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Mobe leaf (*Artocarpus lakoocha Buch. Ham*) ethanol extract's antibacterial activity on *Streptococcus mutans* cell membrane leakage and biofilm formation: An in vitro study

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ABSTRACT

Background: *Using fixed orthodontic appliances inhibits oral hygiene, which can lead to the increased development of biofilms and Streptococcus mutans, a cariogenic bacterium that is well known for causing dental caries, derived from bacteria of the Streptococcus genus. In order to decrease biofilm and the degree of cariogenic bacteria in the oral cavity, a variety of herbal ingredients are used. Among these, mobe (Artocarpus lakoocha Buch. Ham) leaves are herbal ingredients with antibacterial properties.* ***Purpose:*** *This study aimed to investigate the antibacterial activity, antibiofilm, and leakage of DNA and protein from mobe leaves.* ***Methods:*** *The diffusion method was used to assess antibacterial activity and determine the minimum inhibitory concentration. The antibiofilm activity was evaluated with Ultraviolet–visible (UV-Vis) spectrophotometry (600 nm), using violet crystal staining. The detection of DNA and protein leakage was carried out by checking for absorbance values using the UV-Vis spectrophotometry (260 nm and 280 nm). An increase in the absorbance value in the measured cell indicated an increase in the level of cell content produced by the cell. One-way analysis of variance was used statistically analyze the results of this study ($P < 0.05$).* ***Results:*** *Mobe leaf (A. lakoocha) extract's minimum inhibitory concentration level was 3.125 mg/ml, the effective concentration of ethanol extract for inhibiting biofilm formation was 3.125 mg/ml, and the effective concentration of ethanol extract that could cause DNA and protein leakage was 50 mg/ml.* ***Conclusions:*** *Mobe leaf extract has good MIC for S. mutans.*

Keywords: *antibacterial; dentistry; DNA and protein leakage; medicine; Mobe leaves*
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INTRODUCTION

Orthodontic treatment is a prolonged process that addresses a patient's aesthetic and functional teeth problems.[1–3] Orthodontic treatment for managing malocclusion is divided into removable and fixed orthodontic applications.[4–6] Treatment using a fixed orthodontic appliance reduces oral hygiene and increases biofilm retention area.[7–9] Conversely, removable orthodontic appliances provide more convenience for maintaining oral hygiene because they can be removed and cleaned.[9] Using a fixed orthodontic appliance not only increases the presence of biofilm but also increases cariogenic bacteria, i.e., *Streptococcus mutans (S. mutans)*.[7,8,10–14] Patients that receive treatment with orthodontic appliances typically indicate a high level of untreated *S. mutans*.[12] The prevalence of *S. mutans* is typically higher in all orthodontic wires compared with other bacteria.[10]

S. mutans bacteria can convert sucrose into lactic acid, which reduces oral pH to a critical level (5.5), which is slightly acidic. This causes demineralization of the enamel and will proceed to develop dental caries.[14,15] Caries occur when the remineralization process becomes slower than the demineralization process, which causes continuous mineral loss.[13,14,16]

The factors that cause caries in the use of fixed orthodontic appliances are include the host or individual patient characteristics (teeth and saliva), substrate, microorganism, and time.[17] Hosts with fixed orthodontic appliances provide retention areas for biofilms and

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microorganisms on the teeth, particularly in the bracket, wire, and molar band regions. Saliva has a vital function in changing the pH of the biofilm. An abnormally low flow of saliva will cause the pH to become acidic for a long time after exposure to sugary food. Therefore, a higher saliva flow rate (increased volume) and buffer capacity (bicarbonate buffer system) can neutralize acidic conditions. The presence a substrate on the teeth indicates poor oral hygiene, a greater frequency of eating, the consumption of sticky and sweet foods, and an acidic saliva pH. Plaque accumulation comprises a high population of microorganisms. A constant decrease in a below critical pH over a period of time will lead to the demineralization of teeth and caries.[11,13,14,16,18]

S. mutans bacteria are the main cause of caries, as it plays a vital role in biofilm formation.[1,3,19,20] There are two adhesion mechanisms for *S. mutans*, namely, sucrose-independent adhesion and sucrose-dependent adhesion. Sucrose-independent adhesion is a mechanism through which extracellular sucrose is transformed into glucan by cell wall-associated glucosyltransferase in the presence of sucrose and, together with *glucan binding protein* on the bacterial surface, promotes cells aggregation and the subsequent development of dental biofilms on the tooth surface.[13,20,21] In the absence of sucrose, several surface adhesions mediate the bonding of *S. mutans* or other bacteria in dental plaque to the tooth surface.[15,20,22–24]

