Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2025 September; 58(3): 256–261

Original article

Antibacterial and anti-adherence effect of *Laportea interupta* ethanolic extract on *Streptococcus mutans* biofilm

Suryani Hutomo[1], Ceny Gloria Larope[2], Denise Utami Putri[3,4,5], Christiane Marlene Sooai[6], Haryo Dimasto Kristiyanto[1]
[1]Department of Microbiology, Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta, Indonesia
[2]Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta, Indonesia
[3]Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[4]Tuberculosis Center, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan
[5]Department of Internal Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
[6]Department of Parasitology, Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta, Indonesia

ABSTRACT

Background: *Due to its capacity to generate extracellular polymeric substances that aid in biofilm creation, Streptococcus mutans is a leading cause of dental caries. Natural remedies, including traditional plant extracts, are being explored for their antimicrobial potential. Laportea interupta (L. interupta), known as jelatang in Indonesia, is alleged to possess a bacteria-inhibiting effect.* ***Purpose:*** *The purpose of this study is to evaluate the potential use of L. interupta leaf ethanolic extract to inhibit S. mutans proliferation and adherence to hydroxyapatite (HA) discs, simulating the tooth surface.* ***Methods:*** *The broth microdilution approach was utilized to evaluate the minimum concentration of the extract that inhibits bacterial growth. For anti-adherence assessment, HA discs pre-coated with saliva were exposed to different extract concentrations and incubated with S. mutans. Bacterial adhesion was visualized using 0.1% crystal violet staining, quantified spectrophotometrically at 595 nm, and further verified using scanning electron microscopy (SEM).* ***Results:*** *The extract showed inhibitory effects on S. mutans growth at 7,500 µg/ml (MIC). Anti-adherence activity was optimal at 1,500 µg/ml, and SEM analysis confirmed a reduced biofilm formation on extract-treated samples.* ***Conclusion:*** *Laportea interupta ethanolic leaf extract demonstrated both antibacterial and anti-adherence effects against S. mutans, suggesting its potential as a natural anti-biofilm agent for oral health applications.*

Keywords: *Streptococcus mutans; Laportea interupta extract; antibacterial; bacterial adherence; medicine*
Article history: *Received 26 January 2024; Revised 21 May 2024; Accepted 10 July 2024; Online 10 May 2025*

Correspondence: Suryani Hutomo, Department of Microbiology, Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta, Indonesia. Email: suryanihutomo_drg@yahoo.com

INTRODUCTION

The presence of oral microorganisms within dental plaque is often associated with the occurrence of various oral health conditions such as dental caries, periodontal diseases, and even malignancies.[1] Among these, dental caries are strongly correlated with oral hygiene and are acknowledged as a global health problem due to their common prevalence among the general population. In Indonesia, data taken from the Basic Health Research/Riset Kesehatan Dasar in 2018 revealed that nearly 45.3% of the population experienced dental caries, highlighting it as a major public health issue.[2]

The pathogenesis of dental caries includes the involvement of oral microorganisms, including *Streptococcus mutans* (*S. mutans*), which can metabolize carbohydrates as their main food. This bacterium adheres to the enamel surface, which is primarily composed of hydroxyapatite (HA), through adherence to glycoprotein embedded in the pellicle, formed from saliva.[3,4] The body's metabolic processes produce lactic acid, which cause the teeth to experience demineralization and subsequent caries. *S. mutans* will also produce soluble and insoluble glucan, derived from sucrose metabolism. This bacterium has a glucosyltransferase enzyme that can convert salivary sucrose into extracellular polysaccharides, which further facilitates bacterial adherence, matrix formation, and the fermentation of sucrose into a form of acid in biofilm formation.[2] The biofilm on the tooth surface is, in part,

DOI: 10.20473/j.djmkg.v58.i3.p256–261

formed by the production of extracellular polymeric substances (EPS),[5,6] which is also supported by the moist environment within the oral cavity.

