

The Effectiveness of Dayak Onion Bulb Extract Mouthwash (*Eleutherine Bulbosa (Mill.) Urb.*) As Bacterial Growth Inhibitor on *Streptococcus Mutans*, the Main Cause of Dental Caries (In-Vitro and In-Vivo Test)

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Abstract:- Background: Dental caries is still a major problem in Indonesia, where almost 90% of the population has problems with oral health. Dental caries is an infectious disease caused by demineralization of the enamel and dentin layers which is closely related to the consumption of cariogenic foods. The occurrence of dental caries is due to the role of caries-causing bacteria found in the oral *Streptococcus* group which is collectively called *Streptococcus mutans*. Dayak onion bulbs (*Eleutherine bulbosa (Mill.) Urb.*) is one of the plants in Indonesia which based on research can inhibit the growth of a bacteria called *Streptococcus Mutans*.

Objective: To find out and prove the effectiveness of the research and development product of Dayak onion bulbs extract mouthwash as an inhibitor of the growth of *Streptococcus Mutans* bacteria, the main cause of caries.

Method: This research is a true experimental research using a pretest and posttest approach with a control group design. Sampling was done by simple random sampling technique and obtained 14 x 3 groups or 42 respondents. This study was conducted for 7 days, where on the first day a measurement (pretest) was carried out by taking a sample of the respondent's saliva before using the mouthwash in the morning, and on the 8th day (posttest) the respondent's saliva was measured to test the bacterial colonies contained therein. Statistical analysis Pre-Test Post-Test different test using Paired T-Test and One-Way Anova test and Comparative Analysis with Post Hoc Tukey test.

Results: This study shows that the dose of mouthwash in the in-vitro test obtained a potent dose, namely at a concentration of 25% Dayak onion bulb extract, then in the in-vivo test the mouthwash formulation model of Dayak bulb extract (*Eleutherine bulbosa (Mill.) Urb.*) with a potent dose (25%) was effective in inhibiting the growth of *Streptococcus Mutans* in vivo with an average reduction of -4.66×10^6 CFU/ml, with a p value ($0.016 < 0.05$). the + control group was -1.76×10^6 CFU/ml, and the control group - was 0.29×10^6 CFU/ml, but the results of the statistical analysis showed a significant difference in the results before and after treatment in the + control group

(p value = $0.00 > 0.05$) and control – (p value = $0.605 > 0.05$).

Conclusion: Mouthwash formulation model of Dayak bulb extract (*Eleutherine bulbosa (Mill.) Urb.*) with a concentration of 25% is effective in inhibiting the growth of *Streptococcus Mutant* bacteria both in-vitro and in-vivo.

Keywords: *Eleutherine bulbosa*, antibacterial, mouthwash substance.

I. INTRODUCTION

Dental caries is still a major problem in Indonesia, where nearly 90% of the population has problems with oral health. Dental caries is an infectious disease caused by demineralization of the enamel and dentin layers which is closely related to consumption of cariogenic foods. The occurrence of dental caries is due to the role of caries-causing bacteria found in the oral *Streptococcus* group collectively called *Streptococcus Mutans*. Dental caries is a multifactorial process that occurs through interactions between teeth and saliva as hosts, bacteria in the oral cavity, and easily fermentable foods.^[1] The prevalence of caries in Indonesia based on Basic Health Research, abbreviated as RISKESDAS, in 2013 and 2018 increased from 25.9% to 57.6%. This shows that the prevalence in Indonesia is still high. *Streptococcus mutans* is the most common cause of dental caries of all other oral *Streptococci*.^[2]

Mouthwash can be interpreted as a solution with a comfortable taste, is antimicrobial and can also freshen the mouth. Most mouthwashes contain alcohol at a concentration of 5-25%. Mouthwashes containing alcohol can also increase the risk of oral cancer, especially if used continuously.^[3]

Chlorhexidine (CHX) has been commonly used in dental practice as an antiseptic agent since 1970, due to its long-lasting anti-bacterial activity with broad spectrum of action.⁽⁸⁾ *Chlorhexidine* is also an antibacterial compound against most species of bacteria found in the oral cavity.^[4] However, the use of *Chlorhexidine* also has negative effects. The use of *Chlorhexidine* in the long term is not

recommended because the side effects that can occur are taste disturbance, burning sensation, discolored teeth, and mucous membranes, as well as increased calculus formation. Chlorhexidine 0.2% mouthwash will seep into the composite resin material, then degrade the siloxane bonds through hydrolysis reactions and begin to weaken the filler bond at the resin matrix interface resulting in easier water entry into the resin which causes discoloration of the resin fillings. tooth color (composite). As a result, there is controversy about the use of Chlorhexidine for caries prevention.^[5]

Indonesia has long been known as a country producing spices which are very useful as seasonings and medicines. Of the various tribes and ethnicities in Indonesia, there is one tribe on the island of Kalimantan (Borneo) that uses traditional medicine with the Dayak onion plant from the Eleutherine genus. This plant is used by the ancestors of the Dayak tribe on the island of Borneo as a powerful medicine in warding off and treating various diseases that are commonly found in a tribe or community that are commonly found in the interior of the forest. Onion Dayak is often regarded as an Ethnomedicine herbal instrument because it is still unknown to the international community and is only known by the local community.^[6]

Dayak onion bulbs extract is antibacterial because it contains active compounds such as phenols and flavonoids as antibacterial compounds. Dayak onion bulbs extract which can inhibit bacterial growth has advantages in the form of the natural properties of reactive herbal plants.