Herbal ingredients have been formulated as traditional medicines for thousands of years. Numerous herbal extracts have been shown to reduce the number of cariogenic microbes in the oral cavity.[25] Mobe leaves, a species of *Artocarpus* (*Moraceae* family) and known by the locals of Batak as "mobe", are one of these.[26,27] Mobe leaf indicate having antiviral (*herpes simplex virus* and *human immunodeficiency virus*), anthelmintic, antioxidant, antiglycation, antibacterial, antimalarial, antitubercular, antiplasmodial, antiatherosclerotic, anti-fungal, antidiarrheal, and antidiabetic activity, as well as wound-healing, anti-inflammatory, and anticancer properties.[26,28–31] Mobe leaf extract also comprises antibacterial compounds, namely, artocarpin, *oxyresveratrol*, *phenols, flavonoids,* and tannins.[32]

Artocarpin at twice the minimum inhibitory concentration (MIC) (3.9 g/mL) and at four times the MIC (7.8 g/mL) changed the permeability of the S. mutans's membrane and caused membrane damage to S. mutans after 24 hours.[33] Additionally, 30 mg/ ml of oxyresveratrol from A. lakoocha extract had a bactericidal effect on S. mutans.[25,34] The bacteriostatic effect of phenols (an MIC concentration of 62.5–1000 mg/ml) in coffee is higher than 0.2% chlorhexidine or 0.2% fluoride.[35] The impact of flavonoids on S. mutans American Type Culture Collection (ATCC) 25175 bacteria using Albizia myriophylla wood ethanol extract had an antibacterial effect that was almost comparable to chlorhexidine.[36] The antibacterial effect of tannins on S. mutans bacteria using mimba plants could inhibit biofilm formation.[37]

Some of the identified antibacterial mechanisms in antimicrobial agents include inhibiting bacterial cell wall synthesis, interfering with bacterial cell membranes, inhibiting bacterial cell nucleic acid synthesis, and inhibiting bacterial cell protein synthesis.[38] Defining the mechanism of antibacterial actions from the results of antibacterial tests can be achieved by examining protein compounds, nucleic acids, and metal ions in bacterial membrane/cell wall leaks, as well as morphological analysis of bacterial membranes/ cell walls. Bacterial membrane/cell wall leakage can be determined by examining the proteins, nucleic acids, and metal ions measured using an Ultraviolet–visible (UV-Vis) spectrophotometry. Leakage of the bacterial membrane/cell wall as a result of bacteria is characterized by an increase in absorbance value at a 260 nm wavelength for nucleic acids and a 280 nm wavelength for proteins.[39] This study aimed to investigate the antibacterial activity, antibiofilm, and leakage of DNA and protein from S. mutans as a result of using mobe leaf (Artocarpus lakoocha Buch. Ham) ethanol extract.

MATERIAL AND METHODS

This study tested the *S. mutans* ATCC 25175 bacteria, bred in a tryptone-yeast-cysteine-sucrose-bacitracin (TYCSB) (HiMedia, HiMedia Laboratories Pvt. Ltd, Mumbai, India) medium at the Microbiology Laboratory of the Faculty of Pharmacy, University of North Sumatra.

The ethanol extract of mobe leaf was obtained through maceration with 96% ethanol as a solvent and continued with a rotary vacuum evaporator. Mobe leaf ethanol extract was dissolved and diluted with dimethyl sulfoxide (DMSO) (Merck, Merck KGaA, Darmstadt, Germany) to obtain the concentration used (300 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml) and 0.1% chlorhexidine (control group) (Minosep, PT. Minorock, Depok, Indonesia). The following apparatuses were used: an autoclave (All American 25x, Wisconsin Aluminum Foundry Co., Inc, Manitowoc, Wisconsin), incubator (Memmert, Schwabach, Germany), digital scales (Santorius BSA 323 S – CW, Santorius, Göttingen, Germany), 6 mm paper disc (Macherey-Nagel MN827ATD, Macherey-Nagel GmbH & Co. KG, Düren, Germany), Vortex V-1 plus (Biosan, Riga, Latvia), micropipette (Dragonlab, DLAB Scientific Co., Ltd, Beijing, China), centrifuge (Eppendorf centrifuge 5804, Eppendorf, Hamburg, Germany), UV-Visible spectrophotometer (Thermo Scientific™ Orion™ AquaMate, Thermo Fisher Scientific Inc, Waltham, USA), and glassware from the Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia.

