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**INHIBITION ACTIVITY OF *BELIMBING WULUH*(*Averrhoa bilimbi linn*) LEAF  
 EXTRACT TO *Streptococcus mutans* ON ACRYLIC PLATE**

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**ABSTRACT**

**Background:** The leaf of *Averrhoa bilimbi* contains flavonoids and tannins as antibacterial agent against *Streptococcus mutans*. The extract of *Averrhoa bilimbi* leaf can be used as a natural alternative ingredients of denture cleanser. **Purpose:** The main purpose of this research is to know the different antibacterial activities of *Averrhoa bilimbi* leaf extract 6.25%, 12.5%, 25%, 50%, and 100% against *Streptococcus mutans* on acrylic plate. **Methods:** The method of this research was true experimental with posttest-only with control group design that consisted of *Averrhoa bilimbi* leaf extract in 6.25%, 12.5%, 25%, 50%, 100% and chlorhexidine gluconate 0.2%. The antibacterial testing method was using diffusion method. The measurement parameter was the amount of inhibition zone formed in the test medium. The data analysis was using Kruskal-Wallis test which resulted in  $p=0.000$  ( $p<0.05$ ). The result showed that there were differences in the inhibitory activity between each group. The test then continued with Mann Whitney test that results in  $p=0.009$  ( $p<0.05$ ) which showed meaningful differences between each group. **Results:** The smallest inhibitory zone of 6.25% was 10.08 mm, the biggest inhibitory zone of 100% was 23.07 mm, and the inhibitory zone of chlorhexidine gluconate in 0.2% was 25.05 mm. The lower extract concentration inhibitory zone were smaller than the high extract concentration. There was significant increase in inhibitory activity of *Averrhoa bilimbi* leaf extracts cause by the flavonoids and tannins content as antibacterial. **Conclusion:** It can be concluded that there are differences in the inhibitory activity of *Averrhoa bilimbi* leaf extract and chlorhexidine gluconate 0.2%.

**Keywords:** *Averrhoa bilimbi* leaf extract, Chlorhexidine gluconate 0.2%, *Streptococcus mutans*, acrylic plate.

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**INTRODUCTION**

The public's need for dental and oral care is increasing, one of which is the manufacture of artificial teeth. Artificial teeth require an artificial tooth base, a denture base material that is still frequently used is a polymethyl methacrylate acrylic resin plate of heat cure type.<sup>1,2</sup> Selection of an acrylic resin plate as a denture base material was based on the fact that it meets the requirements of physical, functional and esthetic aspects.<sup>3</sup> Acrylic resin plate has advantages and disadvantages.<sup>3</sup> The advantages of acrylic resin plate are relatively cheap, the color resembles gingiva, easy in the manipulation and the making process, can be repaired and changed in small dimension.<sup>3</sup> The disadvantages of acrylic resin plate are easy to get abrasion at the time of cleaning and usage, absorbs

mouth fluids that can cause discoloration, plaque formation, calculus and porosity.<sup>2</sup>

Plaque and calculus formation can occur very quickly in the oral cavity due to bacterial colonization.<sup>4</sup> The most common bacterial colonization is *Streptococcus mutans*. It is the first bacteria attached to the acrylic denture base and produce an extracellular polysaccharide substrate (EPS).<sup>5</sup> Microbial adhesion to the denture surfaces can lead to the proliferation of bacterial colonies resulting in the formation of plaque and bad odor.<sup>6</sup>

Oral hygiene and denture hygiene needs to be maintained from contamination of *Streptococcus mutans* bacteria. The user can soak the denture in a denture cleanser containing a disinfectant solution such as chlorhexidine gluconate 0.2% for 15

minutes per day.<sup>7</sup> Chlorhexidine is a solution that is set as gold standard mouthwash due to its antimicrobial effects, its substantivity and antiseptic properties potentially inhibits *Streptococcus mutans*. The use of long-term chlorhexidine can cause discomfort to the user, discomfort can be caused by yellow or brown stains on the denture and mucosal mouth desquamation.<sup>8,9,10</sup> Natural alternative materials that can be used as an antibacterial denture cleanser which has antibacterial effect such as *Belimbing Wuluh* (*Averrhoa bilimbi linn*) leaf.