Currently, an attempt to control cariogenic biofilm formation is effective by applying chlorhexidine as a mouthwash, which acts as an antibacterial drug. However, this substance can cause tooth discoloration when used long-term.[7] Therefore, a novel approach to control *S. mutans* biofilm formation is of interest.

Indonesia is a country with significant biodiversity. There are ± 30,000 known plant species and about 7,000 of them are widely used as medicines, cosmetics, and pesticides.[8] One of the plant species in Indonesia that can be further developed is *Laportea*, which is known as "jelatang" or *Laportea interupta* (*L. interupta)*. This plant is spread across Asia, Africa, and Europe, comprising 163 species. The plant is easy to obtain because it can grow from sea level to high hills, and is even considered a weed in the yard.[9]

Alkaloids are shown to destroy the peptidoglycan component in bacterial cell membranes, and terpenoids inhibit growth and cell membrane processes;[10] accordingly, these two substances are known to be potent antibacterials. A study conducted by Safitri et al.[8] observed that *L. interupta* leaf extract has potential as an antibacterial. The phytochemical components of the leaf extract contained alkaloids, tannins, saponins, and flavonoids, which can act as antibacterial agents, but no information was provided on the origin of the plants, nor was it mentioned where the plants grew. The authors found no research into the anti-adherence of *S. mutans*. Therefore, in the current study, it is important to discover whether *L. interupta* leaf extracts may inhibit *S. mutans'* growth and reduce its adhesion to HA discs that serve as an in vitro tooth surface model. This study was conducted with the hope of providing insights into the potential use of natural herbs as one measure to control dental biofilm formation and, eventually, prevent dental caries. Therefore, this study aims to evaluate the ability of *L. interupta* ethanolic leaf extract in inhibiting the growth and adhesion of *S. mutans* on hydroxyapatite surfaces, contributing to the development of natural alternatives for oral biofilm control.

MATERIALS AND METHODS

The Health Research Ethics Committee of the Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta, approved this study (approval number 1548/C.16/FK/2023). The *Streptococcus mutans* ATCC 25175 strain was cultured in a brain–heart infusion (BHI) broth (Oxoid Ltd., Basingstoke, Hampshire, England) under incubation at 37°C under microaerophilic conditions. Bacterial suspensions were standardized into 0.5 McFarland turbidity, which is equivalent to approximately 1.5 x 10^8 CFU/ml.

L. interupta leaves were collected and verified by a botanical specialist at a herbal manufacturing company (CV Merapi Farma, Yogyakarta, Indonesia) with reference no. SK-SMPL/MFH/II/2023. The plant material, harvested from Hargobinangun village in Sleman, Yogyakarta, was processed at the Basic Biotechnology Laboratory, Universitas Kristen Duta Wacana.[11] A total of 750 g of dried leaves were macerated and emersed in 1,000 ml of 96% ethanol and maintained at 4°C for a duration of 24 hours. The concentrated mixture was filtered three times using a rotary vacuum evaporator at 40°C to get a paste-like ethanolic extract, which was stored at 4°C. For experimentation, the extract was liquefied in 1% dimethyl sulfoxide (DMSO; Merck, Germany) to prepare a stock solution of 15,000 µg/ml for minimum inhibitory concentration (MIC) analysis and 6,000 µg/ml for adherence evaluation. The sample was passed through a 0.45 µm syringe filter (Sartorius, Germany) prior to dilution with the BHI broth.

Qualitative phytochemical analysis was carried out based on modified standard procedures.[12] Alkaloids were identified using Wagner's and Dragendorff's tests. To carry out Wagner's test, first, to acidify the solution, 2 mg of the extract was treated with 1.5% v/v of hydrochloric acid. Subsequently, a few drops of Wagner's reagent were added. The appearance of a yellow-to-brown precipitate was considered a positive indication of the presence of an alkaloid. Dragendorff's test was performed by putting 2 mg of the extract in a test tube. Then, 5 ml of distilled water was added; 2 mg hydrochloric acid was also added until an acid reaction occurred. To this tube, 1 ml of Dragendorff's reagent was added. An orange/orange-red precipitate indicated the presence of alkaloids.

