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Research Article

IN VITRO ANTIBACTERIAL EFFECT OF BREADFRUIT LEAF EXTRACT AGAINST *STREPTOCOCCUS SANGUINIS* ATCC 10556 BIOFILM

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ABSTRACT

Objective: Evidence indicates that breadfruit leaf extract may have antibacterial properties. In terms of bacterial plaque, *Streptococcus sanguinis* is known as an early agent of its formation. The purpose of this study was to analyze the antibacterial effects of breadfruit leaf extract on the growth of *S. sanguinis*.

Methods: *S. sanguinis* ATCC 10556 was cultured in 96-well plates and was incubated at 37°C for 20 h (the accumulation phase) or 24 h (the maturation phase). Then, breadfruit leaf extract was added at concentrations of 5, 10, 20, 40, 80, and 100%. The viability of *S. sanguinis* was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay at a wavelength of 490 nm.

Results: Our results demonstrated that the viability of *S. sanguinis*, after exposure to the breadfruit leaf extract at all concentrations, during the accumulation and maturation phases was lower than the control group ($p<0.05$). In addition, the viability of *S. sanguinis* after exposure to the breadfruit leaf extract at concentrations of 20, 80, and 100% during the accumulation phase was lower than that observed during the maturation phase.

Conclusion: Collectively, our novel findings should provide insight into the potential of breadfruit leaf extract to positively affect oral and dental health.

Keywords: Breadfruit leaf extract, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, *Streptococcus sanguinis*, Viability.

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INTRODUCTION

Breadfruit (*Artocarpus altilis*) is typically used as an intercropping plant in the yard. The breadfruit plant originated from New Guinea and was later developed in Malaysia and Indonesia [1]. Indeed, breadfruit trees are widely encountered in Indonesia, particularly in hot areas and valleys. In addition to the fact that it is delicious, the leaves of the breadfruit tree are used to treat many types of diseases, including diabetes mellitus and hypertension [2].

Streptococcus sanguinis is a Gram-positive, facultative anaerobic bacteria. *S. sanguinis* directly binds to pellicle on the tooth surface utilizing various mechanisms, one of which involves binding to saliva proteins that are proline rich. Once bound, *S. sanguinis* facilitates other bacteria to colonize on the tooth surface to form biofilms [3]. Then, *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus*, all of which are cariogenic bacteria, are progressively incorporated into the biofilm. The cariogenic bacteria are acidogenic that capable of producing acid from carbohydrates consumed by the host [4]. This acid produced by the bacteria causes a reduction in the pH of plaque, which causes dental caries due to demineralization [5]. Thus, *S. sanguinis* bacteria indirectly contribute to the occurrence of dental caries by forming early colonies of biofilm.

The formation of biofilm formation is divided into three phases: The adhesion phase (0–4 h), the accumulation phase (4–20 h), and the maturation phase (after 20 h) [6]. In the adhesion phase, bacteria such as *Streptococci* (61–78%) and *Actinomyces* (4–30%) are found predominantly attached to the pellicle. Types of *Streptococci* include *S. sanguinis*, *Streptococcus mitis*, and *Streptococcus oralis* [7]. In the accumulation phase, due to accumulation and rapid growth, the amount of bacteria in the biofilm increases exponentially [8]. After 20 h, the maturation phase in which bacterial growth begins to slow down or stop due to limited nutrients occurs [7].

Phytochemical tests of breadfruit leaf extract (*A. altilis*), using the maceration method with 70% ethanol, have indicated that the extract contains active compounds including secondary metabolites that function as antibacterial agents. These include flavonoids, polyphenols, quinones, steroids, saponin, monoterpene, and sesquiterpene [9]. Antibacterial compounds can kill bacteria by suppressing its growth or its ability to reproduce. An ideal antimicrobial agent has selective toxicity, indicating that it is harmful to the parasite but not to the host [10,11]. The results of a recent study have indicated that antibacterial agents kill bacteria effectively at the time the bacteria are growing (i.e., during the accumulation phase) [12].

