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Research Article

EFFECT OF BREADFRUIT LEAF EXTRACT ON THE VIABILITY OF *STREPTOCOCCUS MUTANS* BIOFILM *IN VITRO*

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ABSTRACT

Objective: Breadfruit leaf has potent antibacterial properties that could be used to reduce biofilms in the oral cavity. The purpose of this study was to analyze the antibacterial effect of the breadfruit leaf extract on the growth of *Streptococcus mutans in vitro*.

Methods: *S. mutans* ATCC 25175 was cultured in a 96-well plate and incubated at 37°C for 20 h (accumulation phase) and 24 h (maturation phase). The breadfruit leaf extract was added at the following concentrations: 5%, 10%, 15%, 20%, 40%, 80%, and 100%. The viability of *S. mutans* was tested with the MTT assay at a wavelength of 490 nm. The results were analyzed by one-way analysis of variance.

Results: In the accumulation phase, a significant decrease was found in *S. mutans* viability at different concentrations of the breadfruit leaf extract, but in the maturation phase, a significant decrease was found in the *S. mutans* viability at the 5% concentration. The other groups decreased significantly compared with the control group (*p<0.05). The viability of *S. mutans* after adding the breadfruit leaf extract at all concentrations was lower in the accumulation phase than that in the maturation phase.

Conclusion: In the accumulation phase, breadfruit leaf extract at concentrations of 5%, 10%, 20%, 40%, 80%, and 100% can reduce *S. mutans* biofilm viability.

Keywords: Extract of breadfruit leaf, *Streptococcus mutans*, Viability, MTT assay.

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INTRODUCTION

Medicinal plants are natural materials derived from plants that are traditionally used by communities but have not been scientifically tested. They significantly improve community health when they are used in the correct doses, implementation, and time. A medicinal plant with considerable potential for human health is *Artocarpus altilis*, also known as breadfruit [2].

Breadfruit is distributed throughout the tropics and its tree is sturdy, tall, and leafy [2]. Its fruit is used as food and its leaves have been used to treat various diseases such as kidney disease, diabetes mellitus, high cholesterol, and canker sores [3].

Streptococcus mutans is a Gram-positive, non-motile, and an anaerobic facultative bacterium that plays an important role in the formation of biofilms in the oral cavity [4]. The first phase of biofilm formation is known as the 0–4 h adhesion phase, during which *S. mutans* is able to attract other microorganisms. The maturation phase happens after 20 h, during which *S. mutans* releases a glycosyltransferase enzyme that causes the glucose polymerization of sucrose by releasing fructose. Glucose is then converted to lactic acid. When a large amount of lactic acid is created, they decrease the pH to a critical value of 5.5, causing dental demineralization [5-7].

In dentistry, antibacterial compounds are used to decrease plaque accumulation. Abdassah *et al*. stated that the phytochemical of the breadfruit leaf extract contains active antibacterial compounds, such as flavonoid, polyphenol, quinone, steroid, saponin, monoterpene, and sesquiterpene, making our study important [8]. Breadfruit leaf was extracted by maceration using 70% ethanol as a solvent [8]. The antibacterial activity of breadfruit leaf depends on the concentration and contact time of the antibacterial compound [9]. The purpose of this study was to analyze the antibacterial effect of the breadfruit leaf extract on the viability of *S. mutans* biofilm at different concentrations and contact times.

METHODS

S. mutans ATCC 25175 was obtained from the Oral Biology Laboratory, Faculty of Dentistry, Universitas Indonesia, Indonesia, where the study was conducted. The sample of the breadfruit leaf extract was obtained from the Medicinal and Aromatic Medicine Research Halls (BALITRO), Bogor, Indonesia. Artificial saliva was obtained from the Biochemistry Laboratory of the Faculty of Medicine, Universitas Indonesia, Indonesia. In this study, the positive control used was 0.2% chlorhexidine and BHI broth was added with a ratio of 1:1. The viability of *S. mutans* was tested with the MTT assay at a wavelength of 490 nm.

Preparation of *S. mutans* main solution

The main solution of *S. mutans* was prepared by taking some bacterial colonies grown on BHI agar and placing in a reaction tube containing 10 mL of BHI broth. The reaction tube was placed in an anaerobic jar filled with NO_2 and 5% CO_2 gas and incubated for 24 h at 37°C.

