

## Influence test of *Averrhoa bilimbi* leaf extract as denture cleanser on the growth of *Streptococcus mutans*

Dewi Kristiana\*), Depi Praharani\*\*

\*Department of Prosthodontic Faculty of Dentistry Universitas Jember

\*\*Department of Biomedical Microbiology Faculty of Dentistry Universitas Jember

### ABSTRACT

It has been believed for a long time, especially by Indonesian people, that *Averrhoa Bilimbi* leaves have medical efficacy. *Streptococcus mutans* (*S. mutans*) are bacteria mostly found in plaque. The plaque on denture may cause inflammation in mucosal tissue under the denture namely denture stomatitis. This study was aimed at observing the differences of *Averrhoa Bilimbi* leaf extract efficacy as denture cleanser in concentration of 4%, 8%, 16% for 15 minutes, 30 minutes, 1 hour, dan 8 hours on the growth of *S. mutans*. The samples were made from heat-cured resin acrylic plates with dimension of 10x10x1 mm. Heat-cured resin acrylic plates were immersed in *Averrhoa Bilimbi* leaf extract with concentration of 4%, 8%, 16% for 15 minutes, 30 minutes, 1 hour, dan 8 hours which each concentration contained of 8 samples, and heat-cured resin acrylic plate were immersed in the aquades as control. To observe the inhibiting force of *Averrhoa Bilimbi* leaf extract on the growth of *S. mutans*, it was tested using spectrometer. The data were analyzed using one way ANOVA. The result showed that there were significant differences ( $p < 0.05$ ). Then the data were analyzed using LSD test and it showed that there were significant differences on heat-cured resin acrylic plates immersed in *Averrhoa Bilimbi* leaf extract with concentration of 4%, 8%, 16% for 15 minutes, 30 minutes, 1 hour, dan 8 hours on the growth of *S. mutans*. In conclusion, *Averrhoa Bilimbi* leaf extract in concentration of 16% used to immerse the acrylic plate for 8 hours effectively inhibited the growth of *S. mutans*.

**Key words:** Heat cured acrylic, *Averrhoa bilimbi* leaves, *Streptococcus mutans*

### ABSTRAK

Telah dipercaya untuk waktu yang lama, terutama oleh masyarakat Indonesia, bahwa ekstrak daun *Averrhoa bilimbi* memiliki khasiat medis. *S. mutans* adalah bakteri yang banyak ditemukan dalam plak. Plak pada gigi tiruan dapat menyebabkan peradangan pada jaringan mukosa dibawah gigi tiruan yaitu denture stomatitis. Penelitian ini bertujuan untuk mengamati perbedaan efikasi ekstrak daun *Averrhoa bilimbi* sebagai pembersih gigi tiruan dalam konsentrasi 4%, 8%, 16% selama 15 menit, 30 menit, 1 jam, dan 8 jam pada pertumbuhan *S. mutans*. Sampel dibuat dari pelat resin akrilik heat-cured dengan dimensi 10x10x1 mm. Lempeng resin akrilik heat-cured yang direndam dalam ekstrak daun *Averrhoa bilimbi* dengan konsentrasi 4%, 8%, 16% selama 15 menit, 30 menit, 1 jam, dan 8 jam yang masing-masing berisi dari 8 sampel, dan resin plat akrilik direndam dalam aquades sebagai kontrol. Untuk mengamati

kekuatan menghambat ekstrak daun *Averrhoa bilimbi* terhadap pertumbuhan *S. mutans*, diuji dengan menggunakan spektrometer. Data dianalisis dengan menggunakan ANOVA satu arah. Hasil penelitian menunjukkan bahwa ada perbedaan yang bermakna ( $p < 0.05$ ). Kemudian data dianalisis dengan uji LSD dan menunjukkan bahwa ada perbedaan yang signifikan pada lempeng resin akrilik heat-cured yang direndam dalam ekstrak daun *Averrhoa bilimbi* dengan konsentrasi 4%, 8%, 16% selama 15 menit, 30 menit, 1 jam, dan 8 jam pada pertumbuhan *S. mutans*. Kesimpulannya, ekstrak daun *Averrhoa bilimbi* dalam konsentrasi 16% yang digunakan untuk merendam plat akrilik selama 8 jam efektif menghambat pertumbuhan *S. mutans*.

