ, Maddocks SE, Larson MR, Forsgren N, Persson K, Deivanayagam CC, Jenkinson HF. The changing faces of Streptococcus antigen I/II polypeptide family adhesins. Mol Microbiology. 2010; 77(2): 276-286. doi: 10.1111/j.1365-2958.2010.07212.x
23. Tooy DC, Bernadus JB, Sorisi A. Deteksi plasmodium falciparum dengan menggunakan metode real-time polymerase chain reaction di daerah Likupang dan Bitung. PAAI eBiomedik. 2016; 4(1): 1-9. 10.35790/ebm.v4i1.11057
24. Braga PC, Sasso MD, Sala MT. Sub-MIC concentrations of cefodizime interfere with various factors affecting bacterial virulence. J Antimicrob Chemother. 2000; 45(1): 15-25. doi: 10.1093/jac/45.1.15
25. Kumar SB. Chlorhexidine mouthwash- a review. J. Pharm. Sci. & Res. 2017; 9(9): 1450-1452.
26. Mahendra I, Wardani I, Rochyani L. Daya antibakteri ekstrak ikan teri jengki (Stolephorus insularis) terhadap Enterococcus Faecalis. Denta Jurnal Kedokteran Gigi. 2018; 12(2): 106-116.
27. Yue J, Yang H, Liu S, Song F, Guo J, Huang C. Influence of naringenin on the biofilm formation of Streptococcus mutans. Journal of Dentistry. 2018; 76(2018): 24-31. doi: 10.1016/j.jdent.2018.04.013