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The Effectiveness of Tooth Brushing and Gargling Using Toothpaste and Mouthwash of Beluntas Leaf Ethanol Extract in Reducing Streptococcus mutansBacteria Number in Tooth Plaque Maria Martina Nahak1*, Regina Tedjasulaksana1, I GustiAyu Raiyanti1 Correspondence: *marianahak@rocketmail.com;mobile:+6281236409915; 1Dental Hygienist Department-Polytechnic of Health-Ministry of Health - Denpasar-Indonesia ABSTRACT Background:The aim of this study is to analyze the differences in the effectiveness of toothbrushing and gargling using 10% beluntas leaf ethanol extract toothpaste and mouthwash to reduce the number of colonies of Streptococcus mutans in dental plaque.

Methods: The type of this research is experiment with completely randomized with pre-posttest control group design, with 105 samples divided into three treatment groups and four control groups. Data were analyzed statistically using Repeated Measure to obtain the effectiveness difference of beluntas leaf extract with positive and negative control, before and after treatment.

Results: there is a significant decrease in the amount of Streptococcus mutans in dental plaque after receiving treatment at 95% confidence level with p = 0,00 or p <0,05 and there was no difference in effectiveness of toothbrushing and gargling using 10% ethanol extract of leaves beluntas with 0.12% chlorhexidine and 1% triclosan to decrease the amount of Streptococcus mutans bacteria in dental plaque, with p = 0.166 or p> 0.05.Conclusion: both beluntas leaf ethanol extract gargle and toothpaste group with positive control group using chlorhexidine and triclosan, is equally effective in reducing the number of colonies of Streptococcus mutans in dental plaque, so it can be concluded that the extract of beluntas leaves ethanol effectively decreases the amount of Streptococcus mutans colonies in dental plaque and there is no difference in

effectiveness between the treatment group and the control group.

Keywords: 10% beluntas leaf ethanol extract toothpaste, 10% beluntas leaf ethanol extract gargle, tooth plaque, Streptococcus mutans Introduction Caries is a disease of tooth hard tissue, most commonly found in the oral cavity, can affect all populations regardless of age, gender, race or socio-economic circumstances and is a leading cause of tooth loss.1Caries is caused by microorganism activity by distributing carbohydrates in the mouth, characterized by demineralization of inorganic materials followed by organic material damage from enamel and dentin. Demineralization of hard tissue occurs in the crown of the email and dentin and the root part of cementum and dentin.2

Caries should be addressed by curative effort that is by treating and patching all the teeth that hollow. Promotional efforts are done by providing correct information and education about how to maintain dental health.3 Mechanical preventive measures are performed by brushing, while chemical methods are performed by gargling with antiseptics such as chlorhexidine, or mouthwash from plant extracts to reduce plaque accumulation. 0.12% chlorhexidine, a broad-spectrum antimicrobial, is more effective against Gram-positive bacteria,4 but may have adverse side effects such as staining on teeth and restorations although not permanent.5

Prevention of plaque accumulation using plant extracts or traditional medicines has been widely studied today, one of which is the beluntas leaf which can inhibit the growth of Streptococcusmutans invitro.6Beluntas leaves contain active compounds such as alkaloids, flavonoids, triterpenoids, tannins,and phenols as well as other essential oil derivatives,7,8 which work synergistically to inhibit the growth of Streptococcusmutans.

Mouthwash is a solution or liquid used to rinse the oral cavity with a number of purposes: to exclude pathogenic bacteria, work as an astringent, to remove odor, remove infection and prevent caries, as a topical anti-inflammatory and analgesic agent.9 The mouthwash composition consists of three main components: the active ingredient, the solvent, and the surfactant which serves to remove debris from the tooth and dissolve the other material.9

The mouthwash is effective for reaching the most difficult place to clean with a toothbrush but its use cannot be as a toothbrush substitution.9 Mouthwashes as well as toothpastes have functions can be categorized as cosmetic and or therapeutic.9 Toothpaste is a dental cleaning agent containing abrasive, detergent and one or more therapeutic substances,10 which are useful for maintaining oral health.

According to Soho Global Health's Sales and Marketing for Professional Products Vice

President, In line with the back to nature trend, herbal products have been used by 80 percent of the world's population, while in Indonesia it always increases year by year and in 2012 reaches 13 trillion rupiah or about 2% of the total market of herbal medicine in the world and this makes drug manufacturers interested in continuing to develop herbal medicine.11Beluntas leaf ethanol extract also can be used as one of the active ingredients in mouthwash and toothpaste as a therapeutic agent that serves to prevent plaque accumulation and in the future can be developed into toothpaste products and herbal mouthwash that can be marketed in Indonesia.

