Majalah Kedokteran Gigi Indonesia
Vol 9 No 1 – April 2023
ISSN 2460-0164 (print), ISSN 2442-2576 (online)
Available online at https://jurnal.ugm.ac.id/mkgi
DOI: http://doi.org/10.22146/majkedgiind.83283

RESEARCH ARTICLE

Comparison Effect of Leaves and Bark Extract of Eucalyptus (Melaleuca leucadendra), Sappan (Caesalpinia sappan), and Cinnamon (Cinnamomum zeylanicum) to Reduce Streptococcus mutans Biofilm Formation

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Submitted: 4th November 2022; Revised: 25th March 2023; Accepted: 26th March 2023

ABSTRACT

To evaluate the effect of eucalyptus, sappan, and cinnamon leaf and bark extract on the percentage of *S. mutans* biofilm formation. The test group was divided into a negative control (1% Dimethyl sulfoxide), a treatment group (eucalyptus, sappan, and cinnamon leaves and bark at concentrations of 50%, 25%, and 12.5%, respectively), and a positive control (0.2% chlorhexidine). Each concentration of eucalyptus, sappan, and cinnamon leaves and barks extract, Brain Heart Infusion Broth (BHI-B), bacteria according to the McFarland 0.5 standard, a positive control, and a negative control were added to a 96-well microplate. They were incubated at 37 °C for twenty-four hours before being rinsed with phosphate buffer saline (PBS) and stained with 0.1% crystal violet. The optical density was then measured using a microplate reader with a 540 nm wavelength. The absorbance value is then factored into the percentage of bacterial adhesion inhibition formula. A statistical test revealed a significant difference ($p < 0.05$) in the percentage of inhibition between the three extracts of leaves and bark and the negative control group (1% DMSO). There is a significant difference between all leaves and barks concentration and the positive control group except the 50% concentration of sappan leaf group and 50% concentration of cinnamon leaf group. The sappan leaf and cinnamon leaf at 50% concentration exhibit no significant difference ($p > 0.05$) with the positive control. Eucalyptus, sappan, cinnamon leaves and barks at a concentration of 50% demonstrated effectiveness of the extractant in inhibiting the formation of biofilm masses by *S. mutans* in comparison with the other group.

Keywords: biofilm; caries; cinnamon; eucalyptus, sappan; *S. mutans*

INTRODUCTION

The main oral health problem in Indonesia is dental cavities or caries. According to Indonesian Basic Health Research (Riskesdas) data in 2013 and 2018 it was seen that the DMF-T index for ages 35-44 years increased from 5.4 to 7.02.[1,2] Untreated cavities can have a negative impact on a person's quality of life by limiting their ability to chew, delaying their ability to grow, and impairing their cognitive abilities.[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] Acidogenic and aciduric bacteria including *Streptococcus mutans* (*S. mutans*) further convert carbohydrate to organic acids, so establishing a low-pH microenvironment in the biofilm. This low-pH microenvironment apparently causes dental caries by causing dissolution of the tooth mineral components.[5]

In dentinal caries, the bacteria *S. mutans* American Type Culture Collection (ATCC) 25175 can be found. The ATCC is a bacterium used for laboratory research. The use of these bacteria as the gold standard in research is because these bacteria have passed very meticulous laboratory procedures so that they can be used as quality control.[6] The *S. mutans* ATCC 25175 bacteria have several genes that function the same as the genes in the oral cavity *S. mutans*, namely the spaP

gene which functions in the adhesion of bacteria to teeth. In addition, *S. mutans* ATCC 25175 also expresses brpA and relA genes which function 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.[7,8]