Making of various concentrations of the breadfruit leaf extract

The breadfruit leaf extract was filtered using a 10-mL syringe and a 0.2-mm diameter Minisart. This was necessary to filter the portion of the breadfruit leaf extract that may interfere in reading the OD value. The breadfruit leaf extract was prepared at concentrations of 5%, 10%, 20%, 40%, 80%, and 100%. The artificial saliva was composed of 12 mM NaCl, 3.4 mM KSCN, 17.8 mM $NaHCO_3$, 1.5 mM Na_2HPO_4, 1.5 mM KH_2PO_4, and 2.2 mM urea in an aqueous solvent.

Biofilm formation was performed first in 96-well plates by adding 100 µL of artificial saliva in each well. The plates were then incubated

for 90 min at 37°C. Saliva not attached to the well base was removed and wells were washed with 100 µL PBS. Then, 100 µL of diluted and standardized bacteria with a concentration of 106 CFU/mL=0.061 was added to the 96-well plate, and the plate was placed in 95% NO_2 and 5% CO_2. The plates were incubated for 20 h and a further 24 h at 37°C. Bacteria that were attached to the 96-well plate were rinsed with 100 µL phosphate-buffered saline (PBS).

Exposure of breadfruit leaf extract and MTT test

In each well plate that was washed with PBS, 100 µl of the breadfruit leaf extract was added at various concentrations (5%, 10%, 20%, 40%, 80%, and 100%) to each well plate. For the positive control, 100 µL of 0.1% chlorhexidine (0.2% chlorhexidine mixed with BHI 1:1) was added to each well. For the negative control, BHI broth was added to each well. The well plate was inserted into an anaerobic jar with 90% NO_2 and 5% CO_2 and was incubated for 2 h at 37°C. The MTT test was performed by adding 50 µL into 96-well plate and incubated for 3 h at 37°C. Then, 100 µL of acidified isopropanol was added into each well and the plate was observed on a microplate reader at a wavelength of 490 nm. The percentage of biofilm viability was examined using the following formula:

$$\text{Viability} = \frac{\text{OD value of treated group}}{\text{OD value of control group}} \times 100\%$$

Data analysis

The percentage of each *S. mutans* treated group viability was tested using one-way analysis of variance.

RESULTS

The normality test, Kolmogorov–Smirnov, showed that the data had a normal distribution (p>0.05)

In the accumulation phase (20 h), the viability of *S. mutans* control group was 100% (OD, 1.541±0.035) (Fig. 1). 0.1% chlorhexidine positive control group had a viability of 9% (OD, 0.137±0.010) (Fig. 1). In the breadfruit leaf extract exposure group, at 5% concentration, the viability was 82% (OD, 1.265±0.023). In 10% concentration group, the viability was 79% (OD, 1.217±0.038); in 20% concentration group, the viability was 56% (OD, 0.867±0.037); in 40% concentration group, the viability was 50% (OD, 0.44±0.016); in 80% concentration group, the viability was 36% (OD, 0.77±0.038); and in 100% concentration group, viability was 29% (OD, 0.561±0.031) (Fig. 1). *S. mutans* viability declined with increasing concentration of the breadfruit leaf extract in the accumulation phase (Fig. 1). All the treated groups significantly differed from the control group (*p<0.05).

In the maturation phase (24 h), the viability of *S. mutans* control group was 100% (OD, 0.953±0.061) in the negative control group and 18% (OD, 0.173±0.010) in 0.1% chlorhexidine positive control group (Fig. 2). In the breadfruit leaf extract exposure group, at 5% concentration, *S. mutans* viability was higher than the control group of 117% (OD 1, 119±0.054). *S. mutans* viability was 92% (OD, 0.877±0.059) at a concentration of 10%, 79% (OD, 0.755±0.039) at a concentration of 20%, 68% (OD, 0.647±0.032) at a concentration of 40%, 65% (OD, 0.621±0.027) at a concentration of 80%, and 59% (OD, 0.566±0.047) at a concentration of 100% (Fig. 2). *S. mutans* viability declined with increasing concentration of the breadfruit leaf extract in the maturation phase. All treated groups significantly differed from the negative control group (*p<0.05).