**Kata kunci:** Akrilik heat cured, *Averrhoa bilimbi*, *Streptococcus mutans*

## INTRODUCTION

Polymethyl methacrylate acrylic resin material (PMMA) is still widely used in the field of prosthodontic as a denture base, although it is now widely available denture base material of metal or metal frame denture. Acrylic resin has color and optical properties that remain stable under normal conditions of the oral cavity and physical properties that have been proven suitable for dental applications.<sup>1</sup>

The use of artificial teeth is one of the factors that could cause the increase of *Candida albicans* in the mouth. Closure of the mucosa by the denture base may reduce the self cleansing effect of the saliva. As a result, the food debris and microorganisms accumulate; including *C. albicans*. The number of colony density *C. albicans* in denture users is reported that it depends on the length of using and user habits. When the denture is used continuously, including at night, the density of *C. albicans* will increase and increase the tendency for the occurrence of denture stomatitis.<sup>2</sup> According to Wahyuningtyas and Indrastuti<sup>3</sup>, the accumulation of microorganisms and plaque attached to the denture is the same as that of the natural teeth. *Streptococcus mutans* is the most often bacteria found on the plaque because its main habitat is plaque and colonize on the tooth surface, forming plaque. Denture stomatitis is a common condition found in 35-50% complete denture users. The prevalence of denture stomatitis on the partial denture users is less than complete denture users whose numbers ranged from 10-70%, depending on the study population.<sup>4</sup>

Prevention of denture stomatitis needs to be done by denture users, which could be done by

cleaning it with denture brush or by soaking the dentures in denture cleanser. Removable denture users are instructed to remove the dentures at night or for 6-8 hours a day to provide an opportunity for supporting tissues rest.<sup>4</sup> Denture cleansing by soaking can be done a whole night long or for 1 hour, 30 minutes, or 15 minutes, depends on the cleanser used.<sup>5</sup>

The Indonesian government is currently promoting the use of traditional medicines as an alternative treatment, because Indonesia is rich in medicinal plants. The use of traditional medicines derived from plant is currently based on empirical data, because of the lack of scientific data. Therefore, it is necessary to continue the excavation, research and testing for traditional medicines so that the development of traditional medical plant is medically accountable.<sup>6</sup>

One of the commonly used medicinal plants is the carambola or also known as starfruit (*Averrhoa bilimbi* L). Carambola or starfruit plant parts that can be used as traditional medicine are the leaves, fruit and flowers. Chemical content of its leaves (*Averrhoa bilimbi* L) are saponin, tannin, sulfuric acid, formic acid and peroxide.<sup>7</sup> Several studies on the efficacy of carambola leaves and fruit have been reported. Roosinta<sup>8</sup> observed that star fruit juice with a concentration of 4%, 8%, 16% can reduce the number of oral bacteria significantly. While Linda<sup>9</sup> observed that the methanol extract of leaves of carambola can inhibit the growth of *Staphylococcus albus*, *Staphylococcus aureus*, *Streptococcus hemolyticus*, and *Pseudomonas aeruginosa* in vitro.<sup>9</sup>

There have been several studies on the efficacy of the fruit and leaves of carambola, however, there has been no research on the antibac-

The results of the statistical test of homogeneity  $p > 0.05$  indicated the similar level of diversity of treatment (homogeneous). Furthermore test of normality  $p > 0.05$  is normally done, and then performed statistical analysis using one-way ANOVA test to see differences in the growth of *S. mutans* in the acrylic resin plate after immersion in leaf extracts of Carambola with a concentration of 4%, 8%, 16% for 15 minutes, 30 minutes, 1 hour and 8 hours and sterile distilled water as control.

Based on the results obtained by one-way ANOVA test  $p\text{-value} = 0.00$  ( $p < 0.05$ ), there was a significant difference in the growth of *S. mutans* in acrylic resin plate after immersion in leaf extracts of Carambola with a concentration of 4%, 8%, 16% for 15 minutes, 30 minutes, 1 hour and 8 hours and distilled water as control. LSD test was then performed (Tab. 2) to assess the differences between the concentration of leaf extract of 4%, 8%, 16%, acrylic plate immersion time 15 minutes, 30 minutes, 1 hour, 8 hours and distilled water as control. LSD test results showed that there were significant differences ( $p < 0.05$ ) growth of *S. mutans* in acrylic plate is immersed in all the concentration and duration of submersion, except at a concentration of leaf extract leatherback 4% for 30 minutes, 1 hour, 8 hours with a concentration of 8% for 15 minutes, 30 minutes, and the concentration of 16% for 30 minutes.

