The Effectiveness of *Eugenia polyanta Wight* on Oral Microorganism in Used Toothbrushes

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Abstract. Background : Oral cavity is a reservoir of microorganism as a focus infection. Some of microorganisms are transmissible of pathological disease. Toothbrushes as a medium transfer of oral microorganism has to keep properly to prevent cross contamination or reinfection. Therefore, decontaminated toothbrush is needed to reduce numbers of microorganism. Bay leaf *(Eugenia polyantha Wight)* as an Indonesian's spices is easy to find in the market. It has chemical properties consist of tannin, flavonoid, essential oils, including citric acid and eugenol.
Objective: The purpose of this study was to determine the effectiveness of *Eugenia Polyantha Wight* decoction against oral microorganism collected from used toothbrushes.
Methods: 150gram *Eugenia polyantha Wight* were boiled in 1.5 lt. distilled water in 900C for 20 minutes. It was cooled, filtrated and diluted with distilled water to become 60%, 80% and 100% of decoction. 32 toothbrushes that used by children in primary school were soaked in 3 different concentration of *Eugenia polyantha Wight* (60%, 80%, 100%) and sterile water as a control. All samples and control were diluted with NaCl 0.85%, then inoculated twice on PCA medium using pour plate method. Full covered plates were incubated for 24-48 hours in 37^0C. Colony counter has been used to count the total number of oral microorganisms on 64 PCA discs. The data were analysed using Kruskal-Wallis and Mann-Whitney test.
Result: There were significance differences of numbers of microorganism between three concentration (p = 0.007) including sterile water. The mean of microorganism number in concentration 0%, 60%, 80% and 100% were 22.94, 35.96, 3.44 and 3.88 respectively. There were significance differences between 0% and 60% (p=0,006), 0% and 80% (p=0.009), 0% and 100%(p=0.08). However, there were no significance differences between three consecutive concentration: 60% and 80% (p= 0.123), 60% and 100% (p = 0.164). 80% and 100% (p=0.874).
Conclusion: *Eugenia polyantha Wight* has an effectiveness to reduce numbers of oral microorganism within three concentration compare to the control. However, the most effective concentration of decoction cannot be determined.
Keyword: Used toothbrush, Decoction, Eugenia polyantha Wight, Oral microorganism

Introduction

Oral cavity as a reservoir of million types of oral microorganisms including Streptococcus, Staphylococcus, Neiseria, Candida, Lactobacillus, Veillonella and Coliforms are confirmed. Used toothbrush as a reservoir of oral microorganism may contribute in disease transmission and increase risk of infection. In addition, contaminated water washing rinse, toothbrush storage and dirty hands allows the presence of microorganism outside the oral cavity to be attached to the toothbrush. However, role of toothbrushes as an instrument uses in both community and hospital settings are essentials. Appropriate container and decontamination of toothbrushes to reduce cross contamination in primary school which provide facility and program toothbrushing together program is needed.

The American Dental Association (ADA) acclaims a number of things to avoid or reduce microorganism

contamination on toothbrushes. They suggest to rinse toothbrushes with running water or another soak in antimicrobial solutions such asCetylpyridinium chloride 0.05%[1], Alcohol[2,3], chlorhexidine[2,3,4,5,.]Antimicrobial solutions from herbal to decontaminate toothbrushing have been introduced in sprays or soaked solutions[6,7] There are garlic, green tea and neem fruit [5.]However, most of materials and instrumentsfor decontamination are expensive and not familiar to use at home or rural community area.

Eugenia polyantha Wight or *Eugenia lucidula Miq*.; *Syzygium polyanthu* (*Wight) Walp with classification Plantea (Kingdom),* Kingdom : *Plantea*, division: *Spermatophyta*, Sub division : *Pinophyta*, Class: *Coniferopsida*, family: Eugenia, genus: *Myricales* and spesies: Eugenia polyanthum (Wight) walp.
In Indonesia: Indonesian *bayleaf* atau *Indonesian laurel*. Nama Daerah: Sumatera : ubar serai, meselangan, Jawa : manting, salam, Madura : salam. Eugenia polyantha, a tropical tree belonging to the Myrtaceae family, is also known as Indonesian bay leaf or Salam.