*Averrhoa bilimbi* leaf has some active substances that can inhibit bacterial growth. The content contained in the leaves of *Averrhoa bilimbi* as an inhibitor of bacterial growth is the active compounds of flavonoids, tannins, saponins, and triterpenoid.<sup>11</sup> Nurmaya Sari research (2013) showed that the treatment of extracts of *Averrhoa bilimbi* leaves has the greatest effect (6,2517 mm) and the smallest effect of the treatment of leaf *Averrhoa bilimbi* extract 6.25% (0.8133 mm).<sup>12</sup> Based on the description above, this study aims to determine the difference inhibitory activity of Belimbing Wuluh leaf extract wuluh 6.25%, 12.5%, 25% , 50%, and 100% of *Streptococcus mutans* in vitro on a heat cure acrylic plate.

#### MATERIALS AND METHODS

The method used in this research is a pure laboratory experimental method with post-test only with control group design with six treatments of *Averrhoa bilimbi* leaf extract 6.25%, 12.5%, 25%, 50%, 100% and chlorhexidine gluconate 0.2 % (positive control). The minimum number of repetitions for each treatment group was 5 times. This research was conducted in Microbiology Laboratory of Faculty of Medicine, University of Lambung Mangkurat Banjarmasin, laboratory skills room of Faculty of Dentistry, University of Lambung Mangkurat Banjarmasin and Basic Laboratory of Faculty of Mathematics and Natural Sciences of Universitas Lambung Mangkurat Banjarbaru from June to November 2016.

The tools used in this research are Bowl (rubber bowl), spatula, anatomical tweezers, model knife, cuvet, reaction tube, measuring cylinder, vibrator, Hydraulic Bench Press, stove, pot, Autoclave, Incubator, petri dish, ose, Bunsen lamp, sterile lid cotton, Calliper, Erlenmeyer flask, Witmann No.1 filter paper, Aluminum foil, Laminary flow. The materials used in this research are heat cure acrylic plate, white plaster, blue plaster, red night, sterile aquades, saline solution, *Averrhoa bilimbi* leaf extract with concentration of 6.25%, 12.5%, 25%, 50% , 100% and 0.2% chlorhexidine gluconate, methanol, Mueller Hinton Agar (MHA), *Streptococcus mutans* suspension, and Brain Heart Infusion (BHI) media, sterile blank disc paper, Mc

Alland solution sequences and Could Mold Seal (CMS) .

The manufacture of heat cure acrylic plate was made by making a mold model with red wax measuring 10 mm x 10 mm x 2 mm 6 pieces. The mold space making was by mixing 75 ml water and 250 grams casts, stirred in a rubber bowl with a spatula for 60 seconds. The blend then inserted into the lower quad of the cuvette. Red wax was put on the surface of the mixture and the waited for 15 minutes. The covert surface of the lower cuvette was applied with vaseline and the upper cuvette was mounted which is then given the blended plaster. After the plaster was hardened, the cuvette was opened and red wax was poured with hot water until it was clean. Hot cure acrylic resin was consisted of powder and water ratio 6 gr: 3 ml. After 4 minutes, the mixture would reach the dough stage and inserted into the mold (mold space) where the surface had been applied with Cold Mold Seal (CMS). The cuvette was mounted and pressed under 22 kg/cm Hg pressure using hydraulic press. Curing was done by inserting a cuvette containing hot cure acrylic resin into an aluminum pot containing 15 liters of boiling water (100°C) for 20 minutes. The construction was accomplished by removing the acrylic plate from the cuvette, then the surface of the acrylic plate was rubbed with scratch paper number 600 and 1200. The hot cure acrylic plate was obtained in size 10 mm x 10 mm x 2 mm.

The making of *Averrhoa bilimbi* leaf extract was done by maceration. The leaves were washed with water then dried by drying them under the sun indirectly and not too dry, weighed the leaves that had been dried for 500 grams and mashed it with a blender; then they were inserted into the maceration glass and the methanol was added to the simplicia. Every 4-6 hours once for 24 hours stirred the immersion, after 24 hours filtered using filter paper and storing filtered liquid in another glass did it for 3-4 soaks per 24 hours to separate the liquid extract of *Averrhoa bilimbi* leaf from the powder and obtain the liquid extract which had been dissolved with methanol. The liquid extract that was obtained then put into the rotary evaporator to evaporate the solvent from the mixture comprising the solute and the solvent in order to obtain a concentrated liquid extract and evaporated in the water bath for 6 hours to obtain a thick extract of *Averrhoa bilimbi* leaf.