Based on the active compounds that may act as antibacterial agents present in the leaves of the breadfruit, as well as evidence, suggesting that *S. sanguinis* is an early colonizer that plays a role in the early formation of plaque, the aim of this study was to examine the antibacterial effect of breadfruit extract against the viability of *S. sanguinis* during both the accumulation (20 h) and the maturation (24 h) phases. We expect that our findings will aid in the development and utilization of the breadfruit plant as a traditional medicinal plant that may improve oral and dental health.

METHODS

This study was based on a laboratory experimental design. The research was conducted in the Laboratory of Oral Biology, FKG UI. The bacterial sample used was *S. sanguinis* ATCC 10556.

Preparation of *S. sanguinis* solution

The main solution was prepared by taking several bacterial colonies from the culture on the BHI and inserting them into a 10 mL BHI broth tube. Then, the main solution was inserted into the anaerobic jar and was filled with 95% NO_2 and 5% CO_2. Next, the solution was incubated at 37°C for 24 h.

Determination of the concentration range of the breadfruit leaf extract

Before making the various concentrations, the breadfruit leaf extract was filtered using a Minisart with a diameter of 0.2 mm. The breadfruit leaf extract was made according to the desired concentrations of 5, 10, 20, 40, 80, and 100%.

Biofilm model preparation

For the preparation of the biofilm, 100 µL of artificial saliva was inserted into every 96 well-plate and was incubated at 37°C for 90 min. The saliva that was not attached to the well was removed and was rinsed with 100 µL phosphate-buffered saline (PBS). Furthermore, 100 µL of *S. sanguinis* bacteria at 10^6 CFU/mL standardized suspension was used to expose each well and incubated for 20 h and 24 h at 37°C. Wells with formed biofilms were rinsed with 100 µL PBS solution.

Exposure to the breadfruit leaf extract

In each well plate that had formed a biofilm, we added 100 µL of extract at various concentrations. For the positive control, each well was exposed to 100 µL chlorhexidine (0.1%) (Chl 0.1%). For the negative control, the biofilm model was exposed to BHI without an antibacterial agent. Then, the well plate was inserted into an anaerobic jar containing 95% NO_2 and 5% CO_2 mixed gas and incubated for 2 h at 37°C. Finally, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was performed. 50 µL of MTT solution was added to each 96-well plate and was incubated for 3 h at 37°C. Then, 100 µL of acidified isopropanol was added to each well and placed on the shaker for 1 h. The optical density (OD) values were read on a microplate reader with a wavelength of 490 nm.

The viability of *S. sanguinis* was calculated using the formula:

$$\text{cell viability}(\%)=\frac{\text{Absorbance value of treatment group}}{\text{Absorbance value of control group}}\times 100\%$$

Data analysis

One-way analysis of variance (ANOVA) was used to analyze the viability data for each treatment group of *S. sanguinis*.

RESULTS

In this study, we investigated the antibacterial effect of breadfruit extract against the viability of *S. sanguinis* using the MTT test. The viability was determined by the value of the OD, which was read with a wavelength of 490 nm. The results of the Kolmogorov–Smirnov normality test showed that the data had a normal distribution ($p>0.05$). The data were analyzed by one-way ANOVA.

Fig. 1 shows the viability of *S. sanguinis* control group as 100%, with a mean value of OD 1.094±0.053. Chl 0.1% was used as the positive control, which resulted in *S. sanguinis* viability of 14% (OD 0.157±0.02). In the groups exposed to breadfruit leaf extract at different concentrations, *S. sanguinis* viability was 86% (5% concentration; OD 0.945±0.164), 66% (10% concentration; OD 0.718±0.083), 42% (20% concentration; OD 0.454±0.036), 40% (40% concentration; OD 0.437±0.053), 38% (80% concentration; OD 0.431±0.048), and 36% (100% concentration; OD 0.398±0.053). The statistical results revealed that all the treatment groups were significantly different compared with the control group ($p<0.05$).