The objective of the antibiotic activity test is to establish the MIC of ethanol extract in mobe leaf using a diffusion method. Petri dishes were filled with a bacterial suspension solution (0.1 mL) to TYCSB agar plate. Sterile paper disks (6 mm) that had been dripped with 300 mg/ml, 200 mg/ml,

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100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.1 mg/ml of *A. lakoocha Buch. Ham* leaves extract, as well as 0.1% chlorhexidine (control group) were placed in petri dishes and incubated at 37°C for 24 hours. The MIC result was determined by measuring the inhibitory zone diameter following incubation for 24 hours.[25,40] The procedure was repeated three times.

The aim of the antibiofilm test is to assess the impact of a mobe leaf extract solution on the development of *S. mutans* bacterial biofilms using violet crystal staining. The suspension solution of *S. mutans* bacteria that had been vortexed was taken using a micropipette and 0.1 ml was placed in TYCSB liquid medium well plate. Well plates were put into an incubator for 24 hours at 37°C. Different mobe leaf extract concentrations (300 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.1 mg/ml), and 0.1% chlorhexidine (control group) were put into 2 ml well plates and incubated for 24 hours at 37°C. The well plates were taken out of the incubator after 24 hours and cleaned with an aqua pro injection, stained with violet crystals, incubated for 30 minutes, and cleaned. Once cleaned, the well plate was filled with ethanol using a syringe. Ethanol was taken from the well plate with a syringe and placed into a spectrophotometric cuvette, and measurements were made using UV-Vis spectrophotometry at a wavelength of 600 nm.

The leaking of cellular metabolites, caused by bacteria via the addition of an extract or active chemical compound, can be measured using a UV-Vis spectrophotometer. *S. mutans* bacteria were taken from the agar medium using a sterile ose wire and put into a reaction tube containing the TYCSB medium to create a bacterial suspension solution. The bacterial suspension solution was removed from the incubator and revortexed. Mobe leaf ethanol extract (concentrations of 50 mg/ml, 25 mg/ml, and 3.125 mg/ml) and 0.1 % chlorhexidine were introduced into a 1 ml reaction tube, vortexed, and reintroduced into the incubator for 18–24 hours. The reaction tube was removed from the incubator and placed in the vortex. The reaction tube containing the mobe leaf ethanol extract and chlorhexidine that had been vortexed was inserted into the conical centrifuge tube. The conical centrifuge tube was placed in the centrifuge to separate bacterial cell deposits, extracts, and cell leaks. After centrifuging, a precipitate, separated from the solution, was obtained. The supernatant solution (clear) was taken using a syringe and measured using a UV-Vis spectrophotometer. Leakage of cellular metabolites caused by bacteria was characterized by an increase in absorbance value at a 260 nm wavelength for nucleic acids and a 280 nm wavelength for proteins. The increase in absorbance value in the measured cell indicated an increase in the number of cell content produced by the cell.

The study data are shown in Table 1 and 2 using mean ± standard deviation. The IBM SPSS Statistics for Windows (v.20.0; IBM, Armonk, NY, USA) analysis software was used for this research. In this study, a one-way analysis of variance and a post hoc least significant difference (LSD) test were used for data processing and analysis. Statistical significance was defined as a value of $P < 0.05$.

RESULTS

The highest average value of the bacterial inhibition zone was found in the 300 mg/ml mobe leaf ethanol extract, with an average value of 27 ± 0.361, and the lowest average value was found in the 0.1% chlorhexidine solution, with an average value of 12.267 ± 0.153 (Table 1).

An ethanol extract of mobe leaf at a concentration of 300 mg/ml had the highest percentage of *S. mutans* biofilm inhibition activity, with an average value of 82.04% ± 0.71; the lowest percentage of *S. mutans* biofilm inhibition activity occurred in the 0.1% chlorhexidine with an average value of 50.23% ± 0.153 (Table 2). Table 3 shows the post hoc test data analysis with LSD findings, revealing significant differences between the control groups and all ethanol extracts in terms of *S. mutans* biofilm inhibitory activity ($P < 0.05$). The highest and lowest DNA and protein leaks occurred in the mobe leaf ethanol extract at 50 mg/ml and 3.125 mg/ml concentrations, respectively (Table 2).

Table 1. Diameter of the bacterial inhibition zone (mm) for mobe leaf ethanol extracts against *S. mutans*

Concentration	Mean ± SD
Mobe leaf ethanol extract, 3.125 mg/ml	17.167 ± 0.208
Mobe leaf ethanol extract, 6.25 mg/ml	18.033 ± 0.153
Mobe leaf ethanol extract, 12.5 mg/ml	19.067 ± 0.379
Mobe leaf ethanol extract, 25 mg/ml	20.900 ± 0.361
Mobe leaf ethanol extract, 50 mg/ml	22.633 ± 0.569
Mobe leaf ethanol extract, 100 mg/ml	24.767 ± 0.306
Mobe leaf ethanol extract, 200 mg/ml	25.567 ± 0.379
Mobe leaf ethanol extract, 300 mg/ml	27.000 ± 0.361
Chlorohexidine, 0.1%	12.267 ± 0.153