Indonesian's Bay leaf (*Eugenia polyanta Wight)* or *daunsalam*has long been known as a spice that can be used for traditional healing and easy to find in the market.Itconsists oftannins, flavonoids, saponins and essential oils. Some of them are active compounds and have antimicrobial activity such as disrupting the integrity of bacterial cell membranes or ability to interact with bacterial DNA, thus causing bacterial cell wall permeability (Sabir, 2003). Other than that, bay leaf proven can be as an anti-fungal and anti-worm. Decoction of leaves are easy methods that can practice at home daily. Therefore, based on this data, the efficacy of boiled water *Eugeniapolyantha Wight* against microorganism on used toothbrushes with various concentrations is interesting to determine and it's the aim of this study. The result of this study can be used as reference forantimicrobial solution to decontaminate used toothbrushes.

Materials and Methods

Preparation of decoction: E. polyantha leaf was purchased fromtraditional market, Bandung, Indonesia. The sample was identified and deposited in Laboratory of Natural Products, Institute Technology Bandung.Fresh bay leaves washed, drained, and sliced. Weighed 150g slices of bay leaf, put into 2L beaker, added 1.5L of distilled water andboiled at 90^0C for 20 minutes. Cooled, then filter 600 mL and 800ml of filtrate, diluted with distilled water until 1L of each. No dilution for 100% concentration of decoction. 16 flasks contain 50 mL method such as ultra violet, tetrasodium EDTA and

of distilled water, 16 flasks contain 50 mL of 60% bay leaf, 16 flasks contain 50 mL of 80% bay leaf and 16 flasks contain 50 mL of 100% bay leaf have been prepared.

Media and Reagents: Plate Count Agar (PCA) composed by tryptone, yeast extract, glucose, agar have been made.

Implementation of Tooth Brushing
Elementary students who were selected as participants were each given 4 toothbrushes. Then, given the knowledge of how to brush their teeth properly. Furthermore, under the guidance and supervision, all participants brushed their teeth for 30 seconds. The toothbrush is inserted into each of the flasks prepared according to the concentration. The toothbrush was stirred, then soaked for 20 minutes.

Examination of TotalBacteria:
1ml of each toothbrush immersion put in each tube contain 9ml of 0.85% NaCl (10^{-1} dilution) accordingly, then 10^{-1} dilution put into 10^{-2} dilution until 10^{-3} dilution. The 10^{-3}dilution was inoculated on PCA, then incubated at 37^oC for 24-48 hours. Calculation the total bacteria of each PCA were using colony counter.

Result

The mean of total bacterial colonies on toothbrushes after soaking in various concentrations of bay leaf can be seen in the graph below.

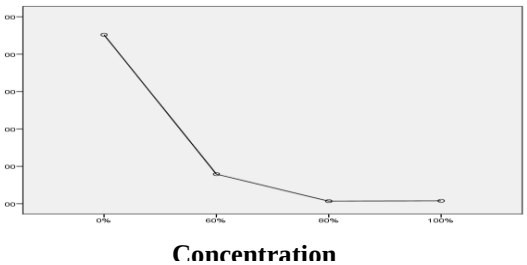

Figure 1. Antimicrobial efficacy of *Eugeniapolyanta Wight* in three concentration compare to distilled water

The picture above shows thatcontrol group the number of bacterial colonies was quite high (average of 225.9, while at concentrations of 60%, 80%, and 100% ware decrease in the number of bacterial colonies

Table 1. Signifince Test Control (0%) and Eugenia polyanta Wight in three concentration.

