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**THE EFFECT OF IRONWOOD STEM BARK EXTRACT (*Eusideroxylon zwageri*)
ON THE GROWTH OF *Streptococcus mutans* ON ACRYLIC RESIN DENTURE
PLATE**

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ABSTRACT

Background: *Streptococcus mutans* is plaque-forming initiator bacteria. Plaque on the surface base of denture can cause color changing, bad breath, inflammation, and infection called denture stomatitis. Denture hygiene must be maintained by denture soaking in 0.2% chlorhexidine gluconate. However, 0.2% chlorhexidine gluconate that used incessantly can cause side effects such as the changing of denture color and the fading of denture base pigmentation. The ironwood stem bark extract can be an alternative for denture cleanser material. **Objectives:** To analyze the effect of ironwood stem bark extract on the growth of *Streptococcus mutans* on heat cured type acrylic resin denture plate. **Methods:** True experimental laboratories research was used with post test only control group design using 9 treatment groups which are 5%, 10%, 20%, 40%, 60%, 80%, 100% concentration of ironwood stem bark extract, 0.2% chlorhexidine gluconate and aquadest. The treatment was performed with 3 times repetition resulting in 27 total samples. Antibacterial activity was measured by calculating the bacteria colony number. **Results:** The 5% and 10% concentration of ironwood stem bark extract were effective in reducing *Streptococcus mutans* with 30.3 CFU/ml and 10.3 CFU/ml average colony number. The ironwood stem bark extracts with 20%, 40%, 60%, 80%, 100% concentration are effective and equivalent to 0.2% chlorhexidine gluconate against *Streptococcus mutans* with 0 CFU/ml average value. **Conclusion:** The 20%, 40%, 60%, 80%, 100% concentration of ironwood stem bark extract is equally effective as 0.2% chlorhexidine gluconate in killing *Streptococcus mutans*, exhibiting potential to be an alternative for denture cleanser material.

Keyword: 0.2% Chlorhexidine gluconate, ironwood stem bark extract, acrylic resin denture plate heat cured type, *Streptococcus mutans*.

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INTRODUCTION

Dental and oral health in Indonesia is an important issue be concerned about. Based on the 2018 Basic Health Research (RISKESDAS) data, South Kalimantan was reported as the top second province with the highest prevalence of dental and oral health problems after South Sulawesi. People of South Kalimantan aged 65 years old or older were outlined to experience tooth loss with a prevalence of 23.48% and around 3.3% of the population installed dentures as the solution for missing tooth.

Denture placement is exceedingly necessary for a person that experiences tooth loss, because it will cause disruption in functional activity, such as chewing, talking, and also can affect their aesthetic.¹ Dentures is an appliance used for tooth loss replacement that can be easily put on and removed by

its user. Removable dentures base material can be made from acrylic, ceramic, and metal resin.²

Heat cured type of acrylic resin is a denture base material that generally used by society.¹ Acrylic resin denture base is commonly used because it has advantages such as close resemblance with gingival color, easy fabrication, easy to repair, and relatively cheaper in price than other kind of materials.³ However, acrylic resin also has disadvantages such as easily damaged at cleaning and usage and also the tendency to absorb oral cavity fluid that facilitates microorganism attachment on the surface of denture base, resulting in the formation of plaque.⁴

Plaque on the surface of denture base can prompt bad breath, color changing, inflammation and infection that is generally known as denture stomatitis.⁵ Plaque formation in the oral cavity can

rapidly occur because of the existence of bacterial colonization. The most common bacterial colony is *Streptococcus mutans*.³ *Streptococcus mutans* is the first bacteria to attach to the denture base and initiate plaque formation process. *Streptococcus mutans* will transform sucrose to extracellular glucose polymer (glucan). Glucan will be used to conduct coaggregation with other microorganism, one of which is *Candida albicans*.^{3,5}

Dentures and oral cavity hygiene should be maintained to prevent *Streptococcus mutans* contamination.³ Dentures should be cleaned after every usage.³ There are few methods that can be done in denture maintenance, which are mechanical method (brushing), chemical method (soaking) or combination of the two methods.⁶ Ultrasonic device and tooth brushing are mechanical methods applied for denture cleaning, meanwhile dentures soaking in disinfectant liquid is a chemicals method to clean the denture.⁴