To inhibit the formation of plaque, antibacterial substances can be applied to surfaces that may serve as plaque growth sites.[7] One of the physical agents commonly used to prevent plaque formation is chlorhexidine, however, its use has several side effects such as tooth staining, dry mouth, and a burning sensation.[9] This side effect of chlorhexidine encourages the development of herbal ingredients as antibacterial agents. Eucalyptus, sappan and cinnamon are some examples of herbal ingredients as antibacterial agents. Both eucalyptus oil (EO) and its primary constituent, 1,8-cineole, possess antibacterial properties that are effective against a wide variety of microorganisms, including Mycobacterium tuberculosis and methicillin-resistant Staphylococcus aureus (MRSA), as well as viruses and fungi (including Candida). Surprisingly for a chemical that kills microbes, it also has actions that are immune-stimulatory, anti-inflammatory, antioxidant, analgesic, and spasmolytic.[10] Meanwhile, sappan is used for wound medicine, bleeding and menstrual functional disorder. It also can be used as sedative agent, dysentery and diarrhea treatment. Cinnamon oil has several benefits such as antimicrobial, antioxidant, antidiabetic and antiallergic effects.[11] Using the natural resources, this study aimed to examine the antibacterial effects of eucalyptus, sappan, and cinnamon barks and leaves by biofilm mass inhibition and to examine the potential of replacing chlorhexidine gluconate as an antibacterial agent in the future. The hypothesis of this study was that both eucalyptus, sappan, and cinnamon barks and leafs can inhibit the biofilm formation by its bioactive compounds through the disturbance in sucrose-dependant pathway.

MATERIALS AND METHODS

The extract was made using the maceration method at the Faculty of Pharmacy of Universitas Gadjah Mada. The collected eucalyptus, sappan, cinnamon leaves, and barks samples were washed under running water and drained. Then the samples were dried in the oven. The leaves and bark were ground into a powder using a blender. The powder was placed in an Erlenmeyer and soaked in 70% ethanol for 72 hours, with intermittent stirring. The products of the soaking were then filtered to produce the filtrate. At a temperature of 35°-40 °C, the filtrate was placed in a vacuum rotary evaporator to evaporate the remaining ethanol until a 100% viscous extract was obtained.

To 8 mL of 1% DMSO was added 4 g of each extract at 100% concentration. Furthermore, the extract was homogeneously dissolved using an ultrasonic instrument. After filtering with 0.45 mm and 0.22 mm Millipore, an extract with a concentration of 50% was obtained. Then as much as 4 mL of 50% concentration of each extract was transferred to a new conical tube. 4 mL of 1% DMSO was added, and the mixture was vortexed to obtain a 25% concentration of each extract. Then, 4 mL of each extract with a concentration of 25% was removed, transferred to a fresh conical tube, and 4 mL of 1% DMSO was added to produce each extract with a concentration of 12.5%.

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. The *S. mutans* bacterial suspension was prepared by taking bacterial colonies using ose sterile then transferred to a conical tube containing BHI broth and incubated for 24 hours. Then the conical tube was centrifuged for 15 minutes at a speed of 3000 rpm. Bacterial colonies were taken using ose sterile 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.

Determination of MIC with microtiter broth method was conducted in flat-bottom 96-well polystyrene microplate filled with 100µl of each extract with concentration of 100% until 6.25%

using serial dilution method. Then in each well was added 100 µL of BHI broth and 100 µL *S. mutans* bacterial suspension. Negative control (DMSO 1%), positive control (Chlorhexidine 0.2%), and blank plate (extracts without bacterial suspension) were included. The subjects then incubated at 37 ºC for 24 hours. All tests were performed in triplicate. Optical Density (OD) readings were taken using a microplate reader at 540 nm. Then the results were reported as the MIC for growth at 24 hours post-inoculation. MIC is minimum concentration of extract that is still able to inhibit the growth of bacteria by 90%.[7] The formula for calculating percent inhibition according to[12] is:

$$\%\ \text{Inhibition} = \left(1 - \left(\frac{OD\ sample - OD\ blank\ sample}{OD\ control - OD\ blank\ control}\right)\right) x\ 100\% \qquad (12)$$

Note:
OD sample: optical density of the extracts group with bacterial suspension
OD blank sample: optical density of the extracts group without bacterial suspension
OD control: optical density of the negative control group with bacterial suspension
OD blank control: optical density of the negative control group without bacterial suspension