Concentration (%)	$\bar{x}$ ± SD	Z	Sig. p <0.05
0	225.94 ± 137.14	-2.759	0.006
60	39.56 ± 105.36		
0	225.94 ± 137.14	-2.598	0.009
80	3.44 ± 3.46		
0	225.94 ± 137.14	-2.648	0.008
100	3.88 ± 4.55		

Based on the data in table 1 above, it can be seen that the average number of bacteria samples with a concentration of 0% = 225.94, 60% = 35.96, 80% = 3.44 and 100% = 3.88. It was seen that the average number of bacteria samples with a concentration of 60%, 80% and 100%, was smaller than the average number of bacteria samples with a concentration of 0%.The results of the calculation of the statistical value of the Z test to test the difference in the number of bacterial samples with concentrations of 0% and 60%, 0% and 80%, and 0% and 100% respectively obtained a test significance value (p-value) <α, ie (0.006, 0.009, and 0.08). There are significant differences in the number of bacteria samples with concentrations of 0% and 60%, 0% and 80%, and 100%.

Table 2. Significancy test within three concentrationof *Eugenia polyantha Wight*

Concentration	Standar Deviation	Z	Sign p < 0,05
60%	39.56 ± 105.36	-1.541	0.123
80%	3.44 ± 3.46		
60%	39.56 ± 105.36	-1.391	0.164
100%	3.88 ± 4.55		
80%	3.44 ± 3.46	-0.159	0.874
100%	3.88 ± 4.55		

Referto the data in table 2, it can be seen that the average number of bacteria samples with a concentration of 60% = 39.56, 80% = 3.44, and 100% = 3.88. It was seen that the average number of bacteria samples with a concentration of 80% was smaller than 100%, and 100% smaller than 60%.The results of the calculation of the statistical value of the Z test to test the differences in the number of bacteria samples with concentrations of 60% and 80%, 60% and 100%, and 80% and 100% respectively obtained a test significance value (p-value)> α, ie (0.123, 0.164, and 0.874)> 0.05.There is no significant difference in the number of bacteria samples with concentrations of 60% and 80%, 60% and 100%, and 80% and 100%

DISCUSSION

Toothbrushing is an effort to clean the oral cavity from plaques and prevent caries and periodontal diseases. A study shows that regular toothbrush can cause cross contamination. In addition, toothbrushes can also be contaminated by microorganisms from the environment out of oral cavity. Humid conditionsin the bathroom can facilitate bacterial growth, especially found through aerosols from toilets, rinse water, contaminated fingers and commensal skin.

Karibasappacit Caudry (1995) through his research stated that has isolated *S. mutans*, Candida albicans, Lactobacillus, Klebsiella, S. pyogenes, S. Aureus, and Pseudomonas on toothbrushes if the toothbrush placed not adjacent to the toilet and Escherichia Coli if near the toilet[8]. Their finding inline to this study, its proven that microorganism in three times dilution of Eugenia polyanta Wight were growing in all concentration after used toothbrushes soaked into the solution.

Based on the results with a concentration of 60%, 80%, and 100% were proven to significantly less numbers of microorganism, that's mean the leaves inhibit the growth of coloniesmicroorganism compare to the control. This supportedwith the research conducted by AdriantoAngger, 2012 who has conducted research on the anti-bacterial bay leaf extract on toothpaste against Streptococcus Mutans. Various literature states that bay leaves have many medicinal properties, including treating diabetes, hypertension, high cholesterol, gastritis, diarrhea, gout, eczema, scabies, and itching [9].

This result shows that decoction of bay leaves has effect of inhibiting the growth of bacterial colonies on toothbrushes by the active substances in bay leaves. Each active substance has a certain mechanism to inhibit microorganism growth, such as essential oils of 0.05% consisting of eugenol and citral[10], Tannin causes protein denaturation by forming a protein complex[10].Flavonoids as an enzyme inhibitor and an antimicrobial, anti-inflammatory effects, stimulate collagen formation, protect blood vessels, antioxidant and anti-carcinogenic [10,11,12].

Even the result shown that the average number of bacteria samples with a concentration of 100% is

less than 80%, and 80% is less than 60%, statistically there were no significance differences. This is probably caused by the bacteria contained in the plaque is very diverse. It seems that eachmicroorganism has a variety of properties and resistanceto bay leaf solution. Probably, due to the difference of effectiveness of bacteria Gram (+) and Gram (-)to disrupt the permeability of a bacterial wallin a certain concentration[13]. Therefore,bay leaf herb extractusing decoction method was able to inhibit colony growth bacteria.

Conclusions

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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