The antibacterial testing of *Streptococcus mutans* on the acrylic plate was done by diffusion. *Streptococcus mutans* isolates were obtained from the cultures which already available at the Microbiology Laboratory of the Faculty of Medicine, Lambung Mangkurat University Banjarmasin. *Streptococcus mutans* cultured bacteria isolates were taken with sterile ose and were inserted by dissolving it into 10 ml of liquid

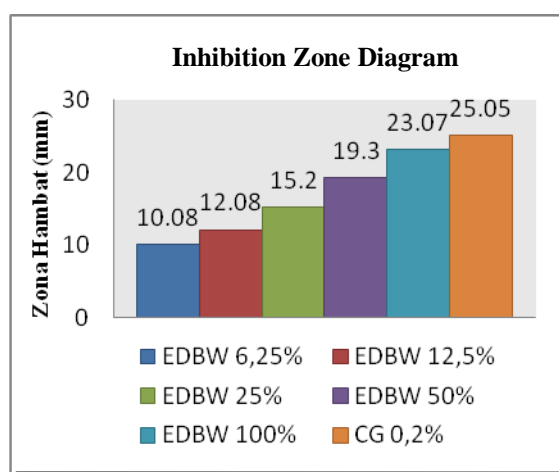
BHI medium, then incubated for 8 hours at 37°C to obtain *Streptococcus mutans* suspension. Suspension of *Streptococcus mutans* was diluted by adding sterile aquades to achieve a certain turbidity according to Mac Farland standard or equivalent bacterial count of  $3 \times 10^8$  CFU / ml.

This research used diffusion method. Conducted by means of acrylic resin with a size of 10mm x 10mm x 2mm, as many as six samples were soaked with aquadest for 48 hours, taken with sterile tweezers, soaked with saline solution for approximately one hour. The acrylic resin was taken with sterile tweezers and then soaked in 10 ml suspension of *Streptococcus mutans* for 24 hours at 37°C in reaction tubes on BHI media adjusted to Mac Farland standard ( $3 \times 10^8$  CFU / ml).

A total of six acrylic resins were divided into six treatment groups: Belimbing leaf extracts of 6.25%, 12.5%, 25%, 50%, 100% and 0.2% chlorhexidine gluconate (positive control) were soaked for 15 minutes. Acrylic resin was removed from each test tube and rinsed with saline and then inserted into a Brain Heart Infusion (BHI) tube and vibrated for 30 seconds and incubated for 8 hours. Prepare 30 paper disks and treatment of *Averrhoa bilimbi* leaf extracts 6.25%, 12.5%, 25%, 50%, 100% and chlorhexidine gluconate 0.2% (positive control). Paper disks were immersed into six treatment groups for 3 hours. Applied *Streptococcus mutans* isolates to each MHA medium (Mueller Hinton Agar) into a petri dish, then incorporated a paper disk which had been soaked in a group of treatments into the MHA medium (Mueller Hinton Agar). The mediums were incubated for 24 hours and then measure the formed zone using the caliper in millimeters.

## RESULTS

The data obtained from the results of research that has been done can be seen in the following bar chart.



**Figure 1.** The diagram of the average inhibitory zone from each treatment against the growth of *Streptococcus mutans*.

The diagram above shows that there are variations in the inhibition zone formed from each treatment. Treatment of *Averrhoa bilimbi* leaf extract concentration of 6.25%, 12.5%, 25%, 50%, 100% respectively resulted in average inhibitory zone to *Streptococcus mutans* of 10.08 mm, 12.08 mm, 15.20 mm, 19.30 mm, 23.07 mm and 0.2% chlorhexidine gluconate treatment resulted in an average inhibitory zone of 25.05 mm.

