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Ability difference of Beluntas Leaf (*Pluchea indica* L) Ethanol Extract and Avocado Leaf (*Persea americana* Mill) Ethanol Extract in Inhibiting caries-causing *Streptococcus mutans* Bacteria Growth Maria Martina Nahak, Regina Tedjasulaksana, Ni Nengah Sumerti Department of Dental Nursing, Health Polytechnic Denpasar, Bali, Indonesia Corresponding: Email: reginatedjasulaksana@yahoo.co.id Abstract Beluntas Leaf (*Pluchea indica* L) has been shown to have antibacterial activity against *Streptococcus mutans*.

The leaf of avocado (*Persea americana* Mill) that have same active substance with beluntas leaf expected to have antibacterial activity against *Streptococcus mutans* so that the purpose of this research is to compare the ability of ethanol extract from avocado leaf and beluntas leaf in inhibiting the growth of *Streptococcus mutans*. This research is experimental type, using completely randomized with posttest only control group design.

Samples were pure isolates of *Streptococcus mutans* ATCC 35668. The sample of 90 divided into four groups: beluntas leaf ethanol extract 25%, beluntas leaf ethanol extract 50%, avocado leaf ethanol extract 25%, avocado leaf ethanol extract 50% and two control groups. The data were analyzed using One-Way ANOVA test followed by Least Significant Difference (LSD) with a 95% confidence level.

The results showed a significant inhibition differences between the control group and the treatment group with a value of  $p = 0.000$  ( $p < 0.05$ ). The ability of 25% ethanol extract of beluntas leaf (*Pluchea indica* L) is equivalent to 50% ethanol extract of beluntas leaf (*Pluchea indica* L) and 50% ethanol extract of leaf of avocado (*Persea americana* Mill) in inhibiting the growth of *Streptococcus mutans*.

It is recommended that further research be conducted to test the mechanism of action of the active substances contained in beluntas and avocado leaf as an antibacterial and test MIC (Minimum Inhibitory Concentration) with an advanced test in determining the MBC (Minimal Bactericidal Concentration). Keywords: beluntas leaf ethanol extract, avocado leaf ethanol extract, inhibition, Streptococcus mutans Background Caries is caused by four factors: substrate containing sucrose-type carbohydrate, oral bacteria, tooth host and time factor.

Streptococcus mutans has specific ability in using sucrose to produce adhesive dextran to allow bacteria to form plaque. Streptococcus mutans, together with Streptococcus sabrinus and Lactobacillus, break down the substrate through the glucanucrase enzyme to produce lactic acid and change the oral environment to more acidic (pH 5.2-5.5) causing the enamel to be demineralized and caries occurs.<sup>1,2</sup> Almost all adults around the world have suffered from caries.<sup>3</sup> One way of chemical caries prevention is with antiseptic gargling with 0.12% chlorhexidine, or gargling plant extracts to reduce the accumulation of plaques that contain a wide variety of bacteria, especially Streptococcus mutans that cause dental caries.<sup>4</sup> 0.12% chlorhexidine can inhibit the growth of Streptococcus mutans bacteria.<sup>5</sup>

