



# FFECTIVENESS OF LIME (*Citrus aurantifolia*) FRUIT EXTRACT IN SOAKING HEAT CURED ACRYLIC RESIN PLATES ON BACTERIA *Streptococcus mutans*

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## Abstract: -

**Background:** Dentures as a prosthesis to overcome tooth loss have several components, one of which is the denture base. Denture bases can be made of both metallic or non-metallic materials such as heat cured acrylic resin. Heat cured acrylic resin has a drawback, namely the occurrence of porosity. As a result of porosity, the acrylic resin plate become a place for bacterial accumulation that it will cause denture stomatitis. Denture cleaning needs to be done so that the health of the oral cavity is maintained. Therefore, the use of herbal ingredients as an alternative to denture cleaning materials continues to be developed. Lime (*Citrus aurantifolia*) contains alkaloids, flavonoids, steroids, phenols, and tannins that have antibacterial effects. **Objective:** To analyze the effect of lime (*Citrus aurantifolia*) fruit extract concentration on immersion of heat cured acrylic resin on *Streptococcus mutans*. **Materials and Methods:** This type of study was a laboratory experimental with post test only control group design. There were 32 samples of heat cured resin acrylic with the size of each sample 10x10x2 mm<sup>3</sup> which were divided into 2 groups, first group was 40% lime fruit extract and the second group was sterile aquades. **Results:** Immersion of heat cured acrylic resin denture base soaked with 40% lime fruit extract showed a significant difference in the number of colonies compared to sterile aquades (p<0,05). **Conclusion:** Soaking a heat cured acrylic resin plate on a 40% lime fruit extract can have an effect on *Streptococcus mutans*.

**Keywords:** Lime fruit, streptococcus mutans, Heat cured resin Acrylic, Antibacterial.

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## Introduction

Partial tooth loss or partial edentulousness occurs when one or more teeth are missing in the dental arch, but not all the teeth are missing. In Indonesia, 14% of denture users are over 15 years old, while the other 54% are over 65 years old, this data was obtained from a survey conducted by GlaxoSmithKline.<sup>1,2</sup> The currently available dentures, namely removable dentures and teeth fixed dentures, but removable dentures made from acrylic resin are the most popular among the public.<sup>1</sup> The use of acrylic resin or polymethyl methacrylate (PMMA) has been used in most denture bases since the mid-1940s. Until now, the use of acrylic resin or polymethyl methacrylate (PMMA) is still widely used in making dentures.<sup>3,4</sup>

The dentures used must be kept clean. There are several methods for cleaning dentures, namely mechanical methods such as brushing, chemical methods by soaking them in cleaning fluid, or by combining the two methods.<sup>5</sup> Herbal ingredients can also be used to clean dentures.<sup>6</sup> Herbal ingredients the one chosen in this research was a lime extract solution.

## Materials and methods

The type of research used was laboratory experimental with a post test only control group design. The place of research was carried out at the PT Laboratory Palapa Muda Perkasa for making lime fruit extract as well as at the Microbiology Center of Research and Education (MiCORE) Laboratory of the Faculty of Dentistry, Trisakti University for cultivating and testing bacteria. The research will be carried out in May - June 2024. Lime fruit provided by the PT Laboratory. Palapa Muda Perkasa which then carried out the extraction process using the maceration method with 98% methanol solvent. The acrylic resin plate used as a research sample measures 10x10x2 mm which has gone through a finishing and polishing process. The number of samples was 32. Immersion of heat cured acrylic resin plates was divided into 2 different groups, namely 1 experimental group and 1 control group. As follows: Group I: this treatment group underwent a soaking process in lime fruit extract with a concentration of 40% for 30 minutes. Group II: this treatment group underwent a process of immersion in distilled water which served as the control group.