Disinfectant material such as 0.2% chlorhexidine gluconate as denture cleaner can reduce microorganism that attaches to the denture.⁷ However, 0.2% chlorhexidine gluconate has side effects if used continually such as the discoloration of the denture. Additionally, the price of 0.2% chlorhexidine gluconate is relatively expensive.^{5,7}

One of the alternative materials that can be used as denture cleaner is the ironwood stem bark extract which is a native plant from Kalimantan. The 20%, 40%, 60%, 80%, 100% concentrations of ironwood stem bark extract, with a Latin name of *Eusideroxylon zwageri*, are proven to inhibit *Staphylococcus aureus*.⁸ Ironwood stem bark contain antibacterial compounds such as 30.48 mg CE/g of flavonoid extract and 31.28 mg GAE/G of phenol, tannin, alkaloid, saponin, terpenoid, and it is proven capable of inhibit *E. Coli* and *S. Typhi* bacterial growth.^{9,10}

Based on the description of problem above, the researcher is further interested to conduct a research on the effect of 5%, 10%, 20%, 40%, 60%, 80%, 100% concentration of ironwood stem bark extract (*Eusideroxylon zwageri*) on *Streptococcus mutans* growth on heat cured type acrylic resin denture plate that can be used as a denture cleanser alternative.

MATERIALS AND METHODS

Before the research conducted, application for ethica clearance was submitted to Health Research Ethics Committee at Faculty of Dentistry Lambung Mangkurat University based on statement letter No. 014/KEPKG-FKGULM/EC/1/2020. This research

was designed as true experimental research employing the post-test only with control group design. The sample of this research was heat cured type acrylic resin with 10 mm x 10 mm x 2 mm size.

The sample was taken using simple random sampling technique. The total sample in this research was 27 that was divided into 9 treatment groups, consisting of 5%, 10%, 20%, 40%, 60%, 80%, 100% concentration of ironwood stem bark extracts and 0.2% chlorhexidine gluconate as the positive control, and aquadest as the negative control. This research was conducted at Microbiology Laboratory Research Center Faculty of Dentistry Airlangga University, Research and Industrial Consultation Hall Surabaya, and FMIPA Laboratory Lambung Mangkurat University Banjarbaru.

Ironwood stem bark extract (*Eusideroxylon zwageri*) was made using maceration method. Two kilogram of ironwood stem bark with a height above 50 cm were taken from Kintap area, Tanah Laut Regency, South Kalimantan, using a knife without harming the cambium. The ironwood stem bark was cleaned from dirt to be sundried, and further dried using an oven. The dried bark was later cut small into ± 2 cm size. The pieces of ironwood stem bark were made into powder and then filtered using mesh screen. The 200 gram of stem bark powder were put into extractor device and 1000 ml of 96% ethanol solvent was added (1:5 ratio).

The extraction process was conducted for 24 hours while mixed using a shaker. After that, the obtained extract was filtered and the obtained residue was extracted again with similar procedures for 4 times. Next, the extract was filtered and obtained the filtrate that was subsequently vaporized using a rotary vacuum evaporator at 59-60°C temperature until the concentrated extract was obtained. The concentrated extract was then heated on the waterbath until all of the solvent was vaporized, resulting in 14 grams of 100% concentration brown-colored liquid residual. Ethanol-free test was conducted by adding a few drops of potassium dichromate ($K_2Cr_2O_7$) to the ethanol extract of ironwood stem bark sample. If no color changing is observed within the sample, then there is no ethanol was contained in the aforementioned solution.