Chlorhexidine has adverse side effects of teeth staining, restorations or on the tongue and interferes with the tongue tasting ability although not permanent.<sup>6</sup> Given the side effects of chlorhexidine, in prevention of plaque accumulation, we can use plant extracts or traditional medicines, such as Beluntas leaf (*Pluchea indica* L.) and avocado leaf (*Persea americana* Mill). The active compounds in beluntas leaves are tannins, terpene, lignin glycosides, flavonoids, and triterpenoids.<sup>7,8</sup> Flavonoids have antibacterial activity through several mechanisms which are first, inhibits bacterial cell wall synthesis; secondly, cause leakage protein as a result of leakage of the bacterial cell wall; thirdly, inhibits bacterial protein synthesis; And fourthly, the possibility of intervening in the function of bacterial cell DNA.<sup>9,10</sup> Phenolic compounds have the ability as antibacterial because it can neutralize harmful free radicals in the body.<sup>11</sup> Tannin has the effect of being anti-microbial by forming complex with proline that is a kind of protein in bacterial cell wall, causing protein leakage, bacterial cell wall damage causing bacterial cell death.<sup>10,12,13</sup> The Nahak study showed that pure beluntas leaf extract could decrease 70% of total bacteria in saliva and there was no significant difference in the reduction of bacterial counts at concentrations of 10%, 20% and 30%.<sup>14</sup> Nahak 2012 study showed ethanol extract of leaves of beluntas concentration of 25% and 50% had inhibitory strength to Streptococcus mutans equivalent to 0.12% chlorhexidine.<sup>15</sup> Another Research by Nahak et al. in 2015, showed gargling of beluntas leaf ethanol extract concentrations of 10%, 20%, and 30% can decrease the amount of

Streptococcus sp.

on dental plaque.<sup>16</sup> Another herb that has antibacterial activity is avocado leaves (*Persea americana* Mill) containing alkaloids, terpenes, tannins, flavonoids, saponins and steroids.<sup>17</sup> Charyadie et al.

(2014), showed an avocado leaf extract with concentrations of 25%, 50% and 100% effective in inhibiting the growth of *Enterococcus faecalis* bacteria in the toothroot canal.<sup>18</sup> Material and methods This study is experimental laboratory (in vitro) with a completely randomized design with posttest only control group design. The study sample was pure isolate of *Streptococcus mutans* bacteria ATCC 35668.

*Streptococcus mutans* was applied evenly to Mueller-Hinton medium plus 5% goat blood which did not contain fibrin (blood agar medium) on a petri dish. Planting of *Streptococcus mutans* bacteria on the media was incubated for 24 hours at 37°C. The paper disc then placed over the agar media which has been inoculated with *Streptococcus mutans* bacteria. Then the test material put on the paper disc.

The sample size was 90 divided into four treatment groups and two control groups. The treatment group consisted of 25% beluntas leaf ethanol extract group, 50% beluntas leaf ethanol extract group, 25% avocado leaf ethanol extract group and 50% avocado leaf ethanol extract group. The control group consisted of a negative control of 96% ethanol solution and a positive control of 0.12% chlorhexidine.

An assessment of the ability of beluntas leaf ethanol extract and avocado leaf ethanol extract on the growth of *Streptococcus mutans* cultures was obtained by measuring the clear zone around the diffusion of the disk by using vertical, horizontal and diagonal thresholds then averaged in millimeters. Data were analyzed statistically by descriptive test, followed by normality test with Kolmogorov-Smirnov and homogeneity test by variance test (Levene's test of variance) and comparability test with One Way Anova. The result of the phytochemical test showed that beluntas leaf ethanol extract had flavonoid content of 4669.67 mg / 100gr QE, phenol 7592.8 mg / 100gr GAE and tannin 24132.85 mg / 100gr TAE. The avocado leaf ethanol extract contained flavonoids of 1550.29 mg / 100gr QE, phenol 2409.31 mg / 100gr GAE and tannin 3430.73 mg / 100gr TAE.

The results showed that the highest growth of bacterial *Streptococcus mutans* inhibited by 25% beluntas leaf ethanol extract group was 11.13 (3.113 mm) and 50% beluntas leaf ethanol extract group is 11.13 (2.231 mm). One Way Anova test results (Figure 2) showed that mean of inhibitory power between 6 groups was significantly different with  $p = 0,000$  ( $p < 0,05$ ).

Further tests of Least Significant Difference Test (LSD) in Figure 3 showed the mean inhibitory power of 25% and 50% beluntas leaf ethanol extract and 50% avocado leaf extract significantly different to positive control. The 25% Beluntas leaf ethanol extract was significantly different to 25% avocado leaf ethanol extract. Figure 1 Mean inhibitory ability of beluntas leaf (*Pluchea Indica* L.) ethanol extract and avocado leaf (*Persea Americana* Mill) ethanol extract against caries-causing *Streptococcus mutans* No. Test material, Inhibitory ability (mm), mean, Standard deviation, and p-value are shown in the table below.