To detect the presence of tannins, 5 mg of the extract was liquified in 5 ml distilled water. After filtration, a few drops of a 1% ferric chloride ($FeCl_3$) solution were added to the filtrate. Tannin was indicated by a blue or green color in the solution. To identify saponins, 5 mg of the extract was combined with 5 ml of warm distilled water. One drop of sodium bicarbonate solution was then added. The mixture was vigorously shaken until foam developed and allowed to stand for 3 minutes. The formation and persistence of foam suggested the presence of saponins in the sample.

Flavonoid content was analyzed using Shinoda's test. To 0.5 ml of the extract, approximately 10 drops of diluted hydrochloric acid were added, followed by a small fragment of magnesium. The appearance of a pink, reddish, or brown coloration indicated a positive result for flavonoids.

A Keller–Kiliani test was conducted to identify the presence of glycosides. A few drops of ferric chloride solution were added to the extract and mixed thoroughly. When a solution of sulfuric acid containing ferric chloride was gradually layered, two distinct layers were observed: a reddish-brown color in the lower layer and a bluish-green tint in the upper acetic acid layer, indicating a positive result. The susceptibility of *S. mutans* ATCC 25175 to *L. interupta* leaf extract was evaluated through the MIC test using the broth microdilution method.[13]

A volume of 10 µl of bacterial culture standardized to a 0.5 McFarland was put into 100 µl of BHI broth

DOI: 10.20473/j.djmkg.v58.i3.p256–261

that contained an ethanolic *L. interupta* leaf extract, with concentrations ranging from 234.375 to 15,000 µg/ml, and distributed into a 96-well microplate (Iwaki, Japan). Each test was performed in triplicate. Ciprofloxacin (Sigma-Aldrich, USA) at 10 µg/ml served as the positive control. After incubation at 37°C for 24 hours, the wells were visually assessed for turbidity. The MIC was defined as the lowest extract concentration at which no visible turbidity was present.

Saliva samples were obtained from three healthy individuals and processed using a modified version of the Yamaguchi method.[11] Prior to collection, participants gargled with a cup of water to minimize any microbial contamination. The collected saliva was heat-treated at 65°C for 30 minutes, followed by centrifugation at 3,000 x g for 15 minutes under refrigerated conditions (4°C). The supernatant resulting from centrifugation was then stored at –80°C for later use.

Hydroxyapatite discs measuring 10 mm in diameter and 1.2 mm in thickness were fabricated by compressing 500 mg of HA powder (sourced from bovine bone provided by the Faculty of Mechanical Engineering, Universitas Gadjah Mada) into molds under 120 MPa pressure. The formed discs were subsequently sintered at 1,450°C for 2 hours. Each disc was immersed in 250 µl of the prepared saliva and incubated within a 24-well plate at 37°C for 30 minutes.

A bacterial adherence assay was conducted following the previously described method.[11] Saliva-coated HA discs were placed in 500 µl BHI broth and treated with 100 µl *L. interupta* leaf ethanolic extract at concentrations ranging from 187.5 to 6,000 µg/ml. The setup was incubated at 37°C for 30 minutes in a 24-well culture plate (Iwaki, Japan). The biofilm formation was initiated by exposing the culture to 100 µl of *S. mutans* suspension, standardized to 1.5 x 10^8 CFU/ml, followed by incubation at 37°C for 24 hours. Post-incubation, the HA discs were gently rinsed with 300 µl of phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich, Germany) and then fixed in 250 µl of absolute methanol for 15 minutes. To visualize bacterial adhesion, the samples were stained with 0.1% (w/v) crystal violet for 10 minutes at a room temperature, then washed twice with PBS. The bound dye, representing adherent bacterial cells, was then solubilized using 96% ethanol and transferred into a fresh 96-well microplate. Absorbance measurements were taken at 595 nm using a microplate reader (Thermo Scientific, USA)

An assessment of the bacterial adherence to the HA discs was carried out using scanning electron microscopy (SEM). The procedure was conducted at the Dental Laboratory Center, Dentistry Faculty, Universitas Gadjah Mada, Yogyakarta. In preparation, HA discs were placed in 500 µl of BHI broth and treated with 100 µl of *L. interupta* ethanolic leaf extract at concentrations of 0 and 1,500 µg/ml. The setup was incubated for 30 minutes at 37°C in a 24-well culture plate (Iwaki, Japan). Subsequently, 100 µl of *S. mutans* suspension (0.5 McFarland standard) was introduced and incubated for 24 hours at 37°C.