Table 2. Biofilm inhibition activity, DNA and protein leakage caused by *S. mutans* in mobe leaf ethanol extracts

Concentration	Biofilm inhibition activity (Mean ± SD)	DNA and protein leakage (Mean ± SD)	
		DNA leakage	Protein leakage
Mobe leaf ethanol extract, 3.125 mg/ml	50.23 % ± 1.62 %	0.0767 ± 0.00306	0.0873 ± 0.00379
Mobe leaf ethanol extract, 25 mg/ml	69.87 % ± 1.36 %	0.5263 ± 0.00404	0.6227 ± 0.00651
Mobe leaf ethanol extract, 50 mg/ml	82.04 % ± 0.71 %	0.7503 ± 0.00808	1.0033 ± 0.12342
Chlorohexidine, 0.1%	72.60 % ± 1.09 %	0.133 ± 0.004	0.0717 ± 0.00874

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Table 3. The post hoc LSD biofilm, DNA, and protein leakage caused by *S. mutans*

Concentration	3.125			25			50			Chlorohexidine, 0.1%		
	B	D	P	B	D	P	B	D	P	B	D	P
Mobe leaf ethanol extract, 3.125 mg/ml	-	-	-	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
Mobe leaf ethanol extract, 25 mg/ml	0.000*	0.000*	0.000*	-	-	-	0.000*	0.000*	0.000*	0.027*	0.000*	0.027*
Mobe leaf ethanol extract, 50 mg/ml	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	-	-	-	0.000*	0.000*	0.000*
Chlorohexidine, 0.1%	0.000*	0.000*	0.765	0.027*	0.000*	0.000*	0.000*	0.000*	0.000*	-	-	-

* B = Biofilm, D = DNA, P = Protein

The post hoc test data analysis with LSD findings in Table 3 shows significant differences in *S. mutans* DNA leakage for all mobe leaf ethanol extracts and control group ($P < 0.05$). The post hoc test data analysis results with LSD findings in Table 3 show that there were insignificant differences in *S. mutans* protein leakage between the ethanol extract group with 3.125 mg/ml and the control group ($P < 0.05$).

DISCUSSION

The use of orthodontic devices inhibits oral cavity hygiene and increases retention area for biofilms, increases the presence of *S. mutans*, and continuously decreases the pH below a critical level over a period of time, resulting in the demineralization of teeth and leads to the formation of caries.[7,13,16,18]

Currently, several herbal extracts are being developed that are believed to reduce the level of cariogenic microorganisms in the oral cavity. The extraction effectiveness of a compound is dissolved in the same polarity, under the principle, i.e., a compound is dissolved in the same solvent with the same properties. Jiratanakittiwat et al.[41] showed that an ethanol solvent is the best diluent for getting the best extraction results; a higher ethanol content and a longer time extraction period will give rise to a higher *oxyresveratrol* content.[42] In this research, an ethanol solvent was used for ethanol extraction using mobe leaves.

The extraction results produced an ethanol extract from mobe leaf that was diluted to several concentrations, i.e., 300 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.1 mg/ml and tested for antibacterial activity to obtain the data on minimum inhibitory levels. According to Ouchari et al.,[43] antibacterial power based on the diameter of the divided inhibition zone can be classified as very strong (inhibition zone of more than 20 mm), strong (inhibition zone of 10–20 mm), medium (inhibition zone of 5–10 mm), and weak (inhibition zone less than 5 mm).

According to the findings of this study's antibacterial activity test, an ethanol extract from mobe leaf at a concentration of 3.125 mg/ml was effective in preventing the growth of *S. mutans* bacteria, with a mean inhibition zone diameter of 17.167 ± 0.208, and its inhibition power have the same effect with 0.1% chlorhexidine (the control group) with an average inhibition zone diameter value of 12.267 ± 0.153. The antibacterial activity test of *S. mutans* at these extract concentrations was strong enough to create a large barrier zone. Teanpaisan et al.[30] discovered that chlorhexidine and aqueous mobe leaf extracts had an effect in killing *S. mutans* at an MIC of 0.1 mg/ml. Study findings by Pumpaluk et al.[25] showed that the inhibition zone obtained from a mobe leaf ethanol extract against *S. mutans* bacteria (24.2 ± 0.58 mm) almost compensated for the inhibition zone obtained from chlorhexidine (29 mm).