The Biofilm mass formation used Crystal Violet Biofilm Assay and carried out using microplate flexible U-bottom PVC 96 wells filled with 100µL of each extract with a concentration of 50%; 25%; and 12.5%. Then in each well was added 90µL of BHI broth containing 2% sucrose and 10µL of *S. mutans* bacterial suspension. The positive control group used 0.2% chlorhexidine gluconate. The negative control group used DMSO 1%. The subjects were then incubated at 37 °C for 24 hours. Then the wells were emptied by removing the media and bacteria that are not attached to the wall of the well. Then the microplate was washed with PBS to remove bacteria that do not adhere to the walls of the well. Furthermore, the microplate was dried and then stained with 200 µL of 0.1% crystal violet and allowed to stand for 15 minutes. The remaining staining is cleaned twice using PBS. The microplate was drained again, and the remaining dye adhering to the cells adhering to the tube walls was cleaned using 200 µL of 100% ethanol. After that, as much as 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 Quave et al., the percentage of biofilm mass formation inhibition was calculated using the formula:[12]

$$\%\ \text{Inhibition} = \left(1 - \left(\frac{OD\ test\ sample}{OD\ control\ sample}\right)\right) x\ 100\%$$

Note:
OD test sample: optical density measurement result of the test group
OD control sample: measurement results of DMSO optical density + bacterial suspension

The findings of the absorbance measurement with a microplate reader at 540 nm were used to calculate the biofilm formation inhibition data. Then, each data point was entered into the formula for the inhibitory percentage of bacterial adhesion. Then, calculations and statistical analysis were performed using SPSS Statistics 26 with a statistical analysis confidence level of 95% (α = 0.05). A one-way ANOVA test was conducted to determine the significance of the effect of the extract concentration of eucalyptus, sappan, and cinnamon leaves and barks on the attachment of *S. mutans* bacteria, followed by a Post Hoc Least Significant Difference (LSD) test to determine the significance of the mean difference between treatment groups.

RESULTS

Microplate 96-wells containing eucalyptus, sappan, cinnamon extracts, negative control, and positive control stained with crystal violet 0.1% were shown in Figure 1. The data for calculating the mean percentage of biofilm mass formation inhibition in the treatment of eucalyptus, sappan, cinnamon leaves, and bark extract with a concentration of 50%, 25%, 12.5%, negative control, and positive control are shown in Table 1. An overview of the mean percentage of bacterial adherence can be seen in the graph in Table 1 that the negative control group (DMSO 1%)

Majalah Kedokteran Gigi Indonesia. April 2023; 9(1): 81-91
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

showed the lowest percentage of biofilm mass inhibition (0.80%) compared to the other treatment groups. Meanwhile, the highest percentage of bacterial inhibition was owned by positive controls (chlorhexidine gluconate 0.2%) which is 89.78%. The percentage of biofilm mass formation of inhibition of *S. mutans* are shown in Table 2. Graph of mean percentage biofilm mass inhibition of *S. mutans* are shown in Figure 2. The result of one-way ANOVA test are shown in Table 3 and it shown that there are significance differences between the effect of the extract concentration of eucalyptus, sappan, and cinnamon leaves and barks on the attachment of S. *mutans* bacteria ($p < 0.05$). The Post-Hoc LSD test was conducted and the result is

Table 1. Comparison of highest concentration of extract substances for biofilm mass formation inhibition Of *S. mutans* ATCC 25175 Bacteria

Treatment		Biofilm Mass Inhibition (%)	
		Avg*	Std**
Positive Control		89.78	0.45
Negative Control		0.80	6.39
Extract of 50% leaf	Eucalyptus	76.59	4.37
	Sappan	83.88	1.67
	Cinnamon	75.37	3.86
Extract of 50% Bark	Eucalyptus	76.26	0.54
	Sappan	82.79	2.40
	Cinnamon	82.82	5.30