Ironwood stem bark extract in a concentration of 100% was diluted to get 5%, 10%, 20%, 40%, 60%, 80%, 100% concentration using this formula:

$$V_1.M_1=V_2.M_2$$

V_1 = Diluted liquid volume (ml)

M_1 = Ironwood stem bark extract that available (%)

V_2 = Desired liquid (water and extract) volume (ml)

M_2 = Ironwood stem bark concentration to be made (%)

Acrylic resin plate samples with 10 mm x 10 mm x 2 mm size were immersed in the sterile aquadest for 48 hours to reduce the residual monomer. The acrylic resin plate was then sterilized by soaking it into 70% alcohol for 30 minutes. The soaking of acrylic resin plate in sterile saliva was later performed for 1 hour to increase bacterial attachment. Further, the plates were rinsed with PBS (Phosphate Buffer Saline) twice. Acrylic resin plates were put into test tubes filled with *Streptococcus mutans* suspension that was obtained from Research Center Microbiology Laboratory Airlangga University Surabaya with 3×10^8 McFarland standard. The samples were then incubated for 24 hours at 37°C temperature.

Next, the acrylic resin plate was put into a test tube filled with ironwood stem bark extract (*Eusideroxylon zwageri*) at 5%, 10%, 20%, 40%, 60%, 80%, 100% concentration, 0.2% Chlorhexidine gluconate as positive control, and aquadest as negative control for 15 minutes. The acrylic resin plates were cleaned using PBS (Phosphate Buffer Saline) 2 times after soaked in respective solution. The acrylic resin plates were inserted into BHIB (Brain Heart Infusion Broth) and then vibrated using a vortex mixer for 30 seconds to eliminate dead bacteria or bacteria that is no longer attached to acrylic resin plate. Each 0,2 ml of sample was taken from BHI tube which then smeared onto Nutrient agar using 96% alcohol sterilized micropipette tip. Inoculated media was then incubated for 48 hours at 37°C temperature in anaerobic state. Next, the *Streptococcus mutans* colony number was calculated and data analysis was conducted.

RESULTS

The result of "The Effect of Ironwood Stem Bark Extract for *Streptococcus mutans* growth on Acrylic Heat Cured Type Resin Denture Plate" based on the calculation of *Streptococcus mutans* colony number after soaked in 5%, 10%, 20%, 40%, 60%, 80%, 100% concentration of ironwood stem bark extracts, 0.2% chlorhexidine gluconate and aquadest obtained an average value as follows:

Table 1. The Mean of *Streptococcus mutans* colony number on Acrylic Heat Cured Type Resin Denture Plate After Soaked in the Ironwood Stem Bark Extract, 0.2% Chlorhexidine Gluconate and Aquadest.

Treatment	N	Mean	Std. Deviation
EKBU 5%	3	30.3	2.08
EKBU 10%	3	10.3	1.52
EKBU 20%	3	0	0
EKBU 40%	3	0	0
EKBU 60%	3	0	0
EKBU 80%	3	0	0
EKBU 100%	3	0	0
CHX 0.2%	3	0	0
Aquadest	3	140.3	5.85

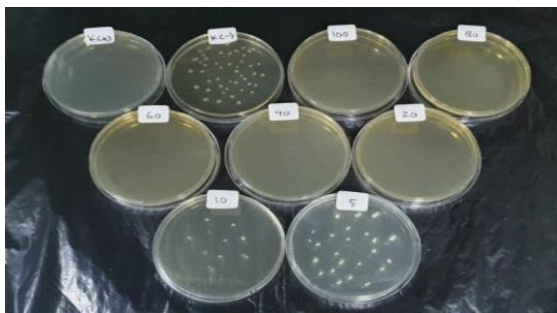
EKBU 5% : 5% Ironwood Stem Bark Extract
 EKBU 10% : 10% Ironwood Stem Bark Extract
 EKBU 20% : 20% Ironwood Stem Bark Extract
 EKBU 40% : 40% Ironwood Stem Bark Extract
 EKBU 60% : 60% Ironwood Stem Bark Extract
 EKBU 80% : 80% Ironwood Stem Bark Extract
 EKBU 100% : 100% Ironwood Stem Bark Extract
 K (+) : Positive Control
 K (-) : Negative Control

Table 1 shows the mean value and standard deviation of various treatment groups. Ironwood stem bark extract at a concentration of 5% obtained a mean value of 30.3 CFU/ml with 2.08 standard deviation. Ironwood stem bark extract at a concentration of 10% obtained a mean value of 10.3 CFU/ml with 1.52 standard deviation. Ironwood stem bark extract at a concentration of 20% obtained a mean value of 0 CFU/ml with 0 standard deviation.