Results

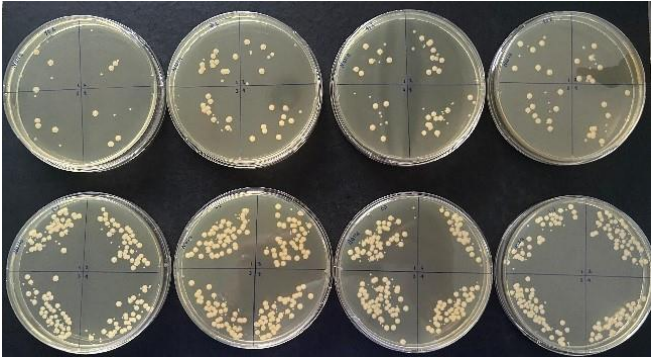


Fig 1.Streptococcus mutans colonies when soaked in 40% lime fruit extract and distilled water

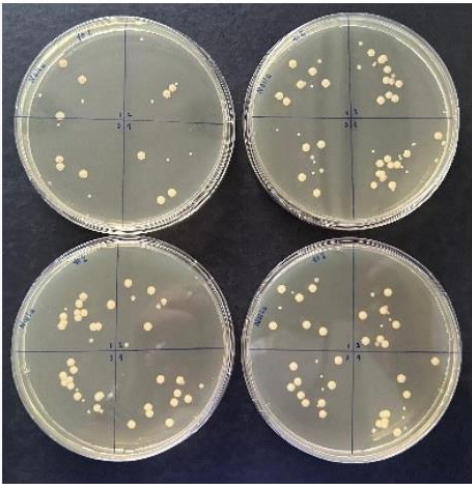


Fig 2.Streptococcus mutans colonies on immersion in 40% lime fruit extract

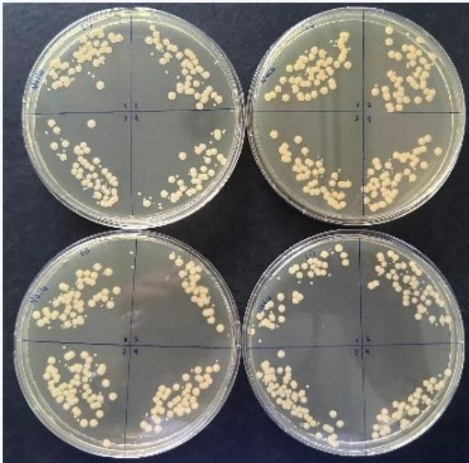


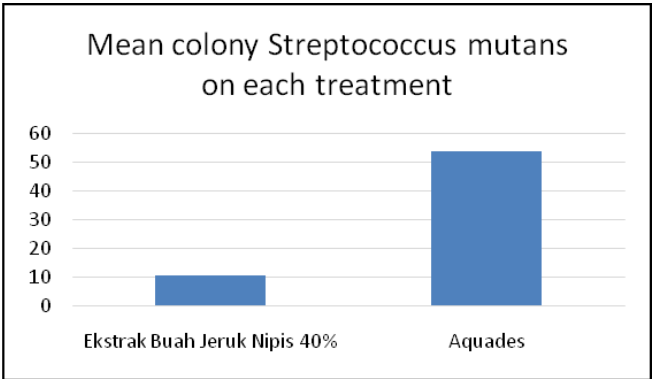
Fig 3.Streptococcus mutans colonies when soaked in distilled water

Descriptive Statistics

The first data analysis carried out was descriptive statistical analysis to determine the characteristics of the data from the results of research that had been carried out previously. The descriptive statistics calculated are the average (mean), median and standard deviation of the data studied. The results of descriptive statistical analysis can be seen in table 1 as follows.

Table 1 Mean, Median, and Standard Deviation of Streptococcus mutans Bacterial Data (CFU/mL)