Following incubation, the discs were rinsed with PBS and fixed in 4% paraformaldehyde for 30 minutes. A dehydration process was then performed using a graded ethanol series: 50% (10 min), 70% (10 min), 95% (10 min), and finally 100% (20 min); the discs were then left to air-dry overnight. To prepare for the SEM analysis, the dried discs were sputter-coated with a thin layer of gold using an auto fine coater (JEC-3000FC, JEOL, Japan) and mounted on carbon double-sided tape for imaging using an SEM device (JEOL JSM IT-200, Japan).

RESULTS

Initial checks were conducted on the phytochemical component in the extract by conducting qualitative analysis. Observations showed that *L. interupta* leaf extract contained alkaloids, tannins, saponins, flavonoids, and glycosides, as shown in Table 1.

On clarifying the bacterial adherence assay, the highest concentration was determined at 6,000 µg/ml, below the concentration of the MIC value (7,500 µg/ml). Then, bacterial cultivation was carried out with increasing doses of *L. interupta* extract, with a gradient concentration of 187.5, 375, 750, 1,500, 3,000, and 6,000 µg/ml. The optical density (OD) values, which reflected the extent of bacterial adherence to the well surface, were compared to the control group. A higher OD reading indicated denser biofilm formation. A dose-dependent decrease in OD was observed with increasing concentrations of *L. interupta* extract. The optimal inhibitory effect on biofilm formation was noted at 1,500 µg/ml (Figure 1), as no significant additional reduction was observed at higher concentrations.

Cultivating the leaf extract with *S. mutans* was carried out to observe whether *L. interupta* may inhibit bacterial growth. The test showed that *L. interupta* leaf ethanolic extract inhibited *S. mutans* growth at a concentration of 7,500 µg/ml (the minimum inhibitory concentration or MIC; see Figure 2). At this concentration, no turbidity was observed in the BHI medium, which was comparable to the ciprofloxacin-treated control (see Column A in Figure 2).

Table 1. Phytochemical results obtained from *L. interupta* leaf extract

Chemical compound	Results
Alkaloid	Wagner test: (+) brown precipitate Dragendorff test: (+) dark red precipitate
Tannins	(+) dark green solution
Saponins	(+) the presence of foam
Flavonoids	(+) brown color
Glycosides	(+) dark green or black color

DOI: 10.20473/j.djmkg.v58.i3.p256–261

The results were validated through three independent experimental repetitions. Statistical analysis was performed using one-way ANOVA with a significance threshold set at 0.05. A statistically significant difference was found between the untreated group (0 µg/ml) and the groups treated with 1,500, 3,000, and 6,000 µg/ml of the extract ($p = 0.000$).

The SEM observations, representing the experimental groups, supported the above findings. Bacterial cells were visible on the extract-treated and untreated discs; however, those on the untreated discs showed a higher density of *S. mutans* colonies compared to those that had been treated with the 1,500 µg/mL extract. Furthermore, the extracellular matrix associated with the *S. mutans* biofilm appeared more abundant in the untreated group (Figure 3).

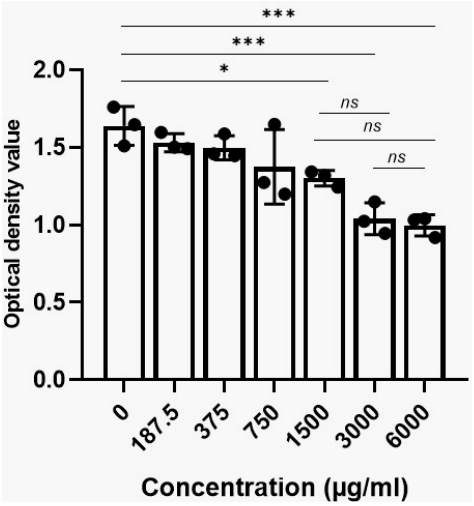

Figure 1. Optical density measurements indicating *S. mutans* adherence across different concentrations (ns: not significant; *p < 0.05; **p < 0.01; ***p < 0.001).