The antibacterial activity test results showed that the mobe leaf ethanol extract had dose-dependent activity, which was reflected by the increasing in concentration following an increase activity. This was also the case in the biofilm inhibitory activity test results. The higher the concentration, the higher the antibacterial power and antibiofilm would be. The biofilm inhibitory activity test conducted for this study showed effectiveness as an antibiofilm against *S. mutans* bacteria.

The findings of this study showed that biofilm inhibition with 25 mg/mL mobe leaf ethanol extract effectively inhibited *S. mutans* biofilm formation and almost same with the effects of 0.1% chlorhexidine. Wu et al.[34] showed that the *S. mutans* biofilm was reduced, along with lactic acid production, and the resistance to acid (acidity) was reduced by 20–30%, while an increase in *oxyresveratrol* concentration occurred. This was because *oxyresveratrol*, a primary ingredient of mobe leaf extract, inhibited *S. mutans* by altering the metabolism of sucrose.

Sucrose is primarily used by *oxyresveratrol* to synthesize water-soluble or decomposed glucans into lactic acid. A decrease in water-insoluble glycan production leads to a weakening of the biofilm structure and its adhesion. Damage to the biofilm structure reduces the adverse conditions caused by bacteria, namely, a low pH in the oral cavity. The research results presented by Teanpaisan et al.[30] show that mobe leaf ethanol extract can act as a dual-function antibiofilm, namely, preventing the formation of biofilm and inhibiting existing biofilm.

Oxyresveratrol may also affect the integrity of bacterial cell walls. In the presence of using mobe leaf ethanol

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extract, *S. mutans* showed an irregular cell wall structure. The reason for this is likely that gram-positive bacteria have a thicker peptidoglycans layer. Gram-positive bacteria have teichoic acid covalently attached to peptidoglycans. This teichoic acid is hydrophilic (soluble in water) and functions as a positively charged ion transport medium for entering and exiting the cell wall. Gram-positive bacteria cell walls are more polarized as a result of this water-soluble property, making them more permeable to antibacterial compounds. The incoming antibacterial compound heightens osmotic pressure in the cell, enabling the bacteria to lyse.[44]

The following are also present in mobe leaf ethanol extracts: flavonoids, tannins, and phenols, which have strong antimicrobial activity. Flavonoid compounds work against bacteria in a number of ways, e.g., preventing the production of nucleic acids, the function of cytoplasmic membranes, preventing bacteria from using oxygen for energy metabolism, and preventing the synthesis of cell membranes by forming intricate compounds with extracellular proteins.[42,45–48] Joycharat et al.[49] studied the antibacterial effect of flavonoids on *S. mutans ATCC* 25175 bacteria using an *Albizia myriophylla* wood ethanol extract. The results showed that *lupinifoline* (from the flavonoid group) had an antibacterial effect that was almost comparable to 0.5 µg/ml chlorhexidine.

Artocarpin inhibits *S. mutans* by changing the permeability of the bacterial membrane, resulting in the release of intracellular material, including nucleic acids as an indicator of cell damage. Septama et al.[33] showed that artocarpin twice the MIC (3.9 g/mL) and four times MIC (7.8 g/mL) changed membrane permeability and caused cell membrane damage in *S. mutans* after 24 hours.

Tannins combine with proteins to form complex covalent and noncovalent compounds that can cross cell membranes, inhibit enzymes like glucosyltransferases, and directly activate microorganisms.[50] The antibacterial effect of tannins on *S. mutans* bacteria was studied by Sudhakar et al.[51] using mimba plants, and the results showed that tannins possessed antibacterial properties and could inhibit biofilm formation.

Phenol eliminates bacteria by denaturing cell proteins so that the permeability of cell walls and cytoplasmic membranes become unbalanced, leading to cell lysis.[21,38,46] Lysis in the cytoplasmic membrane prevents cell growth and cause cell death, the denaturation of proteins and nucleic acids, and the inhibition of protein synthesis.[38,44,46] The antibacterial (bacteriostatic) effect of phenols on *S. mutans* bacteria using a coffee extract was higher than 0.2% chlorohexidine or 0.2% fluoride.[35]

Defining the antibacterial mechanism from the results of antibacterial tests can be done by, among others, analyzing the protein compounds, nucleic acids, and metal ions in bacterial membrane/cell wall leaks, and morphological analysis of bacterial membranes/cell walls. Examining proteins, nucleic acids, and metal ions like Ca2+ and K+ can be used to identify bacterial membrane/cell wall leaks. Leakage due to cell damage can be detected by using absorbance values at 260 nm and 280 nm wavelengths to determine DNA and protein leakage using a UV/VIS spectrophotometer.[39]

According to this study's findings, a 25 mg/ml concentration of mobe leaf ethanol extract can cause protein leakage (abs 280 nm), and a concentration of 3.125 mg/ml and 0.1% chlorhexidine showed the same effectiveness against *S. mutans* bacteria in the protein leakage test (abs 280 nm). This was shown in the way chlorhexidine and mobe leaf ethanol extract at a concentration of 3.125 mg/ml inhibited bacteria, where both interfered with the integrity of the bacterial cell membrane to cause cell leakage and bacterial cells to die. The most effective mobe leaf ethanol extract concentration (causing the highest levels of DNA and protein leakage) was 50 mg/mL.