|                    | Streptococcus mutans Bacterial<br>CFU/mL) |                        |
|--------------------|-------------------------------------------|------------------------|
|                    | lime fruit extract40%                     | Aquades                |
| 1                  | 9x10 <sup>6</sup>                         | 54x10 <sup>6</sup>     |
| 2                  | 13x10 <sup>6</sup>                        | 48x10 <sup>6</sup>     |
| 3                  | 14x10 <sup>6</sup>                        | 46x10 <sup>6</sup>     |
| 4                  | 12x10 <sup>6</sup>                        | 52x10 <sup>6</sup>     |
| 5                  | 5x10 <sup>6</sup>                         | 58x10 <sup>6</sup>     |
| 6                  | 5x10 <sup>6</sup>                         | 22x10 <sup>6</sup>     |
| 7                  | 5x10 <sup>6</sup>                         | 61x10 <sup>6</sup>     |
| 8                  | 5x10 <sup>6</sup>                         | 46x10 <sup>6</sup>     |
| 9                  | 14x10 <sup>6</sup>                        | 51x10 <sup>6</sup>     |
| 10                 | 7x10 <sup>6</sup>                         | 60x10 <sup>6</sup>     |
| 11                 | 15x10 <sup>6</sup>                        | 78x10 <sup>6</sup>     |
| 12                 | 11x10 <sup>6</sup>                        | 52x10 <sup>6</sup>     |
| 13                 | 10x10 <sup>16</sup>                       | 63x10 <sup>6</sup>     |
| 14                 | 16x10 <sup>6</sup>                        | 55x10 <sup>6</sup>     |
| 15                 | 10x10 <sup>6</sup>                        | 67x10 <sup>6</sup>     |
| 16                 | 20x10 <sup>6</sup>                        | 48x10 <sup>6</sup>     |
| Mean               | 10,69x10 <sup>6</sup>                     | 53,81x10 <sup>6</sup>  |
| Median             | 10,50x10 <sup>6</sup>                     | 53x10 <sup>6</sup>     |
| Standar<br>Deviasi | 4,542x10 <sup>6</sup>                     | 12,023x10 <sup>6</sup> |



Data Normality Test

Data on the number of colonies of Streptococcus mutans bacteria that had been collected were tested for data normality. The data normality test was carried out before carrying out parametric tests on the data resulting from calculating the number of colonies. The normality test used the Shapiro Wilk method because the number of research samples was less than 50 samples. This test aims to find out whether the research data is normally distributed or not. By making decisions based on the p-value or significance

value in the SPSS output, if the  $p$ -value  $> 0.05$ , then the research data is normally distributed and if the  $p$ -value  $< 0.05$ , then the research data is not normally distributed. The results of the normality test can be seen in Table 2 as follows.

**Table 2** Data Normality Tests for Number of Streptococcus mutans Bacterial Colonies

| No.                       | UjiNormalitas<br>(Nilai p*) | Keterangan |
|---------------------------|-----------------------------|------------|
| 1. lime fruit extract 40% | 0.307                       | Normal     |
| 2. Aaquades               | 0.174                       | Normal     |

It can be seen in Table 2 above, it is known that the results of the normality test data on the number of colonies of Streptococcus mutans bacteria in each treatment group were normally distributed because both treatment groups had a  $p$ -value or  $p$  value  $> 0.05$ .

### Homogeneity of Variants Test

A homogeneity test also needs to be carried out before carrying out a comparison test on data from calculating the number of Streptococcus mutans colonies. This test was carried out with the aim of finding out whether the data from the variables studied were homogeneous ( $p$  value  $> 0.05$ ) or not homogeneous ( $p$  value  $< 0.05$ ). The homogeneity test carried out was the Levene test. The homogeneity test results can be seen in Table 3 as follows.

**Table 3.** Homogeneity Test Data for Number of Streptococcus mutans Bacterial Colonies

|        | df1 | df2 | Sig.  | Keterangan |
|--------|-----|-----|-------|------------|
| Mean   | 1   | 30  | 0,052 | Homogen    |
| Median | 1   | 30  | 0,053 | Homogen    |

Based on the results of the homogeneity test in Table 3, a significance value ( $p$  value)  $> 0.05$  was obtained, so it can be concluded that the variants of the two groups of data are the same or can be said to be homogeneous data. Then, if the requirements for data analysis or statistical tests have been met, then parametric statistics will be carried out, namely the Independent T-Test.