Materials and Methods This research is experiment with completelyrandomized with pre-posttest control group design. The sample size is 105 respondents, consisting of 3 treatment groups and 4 control groups. How to select the sample is simple random sampling. The research procedure is as follows: a. At first swab plaque using sterile copanswab on the surface of the buccocervical of the maxillary tooth.

Results of plaque swabs were incubated for 24 hours at 37° C, then diluted according to preliminary test results, reproduced in Mueller Hinton solid media on blood agar plates, grounded for 24 hours at 37oC. b. Conducted isolation to separate the bacteria Streptococcus mutans from other bacterial colonies c. Identification test with Gram test, catalase test, oxidase test and identification test using Streptococcus grouping kit to confirm the presence of Streptococcus mutans bacteria and calculate the number of Streptococcus mutans manually. The calculated result is multiplied by the plaque dilution. d.

Respondents in the treatment group: brushing or gargling with 10% beluntas leaf ethanol extracttoothpasteand mouthwashor brushing and gargling with 10% beluntas leaf ethanol extracttoothpaste and mouthwash for 7 days consecutively. e. Respondents in the negative control group, brushing or gargling with a toothpaste base, or a mouthwash base, while for the positive control group, toothbrushes use 1% triclosan toothpaste and gargle with 0.12% chlorhexidine, for 7 days consecutively. f.

On the eighth day, swabs were performed on all respondents, all swabs were treated as pretest stage and then counted the number of Streptococcus mutans bacteria manually. The calculated result is multiplied by dilution. Collected data were analyzed statistically by SPSS, using descriptive test, normality test using Shapiro-Wilk test and homogeneity test using Levene test.

Normality and homogeneity test results showed normal and non-homogeneous distribution data, comparative test between groups of data was done by Repeated Measure test followed by Least Significance Difference test to see which groups were

significantly different. Results Descriptive test results were seen in Table 1 below: Table 1 The Effectiveness of Tooth Brushing and Gargling Using beluntas leaf ethanol extracttoothpaste and mouthwash to Reduce Number of Streptococcus mutans Bacteria in Dental Plaque No _Sample group _Number of Streptococcus mutans before Treatment (Pretest) (CFU/ml) _Number of Streptococcus mutansafter Treatment (Posttest) (CFU/ml) _ _ _ _Min _Max _Mean _SD _Min _Max _Mean _SD _ _1. _P0 _9 _293 _141.8 _93.30 _4 _300 _143.1 _89.74 _ _2. _P1 _8 _300 _102.3 _103.80 _1 _300 _95.7 _90.40 _ _3. _P2 _9 _305 _67 _72.07 _0 _201 _41.9 _70.49 _ _4. _P3 _19 _325 _150.1 _105.03 _0 _305 _43.7 _79.03 _ _5. _P4 _2 _211 _66,7 _66.55 _1 _114 _26.6 _30.89 _ _6. _P5 _13 _300 _102.3 _105.18 _0 _81 _31.5 _29.91 _ _7. _P6 _6 _305 _146.3 _120.23 _0 _300 _103.3 _96.48 _ _ Table 1 above shows that almost all sample group, except P0, a decrease in the number of Streptococcus mutans bacteria after treatment.

The results of the Repeated Measure test are shown in table 2 below: Table 2 Effectiveness difference of Tooth Brushing and Gargling Using beluntas leaf ethanol extracttoothpaste and mouthwashto Reduce Number of Streptococcus mutans Bacteria in Dental Plaque Effect _ _Value _F _p _ _Pre and post test _Pillai's Trace Wilks' Lambda Hotelling's Trace Roy's Largest Root _0.121 0.879 0.138 0.138 _13.503 13.503 13.503 13.503 _0.000 0.000 0.000 0.000 _ _Efectiveness comparison of pretest and posttest material _Pillai's Trace Wilks' Lambda Hotelling's Trace Roy's Largest Root _0.087 0.913 0.096 0.096 _1.565 1.565 1.565 1.565 _0.166 0.166 0.166 0.166 _ _ Table 2 shows that thecalculated F value on each test is the same, and selected variance test is Pillai's Trace because the data is not homogeneous.