## DISCUSSION

Denture acrylic resin can be an accumulation point of stain, tartar and plaque that will adversely affect oral health. Denture is also a place to accumulate food debris. Soft food debris stuck in denture will cause bacteria to increase gradually.<sup>10</sup> *S. mutans* is the most often found bacteria on the plaque because its main habitat is a plaque and colonize the tooth surface, forming plaque formation. Denture plaque can cause inflammation of the mucosal tissue under the denture, causing denture stomatitis. Prevention of denture stomatitis needs to be done by denture users, for example by soaking the dentures in the evening beside maintaining and cleaning action

The results obtained by one-way ANOVA test  $p\text{-value} = 0.00$  ( $p < 0.05$ ) showed a significant difference in the growth of *S. mutans* in acrylic resin plate after immersion in leaf extracts of Caram-

bola with a concentration of 4%, 8%, 16% for 15 minutes, 30 minutes, 1 hour and 8 hours and distilled water as control. Leaf extracts of Carambola contains saponin, tanin, sulfur, format acid and peroxide.<sup>7</sup>

Tannins are astrigent, such research has been done by Fithrony<sup>11</sup> indicating that the material in boiling *gambier* tannins can inhibit the adhesion of *S. mutans* on the denture immersion, is bacteriostatic and bactericide. Wahyuningtyas and Indrastuti<sup>3</sup> show that tannins and flavonoids antibacterial power of the purple leaf extract can reduce *S. mutans*. While, Kozai et al in Fithrony<sup>11</sup>, which conducts research on *Gambier* has a high tannin content, indicating that it is able to inhibit insoluble glucan by glucosyltransferase from sucrose (GTF), which has an important role in the formation of plaques.<sup>11</sup>

Leaf extract solution Carambola with increasing concentration causes a decrease in the growth of *S. mutans*. This is due to the increasing concentrations of the extracts also affects on the increase of the content of tannins. Increased levels of tannins resulting in anti bacterial power will increase as well. Immersion time for 15 minutes, 30 minutes, 1 hour and 8 hours in a solution of leaf extract Carambola also reduced growth *S. mutans*. The increase of contact time increases the antimicrobial effectiveness. Additionally tannins have physiological effects and pharmacological effects due to its ability to form complexes with both proteins and polysaccharides. Complex formation was based on the formation of hydrogen bonds and hydrophobic interactions between tannins (polyphenols group) with the protein.

Antimicrobial ability of tannin compounds based on the ability of these compounds inhibit the action of certain enzymes or the ability to selectively inhibit the binding between a ligand with a receptor. It is possible that tannins are chemicals that most of the spread in the plant is capable of inhibiting bacterial cell wall synthesis and protein synthesis of cells of gram-positive bacteria (*S. mutans*). Tannin compounds existing bond has the ability, among others: as astrigen or material capable of detoxification protein precipitate and form a particular compound, interacts with proteins and form so as to inhibit salivary pelikel *S. mutans* adhesion in vitro, thereby reducing the adhesion of bacteria.<sup>12</sup>

terial effects of extracts of leaves of carambola as a denture cleanser. Therefore, the researchers wanted to conduct research on the efficacy of starfruit leaf extract as a denture cleaning agent with a concentration of 4%, 8%, 16% and an acrylic resin plate long immersion in a solution of the extract for 15 minutes, 30 minutes, 1 hour and 8 hours on the growth of *S. mutans*. The results obtained is expected to provide scientific information about the use of starfruit leaf extract solution as a cleansing agent of acrylic resin denture with the correct quantity and quality, so that it can be used as an alternative of denture cleaning materials to maintain the cleanliness of the denture.

## METHODS

This study was an experimental laboratory study conducted at the Laboratory of Dental Materials Science and Technology Faculty of Dentistry Universitas Jember and Laboratory of Microbiology Faculty of Dentistry, Universitas Airlangga. Materials used in this study were heat cured acrylic resin, starfruit leaf extract, and the suspension of *S. mutans*. Samples were made of acrylic heat cured with a size (10x10x1) mm. All the acrylic plate was immersed in the leaf extract of starfruit with a concentration of 4 groups, 4%, 8%, 16%, respec-

tively for 15 minutes, 30 minutes, 1 hour and 8 hours. As a control, an acrylic plate was soaked in distilled water. Each group consisted of 8 samples. Following the soaking of acrylic plate, rinsing was performed using a solution of 2x PBS, continue with immersion in BHIB, vibration for 30 seconds, then the calculation of the growth of *S. mutans* was conducted with a spectrometer.