DISCUSSION

This study reported the valuable antibacterial and anti-adherence properties of a herbal extract, *L. interupta,* towards *S. mutans,* an important pathogen within the oral cavity. The authors report the MIC test for *L. interupta* leaf ethanolic extract to be at a concentration of 7,500 µg/ml. This result agrees with an existing study involving Gram-positive *Staphylococcus aureus* bacteria. The study, conducted by Safitri et al.[8], indicated that *L. interupta* leaf extract inhibited the growth of *S. aureus* at a concentration of $5x10^4$ ppm, which formed an inhibitory zone of 9 mm. Another study conducted by Pertiwi[14] indicates that at a concentration of 60% *L. interupta* ethanolic extract, there was antibacterial activity against the same bacteria with an intermediate inhibition zone diameter of 15.68 cm. *L. interupta* leaf extract can be used as a daily mouthwash because it has an antimicrobial effect. Like other essential oil mouthwash, the effect of reducing plaque and gingivitis was greater when used together with mechanical plaque control.[15] The MIC value plays a significant role in the optimization of therapy. To fully evaluate the result, a comprehensive analysis should include pharmacokinetic factors such as volume of distribution, elimination half-life, clearance, peak and trough concentrations, as well as the area under the concentration-time curve.[16]

Based on the phytochemical analysis in this study, *L. interupta* leaf ethanolic extract contains organic compounds in the form of alkaloids, tannins, saponins, flavonoids, and glycosides. Although the current study only reports the qualitative measurement of these phytochemicals, evidence of their potent antibacterial activities is abundant. Alkaloids exert their antibacterial activity by inhibiting the adenosine triphosphate (ATP)-dependent transport of compounds in bacterial cell membranes.[16] Alkaloids can also damage the peptidoglycan layer, which causes lysis of bacterial

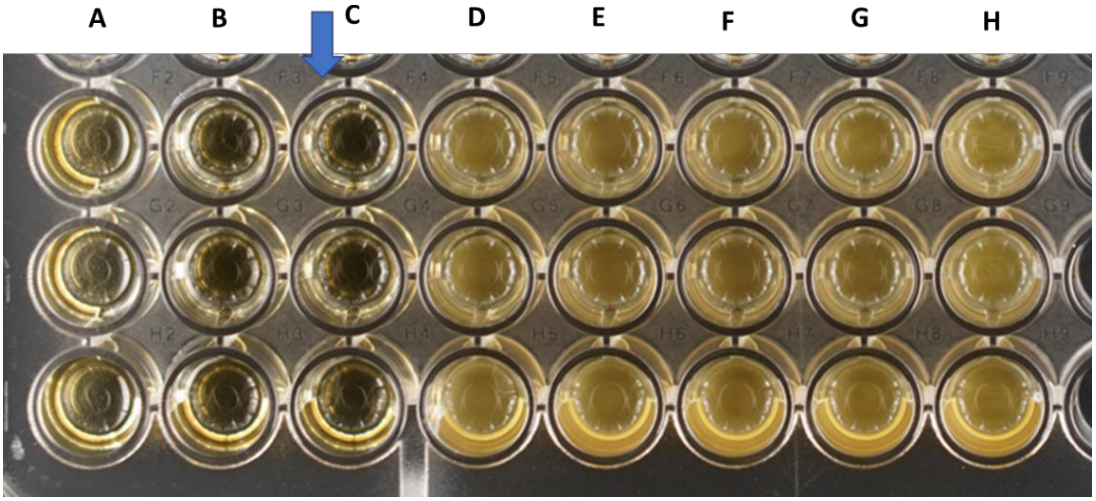

Figure 2. The antibacterial susceptibility assay employed ciprofloxacin as a reference agent, positioned in Column A. The ethanolic extract was tested at concentrations ranging from 15,000 µg/ml (Column B) down to 234.375 µg/ml (Column H). The minimum inhibitory concentration (MIC) was observed at 7,500 µg/ml, indicated in Column C (arrow). Each column represents an individual replication of the experiment.