*Avg : Average ; **Std : Standard deviation

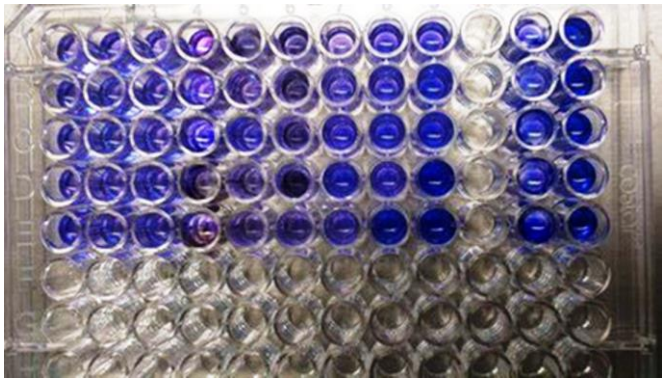
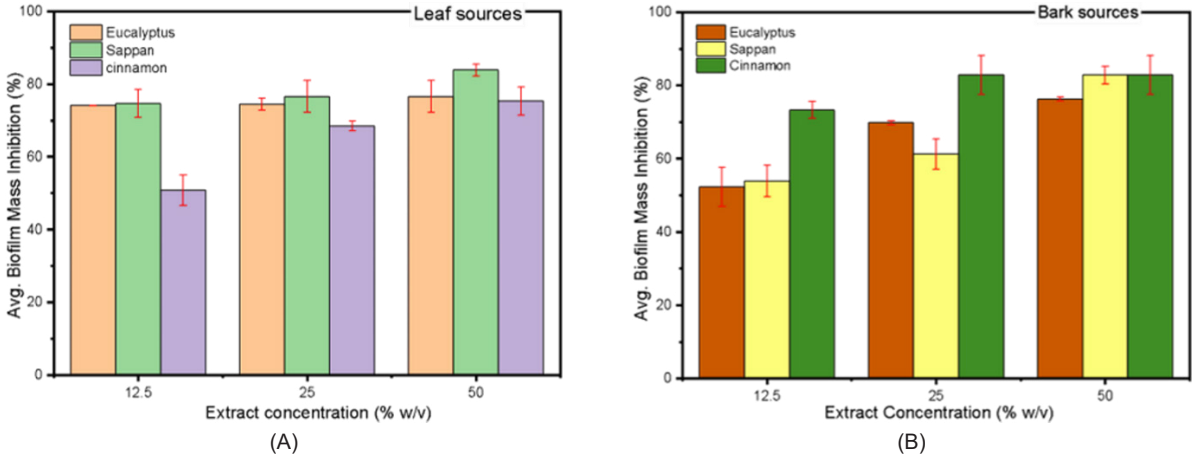


Figure 1. Microplate 96-wells containing eucalyptus, sappan, cinnamon extracts, negative control, and positive control stained with crystal violet 0.1%



(A) (B)

Figure 2. Percentage of biofilm mass formation inhibition of *S.Mutans* ATCC 25175 Bacteria Leaf (A) and Bark (b) of different plant

Table 2. Mean and standard deviation of biofilm mass formation inhibition of S. Mutans ATCC 25175 Bacteria by leaf extracts of different plants

Group	n	Biofilm Mass Inhibition (%)	p- value	Post-Hoc										
				PC	NC	EL1	SL1	CL1	EL2	SL2	CL2	EL3	SL3	CL3
PC	3	89.78±0.45	0.000	-	0.000	0.092	0.149	0.091	0.001	0.003	0.000	0.001	0.002	0.000
NC	3	0.80±6.39	0.000	0.000	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EL1	3	76.59±4.37	0.000	0.092	0.000	-	0.792	0.995	0.045	0.128	0.024	0.045	0.098	0.011
SL1	3	83.88±1.67	0.000	0.149	0.000	0.792	-	0.786	0.046	0.780	0.013	0.026	0.099	0.006
CL1	3	75.37±3.86	0.000	0.091	0.000	0.995	0.786	-	0.046	0.130	0.025	0.076	0.099	0.011
EL2	3	74.44±1.62	0.000	0.001	0.000	0.045	0.046	0.046	-	0.593	0.770	0.797	0.698	0.531
SL2	3	76.58±4.34	0.000	0.003	0.000	0.128	0.780	0.130	0.593	-	0.411	0.593	0.883	0.251
CL2	3	68.43±1.31	0.000	0.000	0.000	0.024	0.013	0.025	0.770	0.441	-	0.781	0.499	0.737
EL3	3	74.09±0.04	0.000	0.001	0.000	0.045	0.026	0.076	0.797	0.593	0.781	-	0.698	0.380
SL3	3	74.62±3.81	0.000	0.002	0.000	0.098	0.099	0.099	0.698	0.883	0.499	0.698	-	0.315
CL3	3	50.82±4.15	0.000	0.000	0.000	0.011	0.006	0.011	0.531	0.251	0.737	0.380	0.315	-