Test material	Inhibitory ability (mm)	mean	Standard deviation	p-value
1_Negative control	0.00	9.00	11.13	11.13
2_Positive control	9.06	10.13	0.000	2.138
3_25% Beluntas leaf ethanol extract	3.113	2.231	2.120	2.996
4_50% Beluntas leaf ethanol extract	2.231	2.120	2.996	
5_25% avocado leaf ethanol extract				
6_50% avocado leaf ethanol extract				

Figure 2 Ability difference of Beluntas Leaf (*Pluchea indica* L) Ethanol Extract and Avocado Leaf (*Persea americana* Mill) Ethanol Extract in Inhibiting caries-causing *Streptococcus mutans* bacteria growth. F, p, Variation between groups, and Variation in groups are shown in the table below.

Comparison	F	p	Variation between groups	Variation in groups	Total
Negative control with Positive control	49,126	0,000			
25% Beluntas leaf ethanol extract					
50% Beluntas leaf ethanol extract					
25% avocado leaf ethanol extract					
50% avocado leaf ethanol extract					

Figure 3 Inhibitory ability differences of 25% and 50% Beluntas leaf ethanol extract and 25% and 50% avocado leaf ethanol extract with control groups against *Streptococcus mutans* growth. Group (I), Comparison group (J), Mean difference (I-J), p, and Negative control with Positive control are shown in the table below.

Comparison	Mean difference (I-J)	p
Negative control with Positive control	-9.000	-11.133
25% Beluntas leaf ethanol extract	-11.133	-9.066
50% Beluntas leaf ethanol extract	-10.133	0.000*
25% avocado leaf ethanol extract	0.000*	0.000*
50% avocado leaf ethanol extract	0.000*	0.000*

Discussion: This study showed 0.12% chlorhexidine test as a positive control, 25% and 50% Beluntas leaf ethanol extract and 25% and 50% avocado leaf ethanol extract have ability to inhibit the growth of caries causing *Streptococcus mutans* bacteria.

Beluntas leaf and avocado leaf contain secondary metabolites namely flavonoids, phenols, and tannins that have the ability as an antibacterial. The 25% and 50% Beluntas leaf ethanol extract had same inhibitory ability against streptococcus mutans bacteria and also had inhibitory ability against the growth of *Streptococcus mutans* bacteria equivalent to 0.12% chlorhexidine.

Beluntas leaf ethanol extract has greater inhibitory ability than the avocado leaf

ethanol extract. Based on the results of phytochemical tests, the content of flavonoids, phenols, and tannins which are antibacterial substances contained more in beluntas leaf than in avocado leaf. 0.12% chlorhexidine inhibitory ability and 25% avocado leaf ethanol extract inhibitory ability in this study were classified as moderate.

Inhibitory ability of 25% and 50% Beluntas leaf ethanol extract and 50% avocado leaf ethanol extract are classified as strong. This means that 25% beluntas leaf ethanol extract can be used as an alternative antiseptic of natural ingredients, while avocado leaf ethanol extract requires a higher concentration to be able to function as an antiseptic equivalent to 0.12% chlorhexidine.

Conclusion There were no significant differences between 25% and 50% beluntas leaf ethanol extract as well as 50% avocado leaf ethanol extract in inhibiting the growth of *Streptococcus mutans* bacteria, but there were significant differences with the 25% avocado leaf ethanol extract. The results of this study prove the ability of 25% beluntas leaf ethanol extract against *Streptococcus mutans* bacteria more effective than other test materials, so it can be used as an alternative ingredient of antiseptic mouthwash from medicinal plant extracts to inhibit the growth of plaque on the teeth because it does not possess side effects. References Vinogradov, A.M., Winston, M., Rupp, C.J., Stoodley, P. 2004.

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