DOI: 10.20473/j.djmkg.v58.i3.p256–261

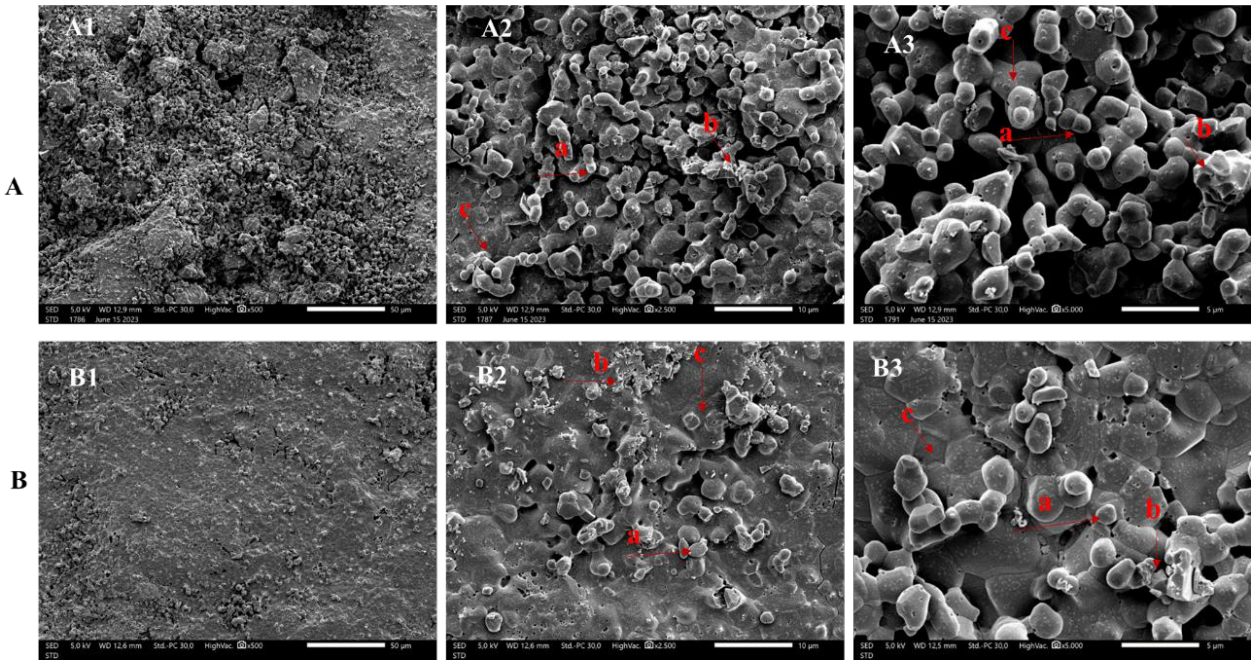

Figure 3. The *S. mutans* biofilm was examined under scanning electron microscopy (SEM). The number of *S. mutans* colonies (a) was greater on the untreated disc (A) compared to the disc treated with 1,500 µg/ml extract (B). The extracellular matrix (b) of the *S. mutans* biofilm was also observed at a higher intensity on the hydroxyapatite (HA) surface (c) of the untreated disc. A1, B1: magnification 500X; A2, B2: magnification 3,000X; A3, B3: magnification 5,000X.

cell walls and leads to cell death. Tannic acids comprise a group of phenolic acids commonly found in plants. They demonstrate the capacity to penetrate bacterial cell walls and interact with the inner membrane to disrupt cellular metabolism. Tannins also help inhibit the attachment of bacteria to surfaces. By reducing this adhesion, it will trigger cell death. The absorption of sugars and amino acids by bacteria is also inhibited by tannins.[17] However, the effectiveness in this context depends on the concentration, pH, temperature, and matrix type.[18] Meanwhile, saponins work by reducing surface tension, thereby enhancing membrane permeability and leading to the leakage of intracellular components.[19] The mechanism of flavonoids as an antibacterial occurs through the inhibition of nucleic acid synthesis. Overall, we propose that the antibacterial effect of *L. interupta* leaf extract may derive from these phytochemical contents.