### Parametric Independent T-Test

**Table 4** Independent T-Test Data on the Number of Streptococcus mutans Bacterial Colonies

|              | df | Sig. (2-tailed) | Diff         |
|--------------|----|-----------------|--------------|
| Data homogen | 30 | $< 0,001$       | -43.125 diff |

The Independent T-Test parametric test was carried out because the data was normally distributed. This test aims to determine the difference in average values between one group and another, where the groups are not paired with each other. The results above show that there is a significant difference between the 40% lime fruit extract treatment group and the negative control with a significance value ( $p$  value)  $< 0.001$  so that the null hypothesis ( $H_0$ ) is rejected and the alternative hypothesis ( $H_a$ ) is accepted because the  $p$  value  $< 0, 05$ . So, in other words, there was a significant difference in the number of Streptococcus mutans bacterial colonies between the 40% lime fruit extract treatment group and the negative control group with distilled water.

## Discussion

This research was carried out with the aim of determining the effect of lime fruit extract (*Citrus aurantifolia*) at a concentration of 40% on immersion in heat cured acrylic resin plates on Streptococcus mutans bacteria. The choice of heat cured acrylic resin as a material for making denture plates is not without reason, this material has properties that are easy to manipulate, small dimensional changes occur, is not toxic, does not irritate tissue and does not dissolve in oral fluids, and meets good aesthetic standards.<sup>7</sup>

The use of acrylic resin is of course inseparable from its drawbacks which include discoloration, leaving residual monomer which can cause allergies in the tissues in the oral cavity, easily absorbing liquids, and the occurrence of porosity. As a result of porosity, food debris easily sticks to the surface of the denture plate, resulting in an accumulation of stain and plaque which will eventually form bacterial colonies on the surface of the denture plate.<sup>1,8,9</sup>

The human oral cavity is the right place for all types of microorganisms to grow and develop because it has perfect humidity. Streptococcus mutans is a bacteria that is easily found in the oral cavity because it lives and colonizes the surface of teeth to form plaque. The presence of plaque on the surface of the denture will cause various problems related to periodontal tissue, discoloration of the denture, and is an etiological factor in the occurrence of denture stomatitis.<sup>10,11</sup>

The herbal plant used in this research is lime fruit (*Citrus aurantifolia*). Lime fruit was chosen because it contains chemical compounds such as saponins, tannins, alkaloids, flavonoids and phenols which have antibacterial properties. According to Jeffrey (2022), the antibacterial properties of lime fruit come from proteolytic and lipolytic properties which are influenced by its contents such as phenols, flavonoids and hydrogen peroxide. The flavonoid content in limes has antibacterial properties because it can damage bacterial cell walls. Several studies have stated that the mechanism of antibacterial activity of lime fruit is through the process of denaturing bacterial cell proteins, disrupting permeability and causing cell wall leakage.<sup>12, 13, 14</sup>

Apart from the fruit, various parts of the lime plant such as the skin, leaves and juice have been studied previously. Research by Adindaputri, et al (2013) regarding 10% concentration of lime peel extract on the activity of the Streptococcus mutans glucosyltransferase (GTF) enzyme, the result of which was that lime peel extract was proven to inhibit the activity of the Streptococcus mutans GTF enzyme. Another study was conducted by Ulya, et al (2018) who also used lime peel extract, stating that there was a killing power of lime peel extract in concentrations of 12.5%, 25%, 50% and 100% against Streptococcus mutans bacteria.<sup>15,16</sup>

The use of lime leaf infusion, which is formulated in the form of a tooth cleaning gel preparation, has been studied by Bachmid, et al (2022). In this study, it was stated that the lime leaf infused tooth cleaning gel preparation had antibacterial activity against Streptococcus mutans bacteria with the strongest inhibitory power at a concentration of 20%. 43 Another study using lime juice was conducted by Fitriani, et al (2016) stated that a combination preparation of 100% lime juice and 50% honey was able to inhibit

the growth rate of Streptococcus mutans bacteria when compared with 0.2% chlorhexidine gluconate.<sup>17</sup>