Visible value of Pillai's Trace = 0.121, with value of calculated F = 13.503 and p-value = 0.000 or p <0.05, hence it is statistically proven that quantity of Streptococcus mutans bacteria before and after treatment in all admission and control group, significantly different and significant with 95% confidence interval. The comparison of effectiveness between the test materials used, the value of Pillai's Trace = 0.087, has the value of calculated F = 1.565 and the value of p = 0.166 (p> 0.05), so there is no significant difference between test materials in decreasing the number of Streptococcus mutans in dental plaque.

Discussion Descriptive test results showed that there was a decrease in the number of Streptococcus mutans in all groups after treatment, except in group P0. The multivariate analysis showed that there was a significant difference between the number of bacteria before and after treatment. Multivariate analysis also showed that all types of mouthwashes or toothpaste used by the study subjects had a significant effect on reducing the amount of Streptococcus mutans bacteria and there is no type of mouthwash or toothpaste that was superior to others.

These results indicate that all test materials have the same effect to decrease the amount of Streptococcus mutans in dental plaque. The results of this study are in accordance with the research conducted by Nahak, et al. (2015), which found that gargling with 10%, 20% and 30% beluntas ethanol extracts and 0.12% chlorhexidine, could decrease the number of Streptococcus sp.

on dental plaque.12 0.12% chlorhexidine mouthwash is an antiseptic that has antibacterial activity. Its ability to reduce aerobic or anaerobic bacteria in the oral cavity reaches 54% - 97%.3 Mechanism of action is allegedly by damaging the bacterial cell wall, inhibits bacterial enzymatic system, removing bacterial lipopolysaccharide causing bacterial cell death.13,14. 1% Triclosan toothpaste has the effect of significantly lowering the number of Streptococcus mutans in dental plaque.

Triclosan is a broad-spectrum non-ionic antiseptic, used in a variety of products, such as soap, mouthwash and toothpaste. The antimicrobial activity was obtained at concentrations of 0.2-2% and was bacteriostatic. Triclosan works by affecting the structure and function of cytoplasmic membranes that cause cells lysis.15 The results of this study also showed that the formula of 10% beluntas leaf ethanol extracttoothpaste and mouthwash could significantly reduce the amount of Streptococcus mutans bacteria in dental plaque. This is allegedly related to the content of bioactive substances.

Tannin is a phenolic compound, has antibacterial activity by forming acomplex with proline that is a kind of protein in bacterial cell wall, causing protein leakage, damaging bacterial cell wall causing bacterial cell death.16,17 Steroids have antibacterial activity allegedly by causing leakage in the liquid part of phosphatidyl ethanolamine in liposome-rich bacteria.18,19 Flavonoid compounds are a group of phenol compounds, have antibacterial activity by causingprotein leakage, resulting in leakage of bacterial cell wall, inhibiting bacterial protein synthesis and possibly interfere with bacterial cell DNA function.18,20 The actual working mechanism of the active substances contained in the beluntas leaf extract is not known with certainty,but it is suspected that the active substances are working synergistically to inhibit bacterial growth in plaque thus decreasing the amount of colony of Streptococcus mutans in dental plaque.

Multivariate test results also showed that no test material had superior efficacy compared to the others to decrease the number of colonies of Streptococcus mutans in dental plaque. In line with the “back to nature” trend, the use of herbal medicine in the world continues to increase as well as in Indonesia.11Beluntas leaf ethanol extract containing antibacterial ingredients can be used as an active ingredient in mouthwash and toothpaste as a therapeutic agent that serves to prevent plaque accumulation and

in the future can be developed into toothpaste products and herbal mouthwash that can be marketed in Indonesia.

Conclusion Beluntas leaf ethanol extracttoothpaste and mouthwash with 10% concentration effective to significantly reduce the amount of Streptococcus mutans in dental plaque and no effectiveness difference between 10% beluntas leaf ethanol extracttoothpaste and mouthwash compared with positive control in lowering the number Streptococcus mutans colonies in dental plaque or in other words, beluntas leaf ethanol extracttoothpaste and mouthwash have the ability to decrease the amount of Streptococcus mutans colony in dental plaque comparable to 1% triclosan toothpaste and 0.12% chlorhexidine mouthwash.

Ethical Clearance: Obtained from the University Committee and Respondent agreement Source of Funding: Indonesian Ministry of Health Refferences Darwita RR, Gityawati C, Andreas P, Setiawati F, Soekanto SA. The Role of Education for 21 Daysin Improving the Effectivenessof Brushing Teeth of Children Age 5 – 8-year-old in Pembeliangan Village North Kalimantan.Journal of International Dental and Medical Research 2017;10(special issue):683-688 BadruddinIA, Kiptiyah NM, Prihartono N, Agtini MD, Musadad DA.

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