The results of this study indicate that *L. interupta* leaf extract inhibits *S. mutans*' formation of biofilm in a concentration-dependent manner. Although inhibition was still observed at 3,000 and 6,000 µg/ml, the OD values at these concentrations were comparable to that at 1,500 µg/ml. This suggests that extract concentrations above 1,500 µg/ml may have reached a saturation point, leading to no further increase in inhibitory effect.

Streptococcus mutans adhere to tooth enamel primarily through its capacity to produce glucan from sucrose. This glucan functions as a major constituent of the extracellular polysaccharide matrix, enhancing the bacterium's ability to attach and establish biofilms.[6,20] *Streptococcus mutans* can also metabolize various carbohydrates into organic acids and can withstand acidic environments to initiate the process of forming dental caries.[21] Some *S. mutans* strains are capable of producing three distinct types of glucosyltransferases (GTFs), namely GtfB, GtfC, and GtfD, which use the glucan portion of sucrose as their substrate. This polymer is what forms the matrix in the biofilm.[22] Continuous glucan synthesis by GtfB and GtfC is an important process, one of which occurs through an increase in the coherence of bacteria and their attachment to the tooth surface; this allows for the final result of a strong, high-density biofilm.[22] It is estimated that the extract studied in this research inhibited glucan synthesis, thereby also inhibiting the polysaccharide matrix formation. Therefore, the successful inhibition of bacterial adherence to the tooth surface will subsequently help to prevent the occurrence of dental caries. The potential of *L. interupta* leaf extract to inhibit biofilm formation by *S. mutans* indicated in this study supports future research for its development as an anti-biofilm agent.

The results of observations in this study using SEM present evidence in the form of different biofilms in cultures that were exposed to the extract. The process of forming an *S. mutans* biofilm mass was hampered by the extracts and a biofilm mass did not form (as was the case in the control group. To the best of our knowledge, this is the first study to report the potential applications of *L. interupta* leaf extract as an antibacterial agent with an anti-biofilm formation effect, despite the study being limited in terms of providing a direct extrapolation of its safety in human mucosa and epithelial cells. Overall, the authors report that *L. interupta* leaf extract shows antibacterial properties

DOI: 10.20473/j.djmkg.v58.i3.p256–261

toward *S. mutans* by directly inhibiting its growth, reducing bacterial adherence, and preventing biofilm formation. It is suggested that future studies clarify the findings in animal models and further standardize extract processing for its potential use in clinical settings.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to Faculty of Medicine Universitas Kristen Duta Wacana, located in Special District of Yogyakarta, Indonesia, for the valuable support that made this research possible.

