Conservative Dentistry Journal Vol. 15 No. 1 January-June 2025; 23-26

Research Report

Effectiveness of Nipah leaf extract (*Nypa fruticans*) against *Streptococcus mutans* biofilm as cavity cleanser

Yosef Kevin Handriutomo[1], Diana Soesilo[2], Aprilia Aprilia[2], Kristanti Parisihni[3], Rafialdi Rayhan[4], Febriastuti Cahyani[5]

[1]Undergraduate Program, Faculty of Dentistry, Universitas Hang Tuah, Surabaya, Indonesia
[2]Department of Conservative Dentistry, Faculty of Dentistry, Universitas Hang Tuah, Surabaya, Indonesia
[3]Department of Microbiology, Faculty of Dentistry, Universitas Hang Tuah, Surabaya, Indonesia
[4]Undergraduate Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[5]Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Background: *Streptococcus mutans is the primary bacterium responsible for dental caries, found in the biofilm on the tooth surface, known as dental plaque. Before restorative treatment or cavity care for carious teeth, a cavity cleanser is necessary to remove residual bacteria that could cause secondary caries or restoration failure. Nipah leaf extract contains phenol, flavonoids, saponins, and steroids, which can destroy bacteria by denaturing proteins and damaging bacterial cell membranes.* ***Purpose:*** *To determine the antibiofilm activity of Nipah leaf extract against Streptococcus mutans biofilm.* ***Methods:*** *The materials used were Nypa fruticans leaf extract prepared using the maceration method at concentrations of 1%, 5%, 10%, and 15%, with chlorhexidine gluconate as the positive control and distilled water as the negative control. Bacteria cultured in BHI media were inoculated into a 96-well flat-bottomed plastic tissue culture plate and incubated for 24 hours at 37°C. Optical Density (OD) was measured using an ELISA reader.* ***Results:*** *KM: 0.039; K (-): 0.887; K (+): 0.085; P1: 0.727; P2: 0.463; P3: 0.347; P4: 0.169. The Mann-Whitney test results showed significant differences between K (+) and P1 (0.000), K (+) and P2 (0.000), K (+) and P3 (0.000), K (+) and P4 (0.005), P1 and P2 (0.000), P1 and P3 (0.000), and P1 and P4 (0.000). The results were significant as $p < 0.05$.* ***Conclusion:*** *Nipah leaf extract (Nypa fruticans) can inhibit the biofilm formation of Streptococcus mutans bacteria.*

Keywords: *Streptococcus mutans, antibiofilm, Nypa fruticans, cavity cleanser, dental plaque*

Correspondence: Diana Soesilo, Department of Conservative Dentistry, Faculty of Dentistry, Universitas Hang Tuah, Jalan Arif Rahman Hakim 150, Sukolilo, Surabaya, Indonesia. Email: diana.soesilo@hangtuah.ac.id

INTRODUCTION

Dental caries occurs due to tooth demineralization, which is caused by acids produced by microorganisms. This demineralization process is indicated by cavities on the surface of enamel, dentin, and cementum.[1] The acidity level and the duration of the acid atmosphere on the tooth surface are closely correlated with the demineralization of tooth structure.

The bacteria that cause caries is *Streptococcus mutans,* the activity of these bacteria will cause demineralization of the hard tissues of the teeth, damage to the pulp tissue, and infection spreading to the apical tissues.[2,3] The ability of *Streptococcus mutans* to form a biofilm, or dental plaque, on the tooth surface is one of its virulence properties that makes it the main causative agent of dental caries in humans. Thus, with the help of glucosyltransferase (GTF) action, the bacteria produce glucans that mediate the attachment of cells to the tooth surface. Furthermore, *Streptococcus mutans* produces several glucan-binding proteins, such as guanylate binding proteins (Gbps), which are thought to enhance adhesion. it is known that *Streptococcus mutans* plays a role in the formation of dental plaque caused by the anti-surface gene c (Pac).[4]

