Propolis *Trigona sp.* Mouthwash Efectiveness in Lowering Anaerobic Gram-Negative Bacteria Colonies

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ABSTRACT

Background: *Periodontal disease is an infectious disease that attacks gingiva and other dental supporting tissue which caused by microbial bacteria in subgingival plaque. The majority bacteria are an anaerobic gram-negative. Propolis is one of natural products as an antimicrobial mouthwash. One type of bee that able to produce propolis in large quantities is Trigona sp. which is common in South Sulawesi.* ***Objective:*** *The aim of this research is to know the effectiveness of propolis Trigona sp. as a mouthwash against some colonies of anaerobic gram-negative bacteria.* ***Method:*** *The type of this research is used pretest-posttest with control group design. A sample size of 28 people was selected with the research criteria who visited the Periodontology Department of Dental Hospital of Hasanuddin University. The treatment group rinse using extract propolis 5% and 10% and aquades as a control. Negative anarobic bacterial carried out from sulcus gingival fluid, then cultivated in medium MacConkey using spread method. Statistical analyzes used were T paired, and Anova test (p<0,05).* ***Result:*** *Paired T-test results showed that there was a difference of the number of anaerobic gram-negative bacteria colonies between before and after 14 days of treatment in the propolis group of 5% and 10% concentration (p = 0,000), when in the control group (aquades) there was no difference (p = 0.057). Anova test results showed that there was a decrease in the number of anaerobic gram-negative bacteria colonies between 5%, 10% propolis and control group (p = 0,000).* ***Conclusion****: Extract propolis Trigona sp. 5% and 10% which is contained in mouthwash is effective in lowering the number of anaerobic gram-negative bacteria colonies. The use of mouthwash with extractives of propolis may be recommended as an alternative therapy for periodontal disease.*
Keywords: Propolis Trigona Sp.; Mouthwash; Anarobic Gram-Negative Bacteria

INTRODUCTION

Health problems identifications, especially for dental and oral health, has been increasing from time to time. Based on the National Health Survey (*Suskernas, Survei Kesehatan Nasional*) in 2003, dental and oral disease was ranked first among the 10 most common diseases in the population. Other than dental caries, periodontal disease is the oral disease that most patients have (Tanjay & Auerkari 2011). In Indonesia, of all oral health problems periodontal disease ranks second prevalence of 96.58% (Lumentut et al. 2013).

Periodontal disease is an infectious disease that attacks the gingiva and other dental supporting tissues. If it is not treated, it will result in tooth loss. (Lumentut et al. 2013; Fedi et al. 2013) Accumulation of bacterial plaque on the tooth surface becomes the main cause of inflammation in the dental supporting tissues that can cause lose of attachment and alveolar bone (Batista et al. 2014). On the other hand, periodontitis is an inflammatory

conditioqn of the gingival tissue that extends to the cementum, periodontal ligament, and alveolar bone which characterized by the formation of periodontal pockets and alveolar bone resorption (Bansal et al. 2012).

Research on the pathogens of periodontal disease has been conducted continuously. Periodontal disease occurs through the growth of microbial bacteria in subgingiva. Bacterial products passing through the junctional epithelium cause inflammation of the tooth supporting tissues. This makes it easier for bacteria to colonize on the subgingival tissue (Kesic 2008). The majority of subgingival plaques consist of Gram-negative anaerobic bacteria, such as *Actinobacillus actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythia (Tf),* and *Fusobacterium nucleatum (Fn)* (Ovadia, Zirdok, Romero, 2017) These bacteria play a major role in severity periodontitis, leading to the formation of periodontal pockets, connective tissue damage, and alveolar bone resorption. (Pereira 2011).

Nowdays, communities are in dire need of safe, effective, and economical prevention and alternative treatment. Dental plaque in the majority of the population has not been cared for effectively. Usage of antimicrobial mouth rinses has been proposed as an effective action in addition to remove or control dental plaque (Parolia 2010). A number of mouthwash chemicals products can suppress the growth of pathogenic microorganism but they have side effects. Therefore, search for alternative products from plants as natural medicine is needed to obtain considerably good alternatives. One of these alternatives is the propolis (Parolia 2010). Propolis is one of the natural products that have been used to treat various diseases and inflammatory conditions as both local and systemic applications. Propolis has antimicrobial characteristic. In its natural form at room temperature, propolis is a sticky substance but becomes hard and brittle at low temperature (Sabir 2005)

Propolis has attracted researchers in the last decade because of some biological and pharmacological properties, such as immunomodulators, antimicrobials, and antioxidants (Anonim 2009). Not all of bee species produce propolis at the same level. One type of bee that is able to produce propolis in large quantities is *Trigona sp.* which is common in South Sulawesi (Anonim 2009). The *Trigona* genus bee has no sting and this is one of the factors that causes the difference in the quantity of propolis produced. (Tomic et al. 2014). The purpose of this research is to investigate effectiveness of mouth rinse containing propolis from *Trigona sp.* against the number of colonies of anaerobic Gram-negative bacteria.