Fig. 2 shows the viability of *S. sanguinis* in the control group of 100%, with a mean value of OD 0.898±0.096. Exposure to Chl 0.1% resulted in *S. sanguinis* viability of 23% (OD 0.21±0.046). In the treatment groups exposed to the breadfruit leaf extract at different concentrations, *S. sanguinis* viability was 75% (5% concentration; OD 0.674±0.082), 54% (10% concentration; OD 0.489±0.029), 47% (20% concentration; OD 0.422±0.062), 38% (40% concentration; OD 0.340±0.027), 50% (80% concentration; OD 0.437±0.045), and 56% (100% concentration; OD 0.498±0.029). Our statistical results revealed that all the treatment groups were significantly different compared with the control group ($p<0.05$).

Fig. 3 shows a significant decrease in the viability of *S. sanguinis* biofilm. The viability of *S. sanguinis* after exposure to Chl 0.1% as the positive control at the 20 h phase was lower than that at the 24 h phase. This was observed also in the groups exposed to the breadfruit leaf extract at 20, 80, and 100%, and there was a significant decrease in the viability of *S. sanguinis* at the 20 h phase compared with the 24 h phase ($p<0.05$). In addition, viability was different in the groups exposed to the breadfruit leaf extract at 5, 10, and 40% at the 20 h phase, which showed a

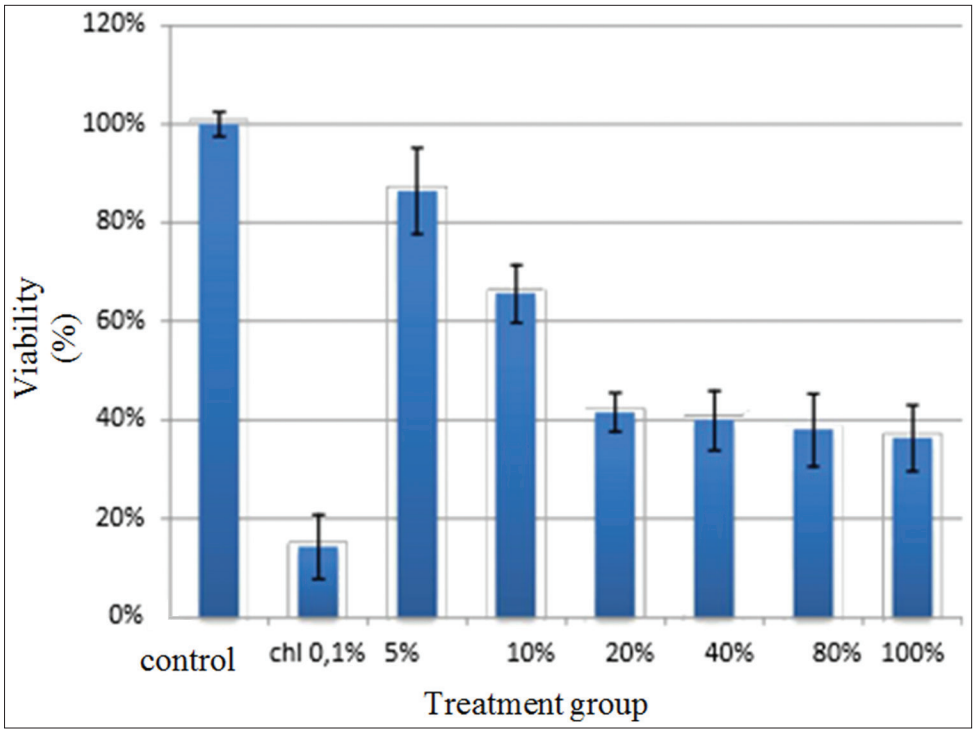

Fig. 1: *Streptococcus sanguinis* viability after exposure to the breadfruit leaf extract during the accumulation phase (20 h)