Cavity cleanser or cavity disinfection removes bacterial debris that can cause secondary caries, postoperative sensitivity, and restoration failure. As much as 40% of cases involving bacterial contamination led to secondary caries. Facts show that the development of cavity cleanser materials can eliminate caries-causing bacteria.[5]

The cavity cleanser materials that are widely used by dentists are sodium hypochlorite (NaOCl), EDTA, and Chlorhexidine digluconate (CHX). Chlorhexidine digluconate (CHX) is a biguanide biocide that inhibits the formation and development of dental plaque and has been used as an oral antimicrobial agent since the 1970s. Chlorhexidine digluconate 2% solution is the most widely used cavity cleanser in clinical dentistry and dental

DOI: https://doi.org/10.20473/cdj.v15i1.2025.23-26

research. Chlorhexidine digluconate is bactericidal at high concentrations and bacteriostatic at low concentrations. At high concentrations, it causes coagulation of intracellular components, leading to cytoplasmic freezing.[6] At low concentrations, Chlorhexidine digluconate damages the cell wall and attacks the cytoplasmic membrane. When used repeatedly, Chlorhexidine digluconate 2% may cause allergic reactions but rarely causes sensitivity.[6]

Nipah (*Nypa fruticans*) is a type of mangrove plant with palm-like characteristics that grows around brackish waters (tidal areas).[7] Phytochemical tests of ethanol extract of Nipah leaves showed that it contains phenols, flavonoids, saponins, and steroids. The active ingredients denature proteins to destroy bacteria and damage the bacterial cell membrane by dissolving fat in the cell wall.[8] The extraction results of Nipah leaves and Nipah fruit seeds show that Nipah leaves produce the most extracts compared to Nipah fruit seeds. These results are due to the primary metabolite network formed in the leaves during photosynthesis.[9] *Nypa fruticans* contains compounds that are believed to have antibacterial effects. Previous studies have shown that Nipah leaves contain active compounds such as alkaloids, flavonoids, phenolics, steroids, saponins, triterpenoids, and tannins.[7]

Microbial cells and extracellular polymeric substances (EPS) form biofilms, accounting for 50%-90% of the total organic carbon biofilm. EPS is also the main biofilm matrix material. If the cells continue to grow and form a thicker layer.[10] The anti-biofilm activity of bacteria tends to naturally regulate the bacterial population in the ecological niche.[11] During their growth, biofilm cells produce EPS, which helps bacteria attach to the surface and form microcolonies. The antibiofilm process works by cell wall destruction and damaging cell proteins, causing cell lysis.[11]

According to Lestari's research (2016), Nipah leaf extract has antibacterial properties against gram-negative and gram-positive bacteria. At concentrations of 0.1%, 0.05%, 0.025%, 0.0125%, and 0.00625%, the n-hexane fraction showed significant differences in inhibition against the positive control of *Bacillus cereus*. At concentrations of 0.1%, 0.05%, 0.025%, and 0.0125%, the ethyl acetate fraction also showed significant differences in inhibition against the positive control of *E. coli*.[7]

MATERIALS AND METHODS

The type of research is true experimental laboratory research. The materials needed for this research are Nipah leaves (*Nypa fruticans*) taken from the Wonorejo mangrove forest, Rungkut sub-district, Surabaya, East Java, Indonesia.