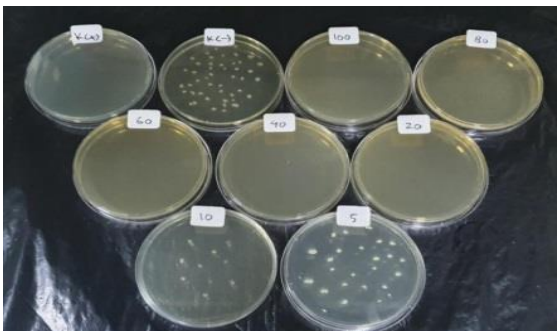
Ironwood stem bark extract at a concentration of 40% obtained a mean value of 0 CFU/ml with 0 standard deviation. Ironwood stem bark extract at a concentration of 60% obtained a mean value of 0 CFU/ml with 0 standard deviation. Ironwood stem bark extract at a concentration of 80% obtained a

mean value of 0 CFU/ml with 0 standard deviation. Ironwood stem bark extract at a concentration of 100% obtained a mean value of 0 CFU/ml with 0 standard deviation. Chlorhexidine gluconate at 0.2% concentration obtained a mean value of 0 CFU/ml with 0 standard deviation. Aquadest obtained a mean value of 140.3 CFU/ml with 5.85 standard deviation. The result of this research shows that the higher the concentration of ironwood stem bark extract, the less *Streptococcus mutans* colony number observed.

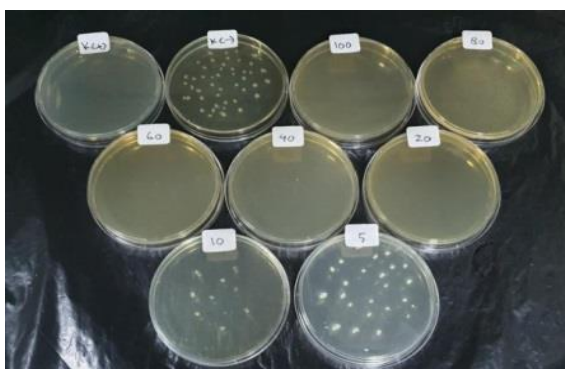
Picture 1. The test result of Ironwood Stem Bark Extract, 0.2% Chlorhexidine Gluconate as Positive Control and Aquadest as Negative Control on *Streptococcus mutans* Bacteria on Acrylic Resin Denture Plate with 3 Times Repetition



1. First treatment



2. Second treatment



3. Third treatment

The obtained data of each treatment was tabulated and a normality test was performed using Saphiro-wilk test. The normality test result obtained $p < 0.05$, so it can be said that the data was not normally distributed. Then, the data were tested using Levene's test that obtained a significance value of 0.006 ($p < 0.05$), so it can be concluded that the data was not homogeneous. The data were then consequently analyzed using Kruskal Wallis non parametric test.

The result test for Kruskal Wallis non parametric test obtained $p = 0.001$ ($p < 0,05$) showing that there was a difference in *Streptococcus mutans* colony number if observed from the given treatment, then the analysis was continued by the Mann Whitney test to find out the group that demonstrate the difference.

Table 2. The Mann Whitney Test Result for Ironwood Stem Bark Extract (*Eusideroxylon zwageri*) Effect on *Streptococcus mutans* on the Acrylic Resin Denture Plate

Treatment	EKB U 5%	EKB U 10%	EKB U 20%	EKB U 40%	EKB U 60%	EKB U 80%	EKB U 100%	CHX 0.2%	Aquadest
EKB U 5%		0.050*	0.037*	0.037*	0.037*	0.037*	0.037*	0.037*	0.050*
EKB U 10%			0.037*	0.037*	0.037*	0.037*	0.037*	0.037*	0.050*
EKB U 20%				1.000	1.000	1.000	1.000	1.000	0.037*
EKB U 40%					1.000	1.000	1.000	1.000	0.037*
EKB U 60%						1.000	1.000	1.000	0.037*
EKB U 80%							1.000	1.000	0.037*
EKB U 100%								1.000	0.037*
CHX 0.2%									0.037*
Aquadest									