PC = Positive control group (Chlorhexidine gluconate 0.2%)
NC = Negative control group (DMSO 1%)
EL1 = Eucalyptus leaf extract in 50% concentration
SL1 = Sappan leaf extract in 50% concentration
CL1 = Cinnamon leaf extract in 50% concentration
EL2 = Eucalyptus leaf extract in 25% concentration
SL2 = Sappan leaf extract in 25% concentration
CL2 = Cinnamon leaf extract in 25% concentration
EL3 = Eucalyptus leaf extract in 12.5% concentration
SL3 = Sappan leaf extract in 12.5% concentration
CL3 = Cinnamon leaf extract in 12.5% concentration

there are significant difference between the effect of of the extract concentration of eucalyptus, sappan, and cinnamon leaves and barks on the attachment of S. *mutans* bacteria with the negative control ($p < 0.05$). However, the test also conducted that there are no significant differences between them with the positive control ($p < 0.05$).

DISCUSSION

Caries can be defined as the localized deterioration of the hard tissues of the teeth by bacterial fermentation of sucrose-containing foods. Caries can occur preceded by the formation of a biofilm. The formation of biofilm includes three stages, which are the formation of a thin layer on the surface of the tooth or pellicle, attachment of bacteria, then the maturation of biofilm.[13] *S. mutans* is the primary colonizer of caries and one of the most found bacteria in the oral environment.[14,15] *S. mutans* has an enzyme called glucosyltransferase (GTF). There are three types of these enzymes found to play a role in causing caries disease, which are type B, C, and D. Type B is responsible for metabolizing sucrose to water-insoluble glucan. This glucan is the core of Extracellular Polymer Matrix (EPM) or biofilm mass. Meanwhile, type C catalyzes the synthesis of a mixture between water-insoluble glucan and alkaline-soluble glucan which is required for plaque or mass formation. Type B has physical properties, that is hydrophilic or polar, and plays a role in bacterial colonization, and type C has hydrophobic or non-polar physical properties and plays more important roles in the attachment between bacteria and the salivary pellicle of teeth. Type D has hydrophilic or polar physical substances and is responsible to produces water-soluble glucan and acts as a primer for glucan produced by type B. Also, this

Majalah Kedokteran Gigi Indonesia. April 2023; 9(1): 81-91
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

Table 3. Mean and standard deviation of biofilm mass formation inhibition of S. Mutans ATCC 25175 bacteria by bark extracts of different plants

Group	n	Biofilm Mass Inhibition (%)	p- value	Post-Hoc										
				PC	NC	EB1	SB1	CB1	EB2	SB2	CB2	EB3	SB3	CB3
PC	3	89.78±0.45	0.000	-	0.000	0.003	0.004	0.002	0.000	0.000	0.000	0.000	0.000	0.000
NC	3	0.80±6.39	0.000	0.000	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EB1	3	76.26±0.54	0.000	0.003	0.000	-	0.920	0.786	0.059	0.000	0.025	0.000	0.000	0.000
SB1	3	82.79±2.40	0.000	0.004	0.000	0.920	-	0.710	0.048	0.000	0.020	0.000	0.000	0.000
CB1	3	82.82±5.30	0.000	0.002	0.000	0.786	0.710	-	0.100	0.000	0.044	0.000	0.000	0.000
EB2	3	69.80±0.53	0.000	0.000	0.000	0.059	0.048	0.100	-	0.015	0.677	0.000	0.000	0.000
SB2	3	61.28±4.19	0.000	0.000	0.000	0.000	0.000	0.000	0.015	-	0.038	0.011	0.032	0.004
CB2	3	82.82±5.30	0.000	0.000	0.000	0.025	0.020	0.055	0.677	0.038	-	0.000	0.000	0.000
EB3	3	52.24±5.31	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	-	0.620	0.668
SB3	3	53.87±4.26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.620	-	0.358
CB3	3	73.27±2.31	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.004	0.668	0.358	-

PC = Positive control group (Chlorhexidine gluconate 0.2%)
NC = Negative control group (DMSO 1%)
EB1 = Eucalyptus bark extract in 50% concentration
SB1 = Sappan bark extract in 50% concentration
CB1 = Cinnamon bark extract in 50% concentration
EB2 = Eucalyptus bark extract in 25% concentration
SB2 = Sappan bark extract in 25% concentration
CB2 = Cinnamon bark extract in 25% concentration
EB3 = Eucalyptus bark extract in 12.5% concentration
SB3 = Sappan bark extract in 12.5% concentration
CB3 = Cinnamon bark extract in 12.5% concentration

glucan can be metabolized by other bacteria present in the biofilm mass.[4,16]