## RESULTS

The result of mean growth of the *S. mutans* in acrylic resin plate after immersion in 4%, 8%, and 16% concentration of starfruit leaf extracts for 15 minutes, 30 minutes, 1 hour, and 8 hours as well as in distilled water to act as a control, can be seen in Table 1.

Table 1. Bacterial growth rates of *S. mutans* in acrylic resin plate after immersion in starfruit leaf extract

Group	Mean (A)			
	15 Minutes	30 Minutes	1 Hour	8 Hour
Starfruit 4 %	18.3	15.15	12.22	9.53
Starfruit 8 %	15.3	12.45	9.34	6.34
Starfruit 16 %	11.85	9.15	6.23	3.38
Aquadest sterile	29.63	29.46	28.65	30.01

Table 2. LSD test on the growth of *S. mutans* in acrylic resin plate after immersion in leaf extracts of *Averrhoa bilimbi* with a concentration of 4%, 8%, 16% for 15 minutes, 30 minutes, 1 hour and 8 hours and distilled water as control

concentration	Time	4% Star fruit leaf extract				8% Star fruit leaf extract				16% Star fruit leaf extract			
		15 min	30 min	1 h	8 h	15 min	30 min	1 h	8 h	15 min	30 min	1 h	8 h
4% Star fruit leaf extract	15 min	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	30 min	0.00		0.00	0.00	0.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1 hour	0.00	0.00		0.00	0.00	0.67	0.00	0.00	0.55	0.00	0.00	0.00
	8 hour	0.00	0.00	0.00		0.00	0.00	0.79	0.00	0.00	0.52	0.00	0.00
8% Star fruit leaf extract	15 min	0.00	0.79	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
	30 min	0.00	0.00	0.69	0.00	0.00		0.00	0.00	0.29	0.00	0.00	0.00
	1 hour	0.00	0.00	0.00	0.79	0.00	0.00		0.00	0.00	0.69	0.00	0.00
	8 hour	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.79	0.00
16% Star fruit leaf extracts	15 min	0.00	0.00	0.55	0.00	0.00	0.29	0.00	0.00		0.00	0.00	0.00
	30 min	0.00	0.00	0.00	0.52	0.00	0.00	0.69	0.00	0.00		0.00	0.00
	1 hour	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.79	0.00	0.00		0.00
	8 hour	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Aquadest		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: Significancey  $p > 0.05$

Phenol has the ability to denaturate protein and cell membrane damage. Phenol binds to the protein through hydrogen bonding resulting in protein structure to be damaged. Most of the structure of the cell wall and cytoplasmic membrane of bacteria contains proteins and fats. Instability in the cell wall and cytoplasmic membrane of bacteria causing the function of selective permeability, active transport function, controlling the composition of proteins from bacterial cells to be disrupted.

LSD test results (Tab. 2) show that there were significant differences ( $p < 0.05$ ) growth of *S. mutans* in acrylic plate was immersed in all the concentration and duration of submersion, except at a concentration of leaf extract leatherback 4% for 30 minutes, 1 hour, 8 hours with a concentration of 8% for 15 minutes, 30 minutes, and the concentration of 16% for 30 minutes. Although the acrylic plate is immersed in the leaf extract with a concentration of 4% Carambola for 30 minutes, 1 hour, 8 hours with a concentration of 8% for 15 minutes, 30 minutes, and the concentration of 16% with a 30-minute immersion time there was no significant difference to the growth of *S. mutans*, but the average amount of growth of *S. mutans* decreased according to increasing concentration and duration of immersion.

In this research, in addition to the treatment group there was also a control group that uses immersion in distilled water. From the data analysis, there was significant differences between the treatment and control groups ( $p < 0.05$ ). Data showed the number of the growth of bacteria *S. mutans* increases with the longer immersion. Increased growth of *S. mutans* on immersion in sterile distilled water comes from a breed *S. mutans* with increased immersion time. Distilled water does not contain chemical compounds that are bacteriostatic and bacteriocide, so that distilled water could not inhibit growth of *S. mutans*.

## CONCLUSION

From these results it could be concluded that the higher concentration of leaf extract solution of Carambola (*Averrhoa bilimbi* L) was 4%, 8%, 16% and the longer time of immersion of acrylic resin plate in a solution of leaf extract

Carambola which was 15 minutes, 30 minutes, 1 hour and 8 hours inhibit the growth of *S. mutans* on the acrylic plate. Concentration of 16% with long of immersion of 8 hours effectively inhibited growth of *S. Mutans*.

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