II. METHODS

The type of research used was a laboratory experiment (in-vitro), in which there were 3 experimental groups in this study, where O1-O3 served as observations. Dayak onion extract (Eleutherine bulbosa (Mill.) Urb.) was given in five treatments as an experimental group namely giving concentrations of 15% (as P1), 20% (as P2), 25% (as P3), K+ (as P4) Chlorhexidine, K- (as P5) Aquadest. The following is the research design scheme, True Experimental Research uses the Posttest-with Control Group Design research design, and the development of a model formulation of Dayak onion bulbs extract mouthwash (Eleutherine bulbosa (Mill.) Urb.) with different concentrations effective against the growth of Streptococcus mutans bacteria in-vivo.

The population in this study are all research objects studied. The population in the in-vivo study were the students of the Darul Ulum Islamic Boarding School, Martapura, Banjar Regency. The sample is part of the population studied by the Darul Ulum Islamic Boarding School students used in this study as many as 42 samples. Samples selected randomly using probability sampling, namely simple random sampling.

III. RESULT

Table 1. Effectiveness test of inhibition of Streptococcus mutans growth in different concentration (in-vitro study).

Consentration	Inhibition	Mean ± SD
25%	R1 : 22,0 mm R2 : 21,10 mm R3 : 21, 50 mm	21.53 ± 0.45
20%	R1 : 19,00 mm R2 : 19,10 mm R3 : 18,60 mm	18.90 ± 0.26
15%	R1 : 17, 00 mm R2 : 17,30 mm R3 : 16,10 mm	16.80 ± 0.62
Clorhexidine (K+)	R1 : 13.00 mm R2 : 12.00 mm R3 : 12.50 mm	12.50 ± 0,50
Aquadest (K-)	R1 : No Inhibition R2 : No Inhibition R3 : No Inhibition	0

Table 1 shows the effectiveness of five groups of solution (and three consentration of mouthwash formulation of Dayak onion bulbs extract). This shows that there are significant differences between the five groups. Therefore, a follow-up in-vivo test will be carry out using the best consentration with potent bacterial inhibition properties, which is 25% consentration.

Table 2. Effectiveness test of inhibition of Streptococcus mutans growth on respondent (in-vivo study).

Variable		Sum of Square	Mean of Square	P. Value
Bacterial Colonies of Streptococcus Mutans	Between Groups	17,812	6,706	0,005
	Within Groups	5,944	0,415	
Total		23,756		
Delta (Δ) Bacterial Colonies of Streptococcus Mutans	Between Groups	25,801	1,201	0,012
	Within Groups	24,276	2,125	
Total		17,812		

*One-Way ANOVA

Table 2 shows that there were significant differences in the number of bacterial colonies in each group, namely in the intervention group and the control group (p = 0.005) and Delta (Δ) Streptococcus Mutans bacterial colonies (p = 0.012) where the p-value (<0 , 05). However, the results of the ANOVA are comprehensive, that is, together they have significant differences. To determine whether or not there is a significant difference between groups, a post-hoc test was carried out.

Table 3. Multicomparative analysis of *Streptococcus Mutans* bacterial colonies between intervention group and control group.

Variables	Groups		Mean Difference	P. Value
Bacterial colonies of <i>Streptococcus Mutans</i>	Intervention	Control (+)	-2.2600*	.001
		Control (-)	-2.3600*	.001
	Control (+)	Intervention	2.2600*	.001
		Control (-)	-.1000	.973
	Control (-)	Intervention	2.3600*	.001
		Control (+)	.1000	.973
Delta (Δ) bacterial colonies of <i>Streptococcus Mutans</i>	Intervention	Control (+)	-.49286*	.005
		Control (-)	-.95714*	.000
	Control (+)	Intervention	.48903	.095
		Control (-)	-.46429	.122
	Control (-)	Intervention	.95714*	.000
		Control (+)	.42649	.122

*Post hoc test : Tukey HSD

Table 3 shows that there were significant differences between the intervention group and the positive control and negative control groups ($p = 0.001 < 0.05$) with a mean difference (-2.2600*), while the positive control and negative control groups did not show significant difference ($p = 0.973 > 0.05$) with the mean difference (-0.1000). In the difference between *Streptococcus Mutans* colonies, there were significant differences between the intervention group and the positive control and negative control groups ($p = 0.005 < 0.05$) with a mean difference (-4.928*), while between the positive control group and the negative control group ($p = 0.945 > 0.05$) with a mean difference (0.48903), where this result indicates that there is no significant difference between the positive and negative control groups.