The ability of these three plant extracts in inhibiting the biofilm mass formation of *S. mutans* is due to the content of active substances in them. The process of making extract was carried out by the maceration method using ethanol 70% solution. This method is the most used for extraction because it has a simple and cheap process. Ethanol solution is an organic solution that is safer than methanol and acetone. Ethanol also can trap both polar and non-polar compound active substances of the plants.[17] According to,[18] ethanol with 70% concentration extracts more active substances compared to ethanol 100% because it contains water that can dissolve or extract polar substances. The water contained in the ethanol solution helped the solvent diffusion in the extraction process.

In this study, 0.2% chlorhexidine was used as a positive control and Dimethyl sulfoxide (DMSO) 1% as a negative control. Chlorhexidine 0.2% 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, also chlorhexidine at low concentrations is known to affect the integrity of the bacterial cell wall and inhibit the attachment of bacteria to a surface so that prevent the formation of biofilms.[19] DMSO 1% 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 50%, 25%, and 12.5%. The use of DMSO as a solvent is because DMSO can dissolve both polar and non-polar compounds. In addition, DMSO < 3% does not affect bacteria, so it can be concluded that the results of the reduction in the formation of biofilm mass are not

influenced by solvents but because of the activity of compounds contained in each plant leaves and barks extract.[20,21]

The result of the biofilm mass formation inhibition test showed that each plant's (eucalyptus, sappan, cinnamon) leaves and barks had the effect of inhibiting biofilm mass formation. Increasing the concentration of extracted substances affected the biofilm formation. Comparison between leaf and bark plants extracted substances had a quite significant value of percentage biofilm mass formation in Table 1. Sappan leaves with a 50% concentration have the highest percentage (83.88%) in the variation of its leaf and bark plants. The sappan leaf and cinnamon leaf at 50% concentration exhibit no significant difference ($p > 0.05$) with the positive control (Chlorhexidine gluconate 0.2%).

The higher percentage of biofilm mass formation for inhibitor by sappan's leaf caused contains an antibacterial active substance which is a flavonoid. Flavonoids are polar compounds that have an antimicrobial response.[22] The other types of antibacterial contained in leaf plants that were extracted are eucalyptol in the eucalyptus leaf and eugenol in cinnamon leaf. The antibacterial substances of leaf and bark plants are different. The α-eusdesmol, Brazzilian, and cinnamaldehyde were found in the eucalyptus, sappan dan cinnamon barks respectively. The activity of antibacterial substances such as flavonoids and eugenol inhibited bacterial infection that is related to their ability to form bonds with proteins before taking a reaction with the lipids.[23] The active functional groups in the substances such as hydroxyl could oxidize/hydrolyze the amino acid side chain. The oxidization of the amino acid side chain from protein will induce disruption or irritation of the bacteria cell membrane.[24] This binding will induce disruption of the bacterial cell wall membrane.[25,26] In addition, flavonoids are also known to have an 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 GTF enzyme so that it inhibits bacterial adhesion.[27,28] Meanwhile, sappan's bark contains brazilin as their most abundant antibacterial active substance. Brazilin is a phenolic color pigment substance of sappan. Besides the antibacterial substances react with the protein, both brazilin and cinnamaldehyde, are due to their ability to dissolve the polar group of the bacterial wall (phosphate), then phospholipid molecule would break down into glycerol, carboxylate acid, and phosphate acid. These changes will cause leakage of the cytoplasm membrane and bacterial growth will be inhibited. Brazilin and eugenol can also dissolve the polar group of the GTF enzyme which is responsible for the biofilm mass formation of the bacteria.[4,11]