One cause of bacterial death is cell leakage, which can be analyzed by looking at the number of cell components that released after adding a mobe leaf ethanol extract (3.125 mg/mL, 25 mg/mL, and 50 mg/mL). The increased amount of DNA and protein that leaked from the cell after adding a mobe leaf ethanol extract (3.125 mg/mL, 25 mg/mL, and 50 mg/mL) indicated that there were cytoplasmic leaks in the bacterial cells. The increase in absorbance value in the measured cell indicated an increase in the number of cell content produced by the cell. In some research that employed several concentrations of extract, the greater the concentration used, the more effective the results were.[45] However, it is important to note that this study's scope was limited by factors such as the temperature of the mobe leaf ethanol extract solution, and bacteria that may have been exposed to well plate when the sample was transferred to a sterile area.

It can be inferred that a mobe leaf ethanol extract (*A. lakoocha*) inhibits the growth and formation of an *S. mutans* bacterial biofilm. The minimum inhibitory concentration of mobe leaf ethanol extract was 3.125 mg/ml. The most effective concentration for inhibiting biofilm formation was 3.125 mg/ ml. The ethanol extract obtained from the mobe leaf caused a decreased in biofilm, and DNA and protein leakage of *S. mutans* bacteria. According to this research, the most effective concentration of mobe leaf ethanol extract for causing DNA and protein leakage is 50 mg/ml. The suggestions for future studies are to continue research on the inhibition of sortase A enzyme, fractionation and antibacterial testing, and evaluating the efficacy of extracts derived from other components of the mobe plant, including its stems, roots, seeds, and fruits.

REFERENCES

1. Ardani IGAW, Budipramana M, Rachmawati E, Nugraha AP, Ardana IKKG, Budhy TI, Hassan R, Listyorini D, Sarno R. COL1A1 and FGFR2 single-nucleotide polymorphisms found in Class II and Class III skeletal malocclusions in Javanese population. Eur J Dent. 2023; 17(1): 183–90.
2. Shirozaki MU, Ferreira JTL, Küchler EC, Matsumoto MAN, Aires CP, Nelson-Filho P, Romano FL. Quantification of Streptococcus