The first step is the extraction of Nipah leaves and powder preparation. Nipah leaves (*Nypa fruticans*) used in this study are the leaves with dark green color. The clean leaves were removed from the stem and then dried in an oven at 50°C for 20 hours. After the leaves are dried, the leaves are ground with a blender until they become powder. Nipah leaves are extracted by mixing 500 grams of Nipah leaf powder with 2500 ml of 100% methanol solvent in an Erlenmeyer. The mixture was then shaken in a water bath at 120 rpm for one hour until homogeneous. The solution was macerated with methanol for 24 hours at room temperature. After the maceration period was complete, the solution was filtered using a Buchner filter to produce a filtering precipitate. The solution was separated from the precipitate, then the solution was stored, and the precipitate was dried at room temperature for 24 hours. The precipitate was re-macerated three times. After three maceration processes, the maceration solutions were mixed one, two, and three, then the solution was concentrated to obtain a concentrated extract using a rotational vacuum evaporator at 60°C for 36 hours. After that, it was diluted with distilled water. KM is the medium control, K (-) is the negative control, K (+) is the positive control. P1 is 1% of Nipah leaf extract obtained from 1g of concentrated extract homogenized with 2 ml of distilled water. P2 is 5% Nipah leaf extract obtained from 1.5 g of concentrated extract, which is homogenized with 2 ml of distilled water. P3 is 10% Nipah leaf extract obtained from 2 g of concentrated extract homogenized with 2 ml distilled water. P4 is 15% Nipah leaf extract obtained from 2.5 g of concentrated extract homogenized with 2 ml distilled water. The extracts that have been obtained will be used for further testing.

RESULTS

The study's results obtained each group's mean value and standard deviation can be seen in Table 1. The results of the normality test with Kolmogorov Smirnov obtained $p > 0.05$, so it is said that the data is normally distributed. The homogeneity test results obtained a significance value of 0.120 or $p>0.05$, so it can be concluded that the data used is homogeneous. Kruskal Wallis test results obtained $p = 0.000$ where the p-value <0.05 indicates a significant difference between groups. Testing was continued with the Mann-Whitney Post Hoc test to determine which groups differed significantly (Table 2).

The results of the *Mann-Whitney Post Hoc test* stated that there was a significant difference if the p-value <0.05. In the K (-) group against K (+), P1, P2, P3 and P4 there are significant differences. There is also a significant difference in the K (+) group against P1, P2, P3, and P4. In the KM group, P1, P2, P3, and P4 against K (-), P1, P2, P3, and P4 there is also a significant difference. There is no significant difference between the KM Group and K (+).

Table 1. Mean OD value of biofilm

Group	Replication	Mean ± SD
KM	8	0.039± 0.017
K (-)	8	0.887 ± 0.048
K (+)	8	0.085 ± 0.044
P1 (1%)	8	0.727 ± 0.055
P2 (5%)	8	0.463 ± 0.031
P3 (10%)	8	0.347 ± 0.034
P4 (15%)	8	0.169 ± 0.037

DOI: https://doi.org/10.20473/cdj.v15i1.2025.23-26

Table 2. Mann–Whitney post hoc test

Groups	KM	K (-)	K (+)	P1	P2	P3	P4
KM		0.000*	0.393	0.000*	0.000*	0.000*	0.000*
K (-)			0.000*	0.000*	0.000*	0.000*	0.000*
K (+)				0.000*	0.000*	0.000*	0.005*
P1					0.000*	0.000*	0.000*
P2						0.000*	0.000*
P3							0.000*
P4							

DISCUSSION

This study tested the effectiveness of Nipah leaf extract (*Nypa fruticans*) as a cavity cleanser against *Streptococcus mutans* biofilm. This study used positive control (Chlorhexidine digluconate 2%) and negative control (distilled water) groups as comparison. The results showed that the negative control had no inhibition against Streptococcus mutans because distilled water does not contain antibacterial compounds so that the bacteria can grow freely with a greater number of colonies.[12]

Streptococcus mutans are non-motile gram-positive bacterium with diameter of 1-2 µm, is facultative anaerobic, round or egg oval shaped (ovoid), arranged like chain without spore.[13] This bacterium is cariogenic, can grow in acidic environment, and can change glucose and sucrose into polysaccharides extracellular that change plaque matrix composition to be like agar, that can facilitate attachment.[7]