METHOD

This study is true experimental study with pretest-posttest with control group design. Mouthwash containing *Trigona sp.* propolis was prepared at Pharmacy Laboratory of Hasanuddin University of Makassar. The mouthwash was applied at Periodontology Departmenet of Dental Hospital of Hasanuddin University, for the treatment of study's subjects. Microbiological evaluation for Gram-negative anaerobic bateria was performed in Microbiology Laboratory, Faculty of Medicine, Hasanuddin University Makassar.

We obtained as many as 30 subjects. However, during the procedure there were 2 people who were excluded from the study because they did not experience the growth of Gram-negative anaerob bacteria so the number of research subjects was only 28 people. The ingredients used to create 5% and 10% mouthwash solution from *Trigona sp.* were extract of *Trigona sp.* propolis, glycerol, propylene glycol, and aquades. Aquades was used as control mouthwash. To evaluate the anaerobic Gram-negative bacteria, we utilized Stuart medium, BHIB medium (Brain Heart Infusion Broth), and medium for MacConkey, petri dish, gasvac, and an incubator.

Procedure

The procedure of this research was:

1. Preparation of mouthwash were contained propolis *Trigona sp.*:
2. Examination of subjects and were taken gingival sulcus fluid
    a. Thirty subjects were randomly selected for either of these three procedures: (1) 10 subjects were treated with 5% propolis mouthwash, (2) 10 subjects were treated with 10% propolis mouthwash, and (3) 10 subjects were treated with aquadest mouthwash as the control group.
    b. The gingival sulcus fluid was taken by inserting the paper point into the sulcus until it no longer moves and then left in the sulcus for 1 minute. Blood-contaminated paper point was excluded. Then the paper point was placed in a tube containing the Stuart medium.
    c. Furthermore, the tube containing the paper point was taken to the microbiology laboratory.
3. Evaluation of Gram Negative Anaerobes

The statistical test used was Kolmogorov-Smirnov test for data normality, Levene test for homogeneity variant, paired T test to differentiate the number of colonies of anaerobic Gram-negative bacteria between before and after 14 days of treatment in all three treatment groups, and Anova test to distinguish the large decrease in the number of colonies of anaerobic Gram-negative bacteria among the three groups. The degree of significance used is $\alpha = 0.05$. (Syeda et al. 2013)

RESULT AND DISCUSSION

Table 1 Distribution of research subject by sex and treatment group

Variable	Frequency (n)	Persentage (%)
Sex		
Male	10	35.7
Female	18	64.3
Treatment group		
Propolis concentrate 5%	9	32.1
Propolis concentrate 10%	10	35.7
Control	9	32.1
Total	28	100

Table 1 shows there were more women than men that participated in this study (18 women, 64.3% and 10 men, 35.7%). Number of subjects in 5% propolis treatment group were 9 (32.1%), in 10% propolis treatment group were 10 (35.7%) subjects, and in the control group were 9 subjects (32.1%).
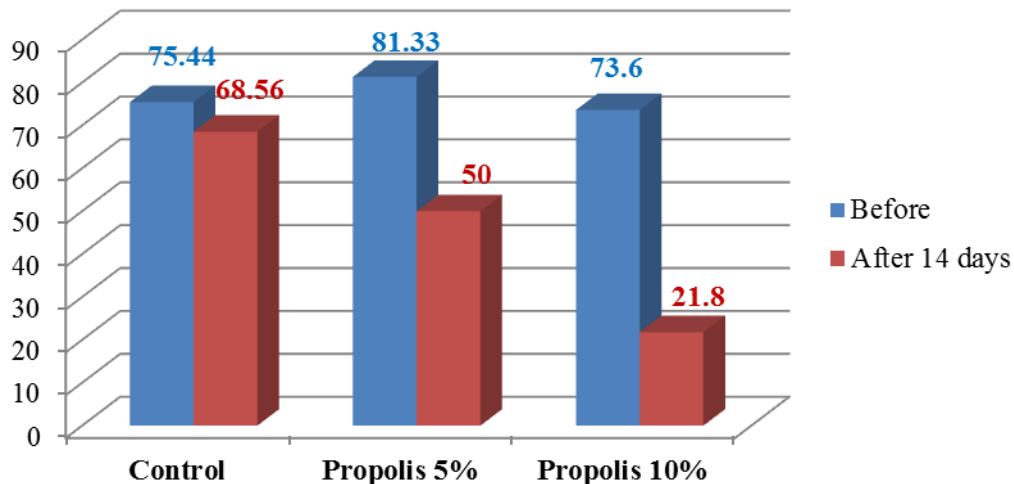
Table 2 Differences in the number of colonies of anaerobic Gram-negative bacteria before and after 14 days with the difference on each treatment group.