DOI: 10.20473/j.djmkg.v56.i4.p226–232

mutans in different types of ligature wires and elastomeric chains. Braz Dent J. 2017; 28(4): 498–503.
3. Mishra S, Routray S, Sahu SK, Nanda SB, Sahu KC. The role and efficacy of herbal antimicrobial agents in orthodontic treatment. J Clin Diagnostic Res. 2014; 8(6): 10–3.
4. Ardani IGAW, Nugraha AP, Vitamamy DG, Gautama S, Alida, Rahmawati D, Hariati IVD, Caesar ADO, Aufa HR, Sugitto MR, Hassan R. Correlation of malocclusion with facial profile in Javanese population: A cephalometric analysis. J Int Dent Med Res. 2023; 16(2): 756–65.
5. Proffit WR, Fields HW, Larson BE, Sarver DM. Contemporary orthodontics. 6th ed. Philadelphia: Mosby Elsevier; 2019. p. 310–51.
6. Cobourne MT, DiBiase AT. Handbok of orthodontics. 2nd ed. Philadelphia: Elsevier; 2016. p. 209–61.
7. Budi HS, Jameel MF, Widjaja G, Alasady MS, Mahmudiono T, Mustafa YF, Fardeeva I, Kuznetsova M. Study on the role of nano antibacterial materials in orthodontics (a review). Brazilian J Biol. 2024; 84: 1–7.
8. Topaloglu-Ak A, Ertugrul F, Eden E, Ates M, Bulut H. Effect of orthodontic appliances on oral microbiota—6 month follow-up. J Clin Pediatr Dent. 2011; 35(4): 433–6.
9. Petrauskiene S, Wanczewska N, Slabsinskiene E, Zemgulyte G. Self-reported changes in oral hygiene habits among adolescents receiving orthodontic treatment. Dent J. 2019; 7(4): 96.
10. Hepyukselen BG, Cesur MG. Comparison of the microbial flora from different orthodontic archwires using a cultivation method and PCR: A prospective study. Orthod Craniofac Res. 2019; 22(4): 354–60.
11. Ren Y, Jongsma MA, Mei L, van der Mei HC, Busscher HJ. Orthodontic treatment with fixed appliances and biofilm formation—a potential public health threat? Clin Oral Investig. 2014; 18(7): 1711–8.
12. Shukla C, Maurya R, Singh V, Tijare M. Evaluation of changes in Streptococcus mutans colonies in microflora of the Indian population with fixed orthodontics appliances. Dent Res J (Isfahan). 2016; 13(4): 309–14.
13. Dianawati N, Setyarini W, Widjiastuti I, Ridwan RD, Kuntaman K. The distribution of Streptococcus mutans and Streptococcus sobrinus in children with dental caries severity level. Dent J. 2020; 53(1): 36–9.
14. Subramaniam SK, Siswomihardjo W, Sunarintyas S. The effect of different concentrations of Neem (Azadiractha indica) leaves extract on the inhibition of Streptococcus mutans (In vitro). Dent J. 2005; 38(4): 176–9.
15. Nuraini P, Pradopo S, Pronorahardjo AS. Sucrose and xylitol-induced Streptococcus mutans biofilm adherence. Pesqui Bras Odontopediatria Clin Integr. 2020; 20: 1–5.
16. Zero DT. Evidence for biofilm acid neutralization by baking soda. J Am Dent Assoc. 2017; 148(11): S10–4.
17. Sunarko SA, Ekasari W, Astuti SD. Antimicrobial effect of pleomeleangustifolia pheophytin A activation with diode laser to streptococcus mutans. J Phys Conf Ser. 2017; 853(1): 012039.
18. Saloom H, Mohammed-Salih H, Rasheed S. The influence of different types of fixed orthodontic appliance on the growth and adherence of microorganisms (in vitro study). J Clin Exp Dent. 2013; 5(1): e36-41.
19. Chhattani S, Shetty PC, Laxmikant S, Ramachandra C. In vitro assessment of photocatalytic titanium oxide surface-modified stainless steel and nickel titanium orthodontic wires for its antiadherent and antibacterial properties against Streptococcus mutans. Singh G, editor. J Indian Orthod Soc. 2014; 48: 82–7.
20. Kriswandini IL, Diyatri I, Putri IA. Density of Streptococcus mutans biofilm protein induced by glucose, lactose, soy protein and iron. Dent J. 2019; 52(2): 86–9.
21. Pribadi N, Yonas Y, Saraswati W. The inhibition of Streptococcus mutans glucosyltransferase enzyme activity by mangosteen pericarp extract. Dent J. 2017; 50(2): 97–101.
22. Hu P, Huang P, Chen MW. Curcumin reduces Streptococcus mutans biofilm formation by inhibiting sortase A activity. Arch Oral Biol. 2013; 58(10): 1343–8.
23. Ahn S-J, Ahn S-J, Wen ZT, Brady LJ, Burne RA. Characteristics of biofilm formation by Streptococcus mutans in the presence of saliva. Infect Immun. 2008; 76(9): 4259–68.
24. Huang P, Hu P, Zhou SY, Li Q, Chen WM. Morin inhibits sortase A and subsequent biofilm formation in Streptococcus mutans. Curr Microbiol. 2014; 68(1): 47–52.
25. Pumpaluk P, Sritularak B, Likhitwitayawuid K, Lapirattanakul J. Antibacterial effect of herbal plants against three cariogenic microorganisms. M Dent J. 2017; 37(1): 71–80.
26. Gautam P, Patel R. Artocarpus lakoocha Roxb: An overview. Eur J Complement Altern Med. 2014; 1(1): 10–4.
27. Povichit N, Phrutivorapongkul A, Suttajit M, Leelapornpisid P. Antiglycation and antioxidant activities of oxyresveratrol extracted from the heartwood of artocarpus lakoocha roxb. Maejo Int J Sci Technol. 2010; 4(3): 454–61.
28. Jagtap UB, Bapat VA. Artocarpus: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 2010; 129(2): 142–66.
29. Saowakon N, Tansatit T, Wanichanon C, Chanakul W, Reutrakul V, Sobhon P. Fasciola gigantica: Anthelmintic effect of the aqueous extract of Artocarpus lakoocha. Exp Parasitol. 2009; 122(4): 289–98.
30. Teanpaisan R, Senapong S, Puripattanavong J. In vitro antimicrobial and antibiofilm activity of Artocarpus lakoocha (Moraceae) extract against some oral pathogens. Trop J Pharm Res. 2014; 13(7): 1149–55.
31. Phoolcharoen W, Sooampon S, Sritularak B, Likhitwitayawuid K, Kuvatanasuchati J, Pavasant P. Anti-periodontal pathogen and a-inflammatory a of oxyresveratrol. Nat Prod Commun. 2013; 8(5): 1934578X1300800.
32. Singhatong S, Leelarungrayub D, Chaiyasut C. Antioxidant and toxicity activities of Artocarpus lakoocha Roxb. heartwood extract. J Med Plants Res. 2010; 4(10): 947–53.
33. Septama AW, Panichayupakaranant P. Artocarpin isolated from Artocarpus heterophyllus heartwoods alters membrane permeability of Streptococcus mutans. J Appl Pharm Sci. 2018; 8(6): 59–64.
34. Wu J, Fan Y, Wang X, Jiang X, Zou J, Huang R. Effects of the natural compound, oxyresveratrol, on the growth of Streptococcus mutans , and on biofilm formation, acid production, and virulence gene expression. Eur J Oral Sci. 2020; 128(1): 18–26.
35. Akhlaghi N, Sadeghi M, Fazeli F, Akhlaghi S, Mehnati M, Sadeghi M. The antibacterial effects of coffee extract, chlorhexidine, and fluoride against Streptococcus mutans and Lactobacillus plantarum: An in vitro study. Dent Res J (Isfahan). 2019; 16(5): 346–53.
36. Lévesque CM, Voronejskaia E, Huang Y-CC, Mair RW, Ellen RP, Cvitkovitch DG. Involvement of sortase anchoring of cell wall proteins in biofilm formation by Streptococcus mutans. Infect Immun. 2005; 73(6): 3773–7.
37. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019; 8(1): 118.
38. Fraise AP, Maillard J-Y, Sattar SA. Russell, Hugo & Ayliffe's: Principles and practice of disinfection, preservation and sterilization. 5th ed. Oxford, UK: Wiley-Blackwell; 2013. p. 5–7.
39. Musini A, Giri A. Investigation of mode of action of anti bacterial activity of Salacia oblonga extract against drug resistant pathogen. Brazilian Arch Biol Technol. 2019; 62: e19180051.
40. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016; 6(2): 71–9.
41. Jiratanakittiwat K, Satirapipathkul C, Charnvanich D. The influences of extraction on the quantity of oxyresveratrol from Artocarpus lakoocha Roxb. Int J Biosci Biochem Bioinforma. 2020; 10(2): 110–6.
42. Juniarti DE, Kusumaningsih T, Juliastuti WS, Soetojo A, Wungsu ND. Phytochemical analysis and antibacterial activity of purple leaf extract [Graptophyllum pictum (L.) Griff] against Streptococcus mutans. Acta Med Philipp. 2021; 55(8): 802–6.
43. Ouchari L, Boukeskasse A, Bouizgarne B, Ouhdouch Y. Antimicrobial potential of actinomycetes isolated from unexplored hot Merzouga desert and their taxonomic diversity. Biol Open. 2018; 8(2): bio035410.