Chlorhexidine digluconate 2% has a broad antibacterial spectrum, especially on gram-positive bacteria, and effectively degrades. *Streptococcus mutans* is the primary cause of dental caries. It works by inhibiting matrix metalloproteinases (MMP) enzymes and cysteine cathepsins, inhibiting pellicle formation as an antiplaque agent, and removing smears that increase surface bonding to the dentine, thus preventing bacterial infection of the dentine.[14]

Biofilms are microbial communities attached to surfaces and encased in a three-dimensional matrix composed of exopolysaccharides, proteins, and nucleic acids.[15] The main components are microbial cells and extracellular polymeric substance (EPS), accounting for 50-90% of total organic carbon. EPS helps bacteria adhere to surfaces and form microcolonies. As the biofilm thickens, microbes in the inner layer lack nutrients and experience an accumulation of toxic products. Plaque in the oral cavity is an example of a biofilm.[16]

The results showed that Nipah leaf extract at all concentrations (1%, 5%, 10%, and 15%) can inhibit the growth of *Streptococcus mutans* biofilm because it contains antibacterial substances such as polyphenols, flavonoids, triterpenoids / steroids, saponins, and alkaloids.[17]

Saponins damage biofilms by affecting the extracellular polymer matrix, reducing polymeric substances and altering the integrity of bacterial cell membranes, causing cell wall instability.[7,18,19] While polyphenols act as toxins in the protoplasm, damaging the cell wall, precipitating bacterial cell proteins, causing cell damage, protein denaturation, enzyme inactivation, and cell leakage.[20-22]

Flavonoids damage biofilms through hydroxyl groups that bind to biofilm proteins, forming complex compounds that cause biofilm denaturation.[22-24] Triterpenoids inhibit oxygen uptake and oxidative phosphorylation, damage bacterial cell membranes and suppress biofilm growth. Cinchonidine (an alkaloid derivative) inhibits *Staphylococcus aureus* biofilm formation and can eradicate mature biofilms at high doses.[25] Phenolic compounds inhibit glucan formation, especially *water-insoluble glucan* (WIG), which plays an important role in the formation of *Streptococcus mutans* biofilm that develops into plaque.[26-28]

The biofilm inhibitory effect of Nipah leaf extract comes from its phytochemical compounds. Flavonoids inhibited *Streptococcus mutans* biofilm through physical interference, interfering with BAP acceptance and polymerization. With their derivative tannins, polyphenols decrease biofilm slime production, reduce icaA and icaD gene expression, inhibit the quorum sensing regulator RNAIII, and interfere with bacterial cell metabolism. Nipah leaf extract contains steroid-type saponins that destabilize the extracellular matrix of polysaccharides and inhibit biofilm formation through binding to the enzyme mannitol dehydrogenase and eDNA that play a role in alginate synthesis. Alkaloids reduce biofilm-forming initiator genes, inhibit quorum sensing, and degrade biofilm-forming regulatory factors.[16]

This inhibition is influenced by the nature of phenolic compounds in Nipah leaf extract (*Nypa fruticans*), which function as antibiofilm. Phenolic compounds react with cell membrane phospholipids, causing changes in membrane permeability so that intracellular components (amino acids, nucleic acids, proteins) come out, reducing the ability of *Streptococcus mutans* to communicate and form EPS. This compound also causes leakage of bacterial cell cytoplasm during the adhesion stage. The decrease in OD values at P1-P4 indicates cytoplasmic leakage, with higher concentrations causing greater cell leakage.

Based on this research, it can be concluded that antibiofilm compounds of Nipah leaf extract (*Nypa fruticans*) at concentrations of 1%, 5%, 10%, and 15% can inhibit *Streptococcus mutans* biofilm. Concentration of 15% is the largest concentration in this research and the most effective one in inhibiting *Streptococcus mutans* biofilm.

DOI: https://doi.org/10.20473/cdj.v15i1.2025.23-26

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DOI: https://doi.org/10.20473/cdj.v15i1.2025.23-26