Statistic test were conducted in order to find whether there is the difference of inhibition activity of each treatment. The statistical tests performed were a data normality test with Saphiro-wilk and homogeneity test data with Levene's test. The result of the data normality test was the data of *Averrhoa bilimbi* leaf extract of 6,25% with p-value = 0,357, 12,5% with p-value = 0,288, 25% with p-value = 0,707, 50% with p-value = 0,402, 100% with p-value = 0,153 and chlorhexidine gluconate 0,2% with p-value = 0,385. The result of data normality test showed normal distributed data because p-value > 0,05. The result of homogeneity test of data showed p-value = 0,026 (p < 0,05), which meant that all data were not homogeneous, possibly happened because there was difference of inhibition zone level significantly between treatment group. Followed by non-parametric test that was Kruskal-wallis, p-value = 0,000 (p < 0,05) was obtained. Further data analysis using Mann-Whitney test obtained p = 0,009 (p < 0,05), which means the hypothesis was accepted so that it can be concluded that there were significant differences in each treatment group.

## DISCUSSION

*Averrhoa bilimbi* leaf extract concentration 6.25%, 12.5%, 25%, 50%, 100% have antibacterial activity against *Streptococcus mutans*. *Averrhoa bilimbi* leaf extract has antibacterial properties because it contains the active compound of flavonoids, tannins, saponins, and triterpenoids.<sup>11</sup> Flavonoids work by interfering with the function of cytoplasmic membranes.<sup>13</sup> Flavonoids at low concentration can damage the cytoplasmic membrane causing the leak of metabolites and inactivating bacterial enzyme systems, high concentration is capable in damaging the cytoplasmic membrane and precipitating the bacterial cell protein.<sup>13</sup> The tannin active compound provides antibacterial properties by destroying the cytoplasmic membrane so that the bacteria will be damaged and die. Tannin also has the ability to inactivate the adhesion of microbial cells (molecules attached to host cells) contained in cell wall polypeptides, because tannins are phenol compounds.<sup>13</sup>

Treatment of 100% *Averrhoa bilimbi* leaf extract has higher inhibitory activity than the treatment below that is 6.25%, 12.5%, 25%, and 50%. According to Hernani (2013), 100% pure extract has higher active ingredient than crude extract. The crude extract contains 20% of the active compound content and it will increase to 60% after being purified.<sup>14</sup> Nur hilal (2015) declared that the combination of active ingredients in medicinal plants shows a synergistic effect and the effect of active ingredients is greater in combination than on the effect of each active ingredient used separately.<sup>15</sup>

According to Mella sari (2014), the dilution of *Averrhoa bilimbi* leaf extract with aquadest reduction of active substances were dissolved in each concentration. The lower the concentration of the extract tested, the smaller the inhibitory diameter is. The higher the concentration of the extract tested, the larger the diameter of the inhibitory activity which is shown by the large difference in the diameter of the inhibitory activity.<sup>16</sup> The inhibitory effect of 100% Belimbing wuluh leaves is still below the 0.2% chlorhexidine gluconate inhibitory effect.

According to Mangundjaja (2011), 0.2% chlorhexidine gluconate is able to precipitate the cytoplasmic acid protein of *Streptococcus mutans* bacteria which leads to changes in the permeability of cell membranes that will eventually cause cell membrane leakage.<sup>17</sup> The structure of the 0.2% chlorhexidine gluconate formula is a lipophilic chlorophenol ring works by seeping into the cell wall and causing leakage of intracellular components.<sup>18,19</sup>

Research by Dina Riwayati (2012) states that *Averrhoa bilimbi* leaf extract has antibacterial activity against bacterium *Bacillus sp* (positive gram bacteria) and do not have antibacterial activity against bacterium *Escherichia coli* (negative gram bacteria).<sup>13</sup> In the cell wall, positive-gram bacteria cell is composed by rigid layer of peptidoglycan (about 20-80 nm) and does not have an outer cell membrane, so that the *Averrhoa bilimbi* leaf extract can work directly on the gram-positive cell wall.<sup>13</sup> It can be concluded that there is a difference of antibacterial activity of each treatment of *Averrhoa bilimbi* leaf extract and chlorhexidine gluconate 0.2% on the growth of *Streptococcus mutans* on the acrylic plate. Leaves extract of *Averrhoa bilimbi* concentration of 100% have greater inhibitory activity but still below 0.2% chlorhexidine gluconate inhibitory effect.

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