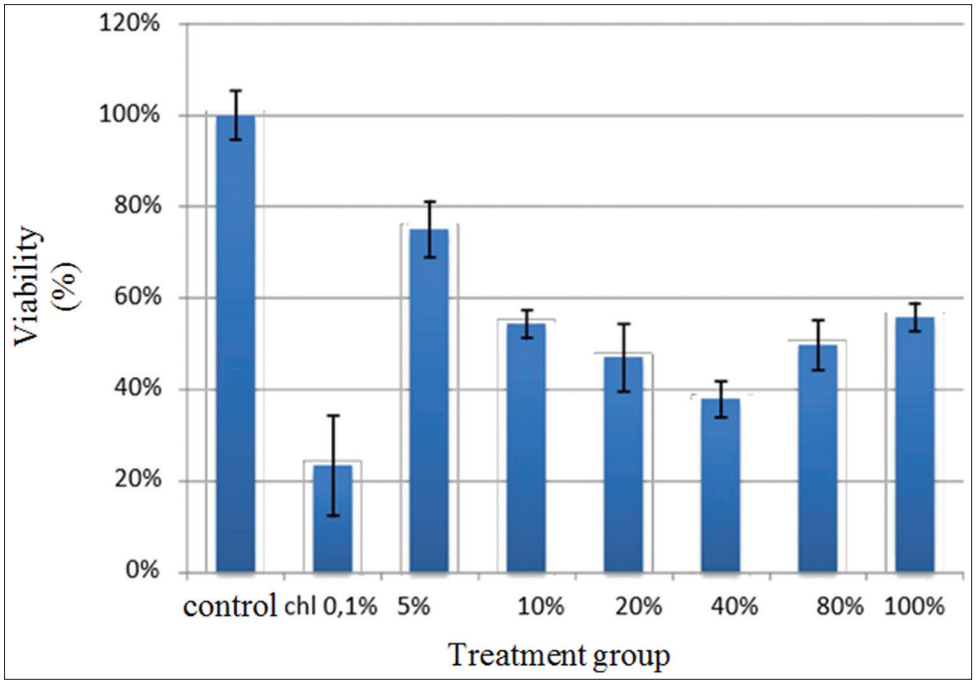

Fig. 2: *Streptococcus sanguinis* viability after exposure to the breadfruit leaf extract during the maturation phase (24 h)

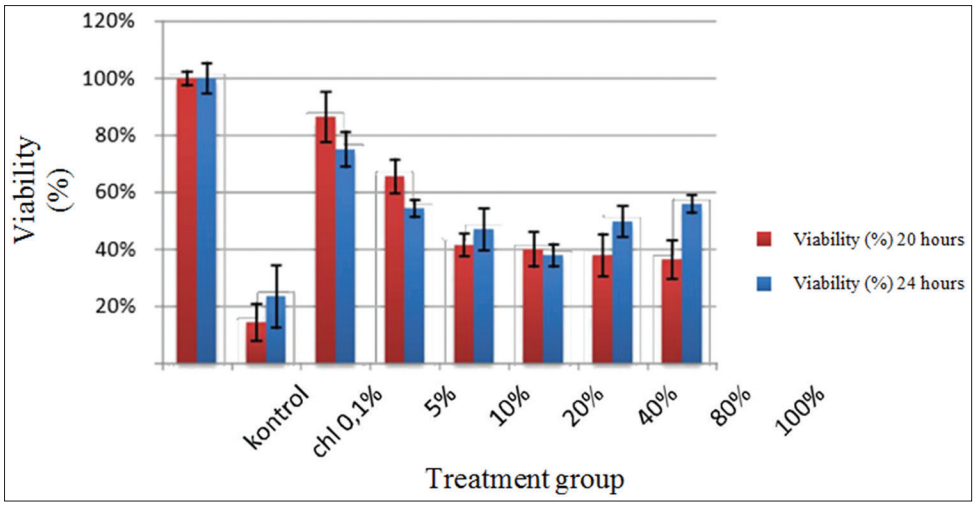

Fig. 3: Comparison of *Streptococcus sanguinis* biofilm viability after exposure to the breadfruit leaf extract for 20 h and 24 h

significant increase in the viability of *S. sanguinis* compared with the 24 h phase ($p<0.05$).