REFERENCES

1. The Lancet. Oral health at a tipping point. Lancet. 2019; 394(10194): 188.
2. Rahman FA, Haniastuti T, Utami TW. The effect of ethanol extract of soursop leaf (*Annona muricata* L.) on adhesion of *Streptococcus mutans* ATCC 35668 to hydroxyapatite discs. Maj Kedokt Gigi Indones. 2018; 4(1): 22–6.
3. Putri DKT, Kriswandini IL, Luthfi M. Characterization of *Streptococcus sanguis* molecular receptors for *Streptococcus mutans* binding molecules. Dent J. 2016; 49(4): 213–6.
4. Syafriza D, Hasanah U, Hidayatullah T, Rifki A, Gani BA. Antibacterial assessment of *Ziziphus mauritiana* Lam on inhibition of the growth and biofilm of *Streptococcus mutans*. Dent J. 2024; 57(3): 189–94.
5. Bahirrah S, Satria D, Yuwanda C. Mobe leaf (*Artocarpus lakoocha Buch*. Ham) ethanol extract's antibacterial activity on *Streptococcus mutans* cell membrane leakage and biofilm formation: An in vitro study. Dent J. 2023; 56(4): 226–32.
6. 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.
7. Walsh T, Oliveira-Neto JM, Moore D. Chlorhexidine treatment for the prevention of dental caries in children and adolescents. Cochrane database Syst Rev. 2015; 2015(4): CD008457.
8. Safitri OM, Nurhamidah N, Amir H. Potensi sitotoksik dan antibakteri ekstrak daun *Laportea interrupta* (L.) Chew (Jelatang ayam) terhadap Staphylococcus aureus. Alotrop. 2019; 2(2): 175–83.
9. Jahan K, Julie AS, Laboni FR, Ahmed MM. Phytochemical screening and evaluation of thrombolytic, membrane stabilizing and cytotoxic activities of *Laportea interrupta* Linn. Pharmacol Online. 2017; 2: 75–80.
10. Marsetyo RI, Andyningtyas SP, Zamrutizahra CS, Fadela IN, Subiwahjudi A, Widjiastuti I. An effective concentration of propolis extract to inhibit the activity of *Streptococcus mutans* glucosyltransferase enzyme. Dent J. 2021; 54(4): 186–9.
11. Hutomo S, Susilowati H, Agustina D, Asmara W. Analysis of anti-*Streptococcus sanguinis* IgY ability to inhibit *Streptococcus sanguinis* adherence. Dent J. 2018; 51(1): 33–6.
12. Pooja S, Vidyasagar GM. Phytochemical screening for secondary metabolites of *Opuntia dillenii* Haw. J Med Plants Stud. 2016; 4(5): 39–43.
13. Hutomo S, Sooai CM, Merry MS, Larope CG, Kristiyanto HD. The effect of brotowali (*Tinospora crispa* L.) stem ethanolic extract on the inhibition of *Candida albicans* biofilm formation. Dent J. 2022; 55(1): 21–5.
14. Pertiwi KK, Fernanda SD. Aktivitas antibakteri herba daun gatal (*Laportea interupta* L. Chew) terhadap *Staphylococcus aureus* dan *Escerichia coli*. J-HESTECH (Journal Heal Educ Sci Technol. 2019; 2(1): 43–50.
15. Brookes Z, Teoh L, Cieplik F, Kumar P. Mouthwash effects on the oral microbiome: are they good, bad, or balanced? Int Dent J. 2023; 73(Suppl 2): S74–81.
16. Mabhiza D, Chitemerere T, Mukanganyama S. Antibacterial properties of alkaloid extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Int J Med Chem. 2016; 2016: 6304163.
17. Pandey A, Chauhan AS, Haware DJ, Negi PS. Proximate and mineral composition of Kadamba (*Neolamarckia cadamba*) fruit and its use in the development of nutraceutical enriched beverage. J Food Sci Technol. 2018; 55(10): 4330–6.
18. Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials—A minireview. Materials (Basel). 2020; 13(14): 3224.
19. Zahro L, Agustini R. Uji afektivitas antibakteri ekstrak kasar saponin jamur tiram putih (*Pleurotus ostreatus*) terhadap *Staphylococcus aureus* dan *Escherichia coli*. UNESA J Chem. 2013; 2(3): 120–9.
20. Zayed SM, Aboulwafa MM, Hashem AM, Saleh SE. Biofilm formation by *Streptococcus mutans* and its inhibition by green tea extracts. AMB Express. 2021; 11(1): 73.
21. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, Abranches J, Brady LJ. The biology of *Streptococcus mutans*. In: Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, Braunstein M, Rood JI, editors. Gram-positive pathogens. 3rd ed. Washington: ASM Press; 2019. p. 435–48.
22. Bowen WH, Koo H. Biology of *Streptococcus mutans*-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res. 2011; 45(1): 69–86.

DOI: 10.20473/j.djmkg.v58.i3.p256–261