S. mutans biofilm viability in 0.1% chlorhexidine group in the accumulation phase was lower than that in the maturation phase (Fig. 3). The same results were observed for the groups exposed to different concentrations of the breadfruit leaf extract (Fig. 3). There was a significant decrease in *S. mutans* viability in the accumulation phase compared with that in the maturation phase.

DISCUSSION

Breadfruit leaf extract at concentrations of 5%, 10%, 20%, 40%, 80%, and 100% decreased *S. mutans* biofilm viability during the accumulation phase (20 h) (Fig. 1). During the maturation phase, breadfruit leaf extract at a concentration of 5% significantly increased the *S. mutans* biofilm viability (Fig. 2).

Based on research conducted by Purnamasari *et al*., antibacterial effects are stronger with higher concentrations of cocoa seed extract due to the greater active compound content of the extract [10]. Breadfruit leaf contains active compounds such as flavonoid, polyphenol, quinone, steroid, saponin, monoterpene, and sesquiterpene [8]. Kusdarwati's study on the antibacterial effect of the adas fruit extract (*Foeniculum vulgare*) on *Micrococcus luteus* bacteria found that the flavonoid compounds saponin and tannin are antibacterial phenolic compounds that have the same chemical structure within plants. The compound exerts its antibacterial mechanism by denaturing the bacterial wall protein so that bacterial metabolism is inhibited, resulting in low bacterial growth [11]. This study differed from the previous study in the use of breadfruit leaf and adas fruit extract. Trijani's study found that if the antibacterial concentration was low, phenol compounds would easily decompose and lead to leakage of bacterial walls. At high concentrations, phenol compounds cause bacterial cell membranes lysis [12].

At the maturation phase, breadfruit leaf extract at a concentration of 5% increased *S. mutans* viability (Fig. 2). Based on the research conducted by Sabir, in the maturation phase, antibacterial compounds at low concentrations would effectively inhibit the growth of *S. mutans* when applied continuously [13]. If they are not applied continuously, it is recommended to use high concentrations [13].

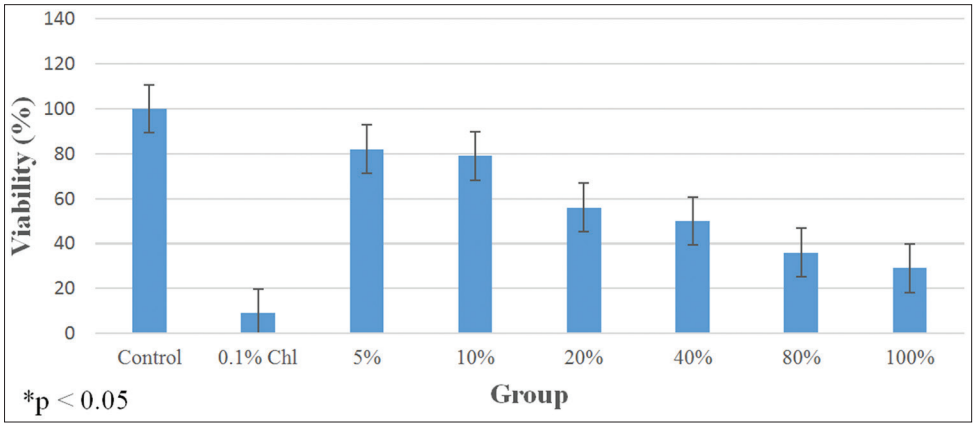

Fig. 1: *Streptococcus mutans* biofilm viability during the accumulation phase (20 h) after exposure to breadfruit leaf extract

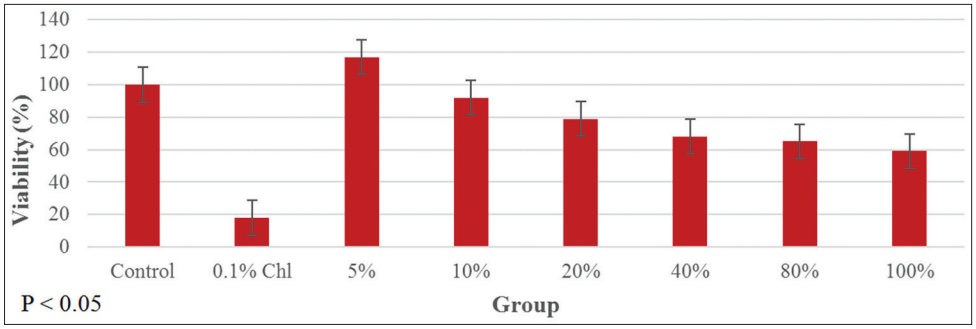

Fig. 2: *Streptococcus mutans* biofilm viability during maturation phase (24 h) after exposure to breadfruit leaf extract