IV. DISCUSSION

Dayak onion bulbs extract mouthwash is a product that can inhibit the growth of *Streptococcus Mutans* which is the main cause of caries. Mouthwash is used because of its ability to effectively reach places that are difficult to clean with a toothbrush and can prevent plaque formation.^[7] Dayak onion bulbs extract (*Eleutherine bulbosa* (Mill.) Urb.) can inhibit the growth of *Streptococcus mutans* as indicated by the formation of an inhibition zone in the form of a clear area around the wells containing Dayak onion bulbs extract.^[8] Making mouthwash from herbal extracts of Dayak onion bulbs is effective in inhibiting the growth of *Streptococcus Mutans* bacteria because the active content of Dayak onion bulbs

contains compounds that are antibacterial. The manufacture of Dayak onion bulbs extract mouthwash includes several stages of testing, namely organoleptic test, pH test, stability test, homogeneity test, hedonic test and bacterial inhibition test.

In the different test between groups there was a significant difference in the inhibition at each given concentration, where the value was ($p = 0.000 < 0.05$). Meanwhile, the analysis of the effectiveness of each intervention showed that the average result between the treatment groups had a probability value ($p < 0.05$) which meant that each group had a significant difference in inhibiting the growth of mutant *Streptococcus* bacteria in vitro with different results. differ from each other by value (p value = < 0.05).

The results above prove that the higher the concentration of Dayak onion bulbs extract, the greater the inhibition zone formed. The inhibition power produced by antibacterial ingredients will be higher if the concentration is also high. Dayak onion bulbs extract has antibacterial activity because it contains alkaloids, saponins, flavonoids, steroids and tannins. Alkaloid compounds have the ability as an antibacterial. The alkaloid mechanism is by interfering with the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed completely and causes cell death. Flavonoids can bind to proteins in the bacterial cell plasma membrane and form complex compounds, so that the bacterial plasma membrane becomes weak and leakage occurs in the plasma membrane. This results in the release of components in the bacterial cell. Steroids work by damaging the plasma membrane of the bacterial cell, causing the cytoplasm to leak so that the cell exits which causes the death of the bacterial cell. Tannins are able to inactivate microbial cell adhesion, inactivate enzymes, and interfere with protein transport in the inner layer of cells. Saponins also have antibacterial activity by leaking proteins and enzymes into cells.^[9]

In the number of bacterial colonies in each group, namely in the intervention group and the control group ($p = 0.005$) and Delta (Δ) *Streptococcus Mutans* bacterial colonies ($p = 0.012$) where the p-value (< 0.05). However, the results of the ANOVA are comprehensive, that is, together they have significant differences. To find out whether or not there were significant differences between groups, a post hoc test was carried out, which showed that there were significant differences between the intervention group and the positive control and negative control groups ($p = 0.001 < 0.05$) with a mean difference (-2.2600*), while between the positive control group and the negative control group did not show a significant difference ($p = 0.973 > 0.05$) with a mean difference (-0.1000). In the difference between *Streptococcus Mutans* colonies, there were significant differences between the intervention group and the positive control group and the negative control group ($p = 0.005 < 0.05$) with a mean difference (-4.928*), while between the positive control group and the negative control group ($p = 0.945 > 0.05$) with a mean difference (0.48903), where this result indicates that there is no significant difference between the positive and negative control groups.

So, it can be concluded that the mouthwash formulation of Dayak onion bulbs extract (*Eleutherine bulbosa* (Mill.) Urb.) has an effect on inhibits the number of *Streptococcus Mutans* bacterial colonies, where a concentration of 25% has the best effect on inhibiting the growth of *Streptococcus Mutans* bacterial colonies, because there's plenty of antimicrobial properties on it.

This is in line with research conducted by Mahfuzun Bone, Yusnita Rifai, Gemini Alam in 2019. This study showed that antimicrobial effect on the bioactive compounds possessed by Dayak onion bulbs extract, including *Streptococcus Mutans* bacteria. The results in this study all the concentration variants of Dayak onion bulbs extract that were tested showed an inhibitory effect on *Streptococcus Mutans* bacteria.

V. CONCLUSION

The potent dosage of Dayak onion bulbs extract mouthwash is at the dose with the highest concentration of Dayak onion extract, namely 25%, where the diameter of the inhibition zone is the highest, namely 22.0 mm with an average increase of 21.5 mm, where these results indicate the zone category. inhibition including the classification of very strong inhibitory zones (> 21 mm) in inhibiting the growth of *Streptococcus Mutans* bacteria.

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