DISCUSSION

Our results showed that the breadfruit leaf extract at concentrations of 5, 10, 20, 40, 80, and 100% in the accumulation phase (20 h) and maturation phase (24 h) can decrease the viability of *S. sanguinis* compared with the control group. This decrease in the viability of *S. sanguinis* after exposure to the breadfruit leaf extract was suspected because the breadfruit leaf extract has been reported to have antibacterial properties due to the phenolic content consisting of tannins, saponins, and flavonoids. Indeed, tannin is believed to interfere with bacterial cell permeability, resulting in the inhibition of bacterial growth [13]. Similarly, saponins have antibacterial activity by disrupting cell permeability by inhibiting ionic membranes. Flavonoids are phenolic compounds that act as disinfectants and that work by denaturing proteins, which cause bacterial cell metabolism to stop resulting in cell death. Flavonoids are also bacteriostatic and serve to inhibit bacterial cell wall synthesis [14].

Our results showed that the breadfruit extract at a low concentration of 5% was able to decrease the viability of *S. sanguinis* at the 20 h phase (Fig. 1). This is in accordance with other researches that have tested the breadfruit leaf extract against the Gram-positive bacteria *Bacillus subtilis*. The results of this previous study showed that the higher the concentration of the breadfruit leaf extract, the greater the inhibition, with a concentration of 5% being able to inhibit the growth of Gram-positive bacteria [15]. Our results are also in accordance with other studies using the MTT method to test the viability of *S. sanguinis* with a temulawak ethanol extract. The reported results showed a decrease in the viability of *S. sanguinis* with increasing concentrations of extract in the accumulation phase (20 h) [16].

Fig. 2 shows a decrease in the viability of *S. sanguinis*, along with increasing concentrations of the breadfruit leaf extract, from 5% to 40%, in the maturation phase (24 h). However, at concentrations of 80% and 100%, there was an increase in viability again. This may be because, during the maturation phase, the biofilm that has been formed can increase resistance to antibacterial agents. Indeed, evidence suggests that the thicker the biofilm, the more difficult it is for antibacterial

agents to penetrate the biofilm [17]. During the maturation phase, the best optimum dose to decrease the viability of *S. sanguinis* appears to be 40% concentration of breadfruit leaf extract. It appears that at higher concentrations of breadfruit leaf extract, the viability decreases. However, after reaching an optimum point, the effectiveness of the breadfruit leaf extract decreases as a result of the increased viability of *S. sanguinis* [18]. While the previous study that examined the temulawak ethanol extract on the viability of *S. sanguinis* initially reported a decreased viability with a concentration of 5%, viability then increased again at a concentration of 15%, with a subsequent decrease again at a concentration of 25%. The cause of this is not known; however, the authors suspected that the temulawak ethanol extract has different effects on the viability of *S. sanguinis* depending on the dosage of the extract used [16].

In Fig. 3, the exposure to the breadfruit leaf extract at 20, 80, and 100% showed that the viability of *S. sanguinis* at 20 h was lower than that at 24 h. At the 24 h phase with the concentrations of 20, 80, and 100%, *S. sanguinis* bacteria appeared to be resistant to the breadfruit leaf extract. It is possible that in the accumulation phase (20 h), bacterial growth is still active and facilitates antibacterial substances to work optimally. In contrast, during the maturation phase, the biofilm formed is thicker than in the accumulation phase; thus, antibacterial substances may have greater difficulty penetrating into biofilms [8,17].

CONCLUSION

Breadfruit leaf extract at concentrations of 5, 10, 20, 40, 80, and 100% decreases the viability of *S. sanguinis* biofilms in the accumulation phase (20 h) and maturation phase (24 h). However, the viability of *S. sanguinis* biofilms after exposure to the breadfruit leaf extract at 20, 80, and 100% in the accumulation phase (20 h) was lower than that in the maturation phase (24 h).

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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