Unlike the case of flavonoids and eugenol, eucalyptol is a non-polar substance because the methyl group can enter the aqueous phase then bond with the lipid component of the bacterial cell membrane and cause leakage of the cell membrane.[29] This will cause the decrease of bacteria's nutrition intake and inhibit bacterial growth.[30] Eucalyptol can also bond with the nonpolar group of the GTF enzyme, and it can destroy the structure of this enzyme. This will disrupt the ability of biofilm mass formation of the bacteria.[4,31] α-eudesmol is a eudesmane sesquiterpenoid and has a non-polar physical property.[32] The mechanism of antibacterial activity of this substance like eugenol [33] which is by adhering to the cell membrane of the bacteria and the nutritional intake of the bacteria will be decreased. This activity causes the osmotic tension of the bacteria increasing and then the bacteria cell membrane will be destroyed. and the nutritional intake of the bacteria will be decreased.[34] Eugenol is a nonpolar phenylpropanoid aromatic substance.[35] This will cause the cell membrane of bacteria will be damaged and inhibiting bacterial respiration.[33] Eugenol could damage the GTF enzyme by using the methyl group of eugenols to bond with a nonpolar group of the GTF enzyme.[31]

The result of the research on the biofilm mass formation of *S. mutans* ATCC 25175 showed that each of the leaves and bark extract can inhibit the formation of biofilm mass. The significant difference between the treatment group (leaves and bark extract) and the negative control (DMSO 1%) showed that there is a significant

difference of mean between these two groups. The sappan's leaf and cinnamon's leaf had similarities namely they have hydrophilic components as the major constituents.[25,36] According to,[3] extract that has hydrophilic or polar physical properties, are more effectively inhibits biofilm mass formation of *S. mutans*, because they can bind the hydrophilic GTF type B which is responsible for bacteria colonization due to its ability to metabolize the core of biofilm mass or Extracellular Polymer Matrix and responsible for bacterial colonization and inhibit the formation of biofilm mass. The sappan's bark 50% not as effective as its leaf, probably because brazilin in sappan's bark is more unstable than flavonoids contained in sappan's leaves and cannot be extracted as perfectly as its leaf.[36] Eucalyptus's leaf has a higher percentage than its bark is probably due to its major substances. Eucalyptol in its leaf has higher antibacterial activities than α-eudesmol in its bark, therefore eucalyptus's leaf has higher antibacterial activities compared to eucalyptus's bark.[34] The previous research is also have a similar statement that the sappan leaf extracts has antibacterial activity towards *S.mutans* bacteria due to its synergistic effects of Brazilin and the other existing compound in sappan leaf.[37]

Bacteria that are not attached to the surface of the teeth will remain in the oral cavity because these bacteria are natural 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 biofilm mass.[38] Therefore, prevention of bacterial colonization and adhesion through inhibition of biofilm mass formation needs to be done so that bacterial growth to form biofilm mass is inhibited and reduces the risk of caries.[39] Plants are considered as a rich natural resources of anti-quorum sensing agent. The anti-biofilm mechanism of *S. mutans* has the possibility of being related to anti-quorum sensing pathway.[40]

The antimicrobial properties of eucalyptus, sappan, and cinnamon should be investigated further in order to identify their active constituents. Additionally, research should be conducted on the bacteriostatic and bactericidal effects of eucalyptus, sappan, and cinnamon on other significant clinical pathogens.

CONCLUSION

The potential of sappan, eucalyptus, and cinnamon leaves and bark as biofilm formation inhibitors was evaluated. The result of the biofilm mass formation inhibition test showed that each plant's (eucalyptus, sappan, cinnamon) leaves and barks had the effect of inhibiting biofilm mass formation. Increasing the concentration of extracted substances affected the biofilm formation. Eucalyptus, sappan, cinnamon leaves and barks at a concentration of 50% demonstrated effectiveness of the extractant in inhibiting the formation of biofilm masses by *S. mutans* in comparison with the other group.

ACKNOWLEDGEMENTS

The data of leaves extract sourced from Bernadetha Nathania Ekananda's thesis in 2021 from Universitas Gadjah Mada with the title "The effect of Eucalyptus leaf extract (*Melaleuca leucadendra*), Sappan leaf extract (*Caesalpinia sappan*) and Cinnamon leaf extract (*Cinnamomum Zeylanicum*) against biofilm mass inhibition of *Streptococcus mutans* bacteria ATCC 25175".

CONFLICT OF INTEREST

The authors declare no conflict of interest with the data contained in the manuscript.

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