DOI: 10.20473/j.djmkg.v56.i4.p226–232

44. Zaini WS. Antibacterial effectiveness of Morinda citrifolia L. extract on Salmonella typhi bacteria using serial dilution method with 15 - 60 minutes contact time. Pharmacogn J. 2021; 13(4): 839–43.
45. Pargaputri AF, Munadziroh E, Indrawati R. Antibacterial effects of Pluchea indica Less leaf extract on E. faecalis and Fusobacterium nucleatum (in vitro). Dent J. 2016; 49(2): 93–8.
46. Moldenhauer J. Disinfection and decontamination: A practical handbook. Boca Raton: Taylor & Francis: CRC Press; 2018. p. 12–3.
47. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Curr Med Chem. 2014; 22(1): 132–49.
48. Wedagama DM, Wahjuningrum DA, Subiyanto A, Pangestika FW, Guspiari K, Goenharto S, Gopikrishna V. Antibacterial activity of Red Pine (Pinus densiflora) and Sumatran Pine (Pinus merkusii) leaf extracts against oral pathogens. J Int Dent Med Res. 2021; 14(2): 559–62.
49. Joycharat N, Thammavong S, Limsuwan S, Homlaead S, Voravuthikunchai SP, Yingyongnarongkul B, Dej-adisai S, Subhadhirasakul S. Antibacterial substances from Albizia myriophylla wood against cariogenic Streptococcus mutans. Arch Pharm Res. 2013; 36(6): 723–30.
50. Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials—A minireview. Materials (Basel). 2020; 13(14): 3224.
51. Sudhakar M, Venkata Ra B. Bactericidal and anti-biofilm activity of tannin fractions derived from Azadirachta against Streptococcus mutans. Asian J Appl Sci. 2020; 13(3): 132–43.

DOI: 10.20473/j.djmkg.v56.i4.p226–232