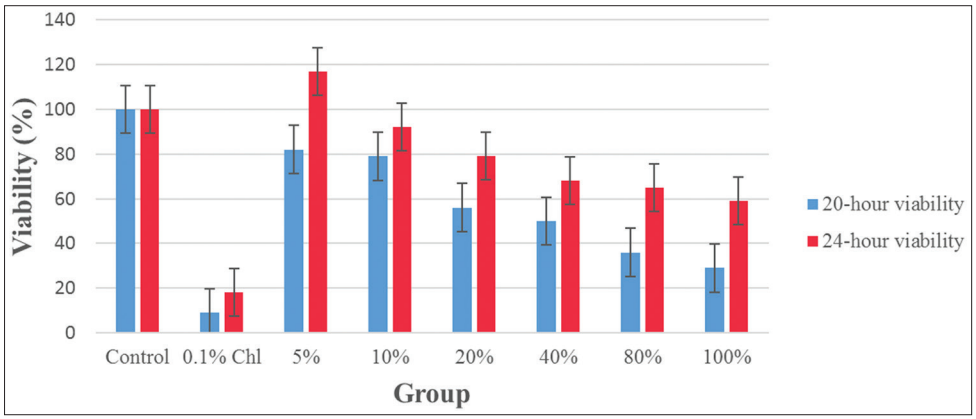

Fig. 3: *Streptococcus mutans* biofilm viability 20 h (accumulation phase) and 24 h (maturation phase) after exposure to breadfruit leaf extract

This result may be influenced by the extracellular matrix of bacteria found on biofilms so it will limit the penetration of antibacterial agents. The thicker the biofilm, the more slowly the antibacterial compound diffuses into the biofilm, thus causing the occurrence of antibacterial resistance at the maturation phase [14]. We found that in the maturation phase, the breadfruit leaf extract was not capable of reducing *S. mutans* viability. Factors that may affect the antibacterial activity are environmental pH, seed components, active substance stability, and bacterial metabolic activity [15].

In the maturation phase, there can be a reduction in the bacterial activity during the process of biofilm formation due to nutritional deficiency [5]. Based on the research conducted by Hidayaningtias, viability would increase in the maturation phase if bacteria were given a medium for growth [16]. In this study, the breadfruit leaf extract was mixed with BHI broth. At 5% breadfruit leaf extract concentration, the amount of BHI broth was higher than the amount of breadfruit leaf extract; therefore, the antibacterial compound could not work optimally. As a result, the biofilm viability increased.

The positive control used in this study was 0.1% chlorhexidine, which prevents caries by inhibiting plaque formation [15]. Rachmawati stated that the antibacterial effect of temulawak ethanol extract on *S. mutans* biofilm was equivalent to that of chlorhexidine [16]. In this study, *S. mutans* viability when exposed to breadfruit leaf extract was higher than that with 0.1% chlorhexidine.

The results of the MTT test with breadfruit leaf extract exposure at various concentrations demonstrated that *S. mutans* viability in the accumulation phase was lower than that in the maturation phase (Fig. 3). Based on the research conducted by Hojo, antibacterial compounds effectively inhibit the growth of bacteria in the accumulation phase [10]. In the accumulation phase, the microorganisms have a solid matrix structure, which is primarily composed of polysaccharide. According to the study conducted by Rachmawati *et al*., the Gram-positive cell wall structure is more simple and single layered with a low lipid content of 1%–4% that allows bioactive materials into cells [17].

CONCLUSION

In the accumulation phase, breadfruit leaf extract at concentrations of 5%, 10%, 20%, 40%, 80%, and 100% can reduce *S. mutans* biofilm viability. In the maturation phase, breadfruit leaf extract at concentrations of 10%, 20%, 40%, 80%, and 100% can reduce *S. mutans* biofilm viability. *S. mutans* biofilm viability in the accumulation phase was lower than that in the maturation phase for all exposure groups. *S. mutans* viability at 5% breadfruit leaf extract concentration increased in the maturation phase.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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