Table 2 is the Pos Hoc result using Mann Whitney test for every treatment group. Ironwood stem bark extract at the concentration of 5% when compared with 20%, 40%, 60%, 80%, 100% concentration, 0.2% chlorhexidine gluconate as positive control, and aquadest as negative control has $p < 0.05$ which stipulates the significant difference. Ironwood stem bark extract at the concentration of 5% when compared with 10% concentration and negative control has $p = 0.05$, thus it did not present any significant difference. Ironwood stem bark extract at the concentration of 10% when compared with 20%, 60%, 80%, 100% concentration, and 0.2% chlorhexidine gluconate as positive control has

$p < 0.05$ which indicates significant difference. Ironwood stem bark extract at the concentration of 10% when compared with negative control has $p = 0.05$, so it does not specify the significant difference. Ironwood stem bark extract at the concentration of 20% when compared with 40%, 60%, 80%, 100% concentration and 0.2% chlorhexidine gluconate as control positive has $p = 1.000$ which means $p > 0.05$, so it did not present significant difference.

Ironwood stem bark extract at the concentration of 20% when compared with aquadest as control negative has $p < 0.05$, so significant difference was obtained. Ironwood stem bark extract at the concentration of 40% when compared with 60%, 80%, 100% concentration and 0.2% chlorhexidine gluconate as positive control has $p = 1.000$ which means $p > 0.05$, so significant difference was obtained. Ironwood stem bark extract at the concentration of 40% when compared with aquadest as negative control has $p < 0.05$, so significant difference was obtained. Ironwood stem bark extract at the concentration of 60% when compared with 80% and 100% concentration and 0.2% chlorhexidine gluconate as positive control has $p = 1.000$ which means $p > 0.05$, so significant difference was obtained. Ironwood stem bark extract at the concentration of 80% when compared with 100% concentration and 0.2% chlorhexidine gluconate as positive control has $p > 0.05$ which means it did not obtain any significant difference. Ironwood stem bark extract at the concentration of 80% when compared with aquadest as negative control has $p \text{ value} < 0.05$, so significant difference was obtained. Ironwood stem bark extract at the concentration of 100% when compared with 0.2% chlorhexidine gluconate as positive control has $p > 0.05$ which means it did not obtain any significant difference. Ironwood stem bark extract at the concentration of 100% when compared with aquadest as negative control has $p < 0.05$, so significant difference was obtained.

Based on this research, it is known that the 20% concentration of ironwood stem bark extract is equally capable to 0.2% chlorhexidine gluconate in killing *Streptococcus mutans* bacteria proven by Mann Whitney test result of ironwood stem bark extract at 20% concentration which indicates no significant difference when compared to 0.2% chlorhexidine gluconate as positive control.

DISCUSSION

Based on this research, it is known that 5%, 10%, 20%, 40%, 60%, 80%, 100% concentration of ironwood stem bark extract have antibacterial activity

to *Streptococcus mutans* which is marked by the growth of *Streptococcus mutans* colony number in 5% concentration of ironwood stem bark extract and reduction of bacteria colony number at 10% concentration of the ironwood stem bark extract.