This research used samples in the form of 32 heat cured acrylic resin plates measuring 10x10x2 mm<sup>3</sup>. The samples to be used are sterilized first before being immersed in an artificial saliva solution for 1 hour. Artificial saliva is made as closely as possible to the function of natural human saliva. This is done considering that the function of saliva in the oral cavity is important in the mucosal defense system against various microorganism infections.<sup>18</sup> According to Gani, et al (2013) there is an interaction between the bacteria *S. mutans*, *C. albicans*, and *A. actinomycetemcomitans* on changes in saliva pH.<sup>19</sup> Next, the soaking samples were divided into two test groups, namely a 40% concentration of lime fruit extract solution and sterile distilled water with a soaking time of 30 minutes in each test group. This is supported by research conducted by Fajriani, et al (2015) which explains that there was a decrease in the number of Streptococcus mutans bacterial colonies after gargling with a lime extract solution for 30 minutes.

First, an extraction process is carried out to obtain the lime fruit extract that will be used. The extraction process uses the maceration method, which is the simplest method, but is not efficient in terms of processing time. The working mechanism of maceration is by placing herbal plant powder and the solvent used in a tightly closed container at room temperature.<sup>20</sup>

This research uses the Total Plate Count (TPC) testing method which is a method for estimating the number of microorganisms (fungi, yeast and bacteria) in a material. 48 TPC analysis uses Tryptic Soy Agar (TSA) media by streaking a diluted bacterial suspension onto in a petri dish, then incubated. The calculation results using this method are in CFU/mL units. The choice of TSA as a bacterial growth medium in this study was not without reason. TSA media was chosen because this media is recommended for the isolation and culture of aerobic and facultative anaerobic bacteria such as the Streptococcus mutans bacteria used in this study.<sup>21</sup>

Based on research conducted by researchers, it is known that the results of immersing heat cured acrylic resin plates in a 40% lime fruit extract solution compared with sterile distilled water as a control group showed a decrease in the number of Streptococcus mutans bacterial colonies (Table 1). In the results of calculating the number of colonies of Streptococcus mutans bacteria, it was found that the average number of colonies formed in the 40% lime fruit extract solution treatment was 10.69x106 CFU/mL, this number was lower when compared to the average number of colonies formed in the distilled water treatment. sterile, namely 53.81x106 CFU/mL. Then, the results of the parametric test showed that there was a significant difference between the two treatment groups, namely 40% concentration of lime fruit extract with sterile distilled water. Thus, it can be concluded that 40% concentration of lime fruit extract is effective in reducing the number of Streptococcus mutans bacterial colonies on heat cured acrylic resin denture plates.

Based on the experience that the researcher had in conducting this research, there were several limitations experienced by the researcher that need to be taken into account by other future researchers. The limitations of this research include not knowing the exact chemical compounds contained in the lime fruit extract used because the researchers did not carry out phytochemical tests before the extraction process. The chemical compound content

used as a reference in this research was taken from previous studies. Therefore, it is not known with certainty the chemical compounds contained in the lime fruit used in this research.

## Conclusion

Based on the results of research and data analysis regarding the effect of lime fruit extract solution (*Citrus aurantifolia*) on immersing heat cured acrylic resin plates on Streptococcus mutans bacteria, it can be concluded that:

1. Lime fruit extract (*Citrus aurantifolia*) with a concentration of 40% has an effect on reducing the number of Streptococcus mutans bacterial colonies on heat cured acrylic resin denture plates.
2. Lime fruit extract (*Citrus aurantifolia*) with a concentration of 40% has an effect on reducing the number of Streptococcus mutans bacterial colonies when compared with sterile distilled water on heat cured acrylic resin denture plates.

## Suggestion

After completing the research and knowing all the limitations that exist in this research, suggestions that can be given by researchers include:

1. Further research is needed regarding the effect of lime fruit extract (*Citrus aurantifolia*) on the number of Streptococcus mutans bacterial colonies with different concentrations, types of acrylic resin and soaking duration.
2. Further research is needed regarding the active compounds contained in lime fruit extract (*Citrus aurantifolia*) which have the best antibacterial properties and are able to reduce the number of Streptococcus mutans bacterial colonies to the maximum.

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