Based on the statistic data of this research, ironwood stem bark extract starting from 20% concentration has no significant difference to 0.2% chlorhexidine gluconate as positive control, resulting in the equal capability of antibacterial activity with 0.2% chlorhexidine gluconate because no bacteria colony growth was observed starting at the referred concentration. This matter can happen because of the ironwood stem bark extract (*Eusideroxylon zwageri*) contains antibacterial compounds such as flavonoid, phenolic, tannin, alkaloid, terpenoid, and saponin.⁹ The said contents are almost equal with starfruit leaf extract contents that was proven capable of inhibiting *Streptococcus mutans* bacteria growth on the acrylic resin plate.³

Flavonoid compound exhibits antibacterial activity through 3 mechanisms that are inhibiting cell membrane function, inhibiting energy metabolism, and inhibiting nucleic acid synthesis. Positive-gram bacteria (*S. mutans*) contain theicoid acid that is hydrophylic (dissolved in water) and function as a positive ion transport medium into the cell wall. The said function cause positive-gram bacteria to be more polar, facilitating the entry of flavonoid compound into *Streptococcus mutans* cell wall.¹¹

Phenol is an antibacterial compound with the mechanism of damaging peptidoglycan structure on the *Streptococcus* cell wall so that its integrity damaged and its layer can not perfectly formed. The damage of *Streptococcus mutans* cell wall can cause phenol and other antibacterial compound to get through deeper into the cell so it can damage the cell membrane. The damaged *Streptococcus mutans* cell membrane can happen because of phenolic compound that forms a protein complex through the hydrogen H^+ bond that attacks the phosphate group, resulting in the substances in cell membranes such as organic enzyme ion, amino acid experiencing leaking and *Streptococcus mutans* metabolism is disturbed, then lysis the bacteria.¹²

The tannin compound content in the ironwood stem bark extract has antibacterial action that connects with its capability to deactivate bacteria adhesion to inhibit enzyme function and inhibit protein transportation in cell cover. The growing bacteria in an anaerobic state need iron for various functions, one of which is DNA ribonucleotide precursor reduction. The bond between tannin and iron will cause bacteria function disturbed.¹¹

This research used 0.2% chlorhexidine gluconate as positive control. The selection of 0.2% chlorhexidine gluconate as positive control was based of the said characteristics. Denture cleanser which contains 0.2% chlorhexidine gluconate is a disinfectant liquid that many society used for their denture.¹³ Disinfectant is a substance that can inhibit or destroy microorganisms in inanimate objects, meanwhile antiseptic used in living tissue.¹⁴ Bacteriostatic and bactericidal of 0.2% chlorhexidine gluconate is their way of disturbing bacteria membrane cell.¹⁵

Based on this research result, there is no growth of *Streptococcus mutans* bacteria after being tested with 0.2% chlorhexidine gluconate. The control positive of this reasearch (0.2% chlorhexidine gluconate) is more effective than povidone iodine and fluoride with zinc supplementation against positive-gram bacteria (*Streptococcus mutans*). This was depicted by the average value of inhibition zone diameter that was resulted in the research groups. The average zone diameter in *Streptococcus mutans* colony is wider than the average inhibition zone of other positive-gram bacteria (*Porphyromonas gingivalis*).¹⁶

The antibacterial mechanism of phenolic compound in ironwood stem bark extract is similar to 0.2% chlorhexidine gluconate that works in bacterial cell walls and affects cell permeability of component with low molecules weight from inside the cell through cell membrane, resulting in the death of the bacteria.¹⁶

The 20%, 30%, 40%, 60%, 80%, 100% concentration of Ironwood stem bark extract and 0.2% chlorhexidine gluconate as positive control shows that there was no growth of bacteria colony found. The said matter shows that the ironwood stem bark extract can be an alternative material for denture cleanser because it is capable to kill *Streptococcus mutans* bacteria at 20% concentration. This result corresponds to Peclzar and Chan (1986) confirming that the higher the concentration of an antibacterial then the stronger the antibacterial effect will be.

It can be concluded that the *Streptococcus mutans* colony number on acrylic resin denture plate after it was soaked in 5% concentration of the ironwood stem bark extract obtained 30.3 CFU/ml mean value. Acrylic resin denture plate after soaking in 10% concentration of the ironwood stem bark extract obtained 10.3 CFU/ml mean value which means that the extract has an effective capability to reduce *Streptococcus mutans* growth. Ironwood stem bark extract at a concentration of 20%, 40%, 60%, 80%, 100% have 0 CFU/ml mean value which means

that they have effective capability in killing *Streptococcus mutans*. The ironwood stem bark extract has potential and can be an alternative material for denture cleanser.

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