Treatment group	Number of colonies of anaerobic Gram-negative bacteria (CFU/ml)				
	Before	After 14 days	p-value	Deviation	p-value
	Mean ± SD	*Mean ± SD*		*Mean ± SD*	
Control	75.44 ± 41.95[a]	68.56 ± 37.44[a]	0.057	6.88 ± 9.29[a]	
Propolis 5%	81.33 ± 33.29 [a]	50.00 ± 30.73[a]	*0.000*[b]	31.33 ± 16.26[a]	*0.000*[c]
Propolis 10%	73.60 ± 26.39 [a]	21.80 ± 17.91[a]	*0.000*[b]	51.80 ± 10.70[a]	

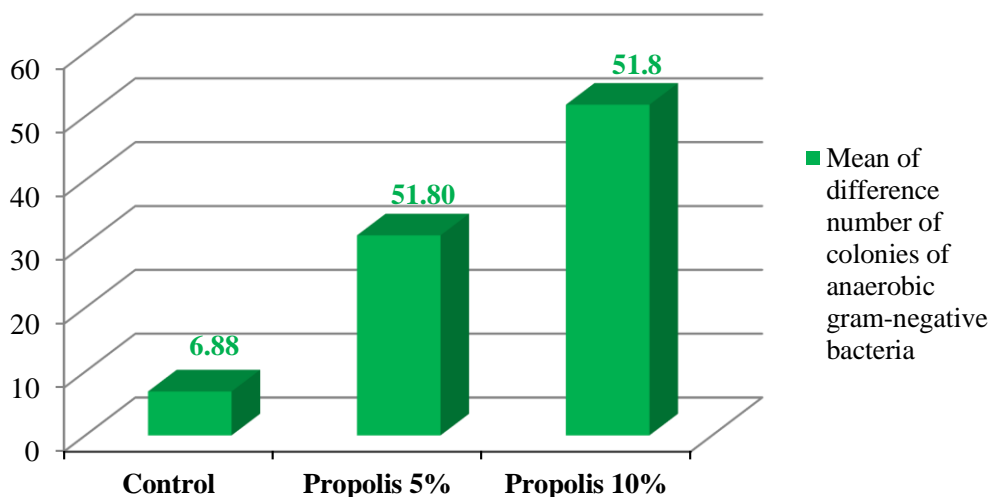[a]Normality data test: *Kolmogorov-Smirnov* test; *p>0.05*; data is normally distributed
[b]*Paired t-test sample: p<0.05; significant*
[c]*One way Anova test: p<0.05; significant*

Figure 1 Distribution of mean of number of colonies of anaerobic Gram-negative bacteria before and after 14 days treatment in the control group, propolis 5%, and propolis 10%

Figure 2 Distribution of mean of deviation of number of colonies of anaerobic Gram-negative bacteria before and after 14 days of treatment in the control group, propolis 5%, and propolis 10%.

Distribution and difference in the number of colonies of anaerobic Gram-negative bacteria before and after 14 days of treatment in each group were shown in Table 2 and Figure 1. The results showed that there was a decrease in the number of colonies of anaerobic Gram-negative bacteria in all treatment groups. In the 5% propolis group, the

number of bacterial colonies before treatment reached 81.33 CFU / mL and after 14 days of treatment decreased to 50 CFU / mL. In the 10% propolis group, the number of colonies before treatment reached 73.60 CFU / mL, but after 14 days of treatment decreased to 21.80 CFU / ml. The same is seen in the control group decreasing from 75.44 CFU / ml to 68.56 CFU / ml. Normality test results show that the data is normally distributed, so the parametric test can be used. Based on the result of statistical test, obtained p value = 0.000 (p <0.05) on propolis group 5% and propolis 10%. This showed that there is a significant difference in the number of colonies of anaerobic Gram-negative bacteria between before and after 14 days of treatment in the 5% and 10% propolis groups. Meanwhile, in the control group, (p> 0.05) which means that there was no significant difference of colony count between before and after 14 days.

The result of data normality test, Kolmogorov-Smirnov shows p> 0.05, in all three groups meaning the data is normally distributed. This is also followed by Levene's homogenity test result that showed the p value > 0.05 meaning homogeneous data variance was observed. Thus, Anova's parametric test requirements are met and can be used. Based on one-way Anova test, p value was 0.000 (p <0.05), which means that there is difference of effectiveness of decreasing number of significant anaerobic Gram bacteria colony among the 5% propolis solution group, propolis solution 10%, and control solution group.

Table 3 Further Difference test results deviantion of the mean number of colonies of anaerobic Gram-negative bacteria before and after 14 days of treatment between control group, propolis 5%, and propolis 10%.

Treatment (i)	Comparison (j)	Mean Difference (i-j)	p-value
Propolis 5%	Propolis 10%	20.466	0.004*
	Control	24.444	0.001*
Propolis 10%	Control	44.911	0.000*

Pos Hoc Test: Tukey's Significant Difference (HSD) test: p<0.05: significant

Table 3 shows difference test results showed p value <0.05 on all differences between groups. Thus, there were a difference in the number of anaerobic Gram-negative colonies between the 5% propolis group and the propolis 10%, between the 5% propolis group and the control, and between the 10% propolis and the control group.

Most oral diseases are caused by bacteria in the oral cavity. Periodontitis is the most common pathological condition in the community and caused by dental plaque bacteria. Antimicrobial agents are needed for the prevention and treatment of periodontal disease because it can inhibit the formation of dental plaque bacteria effectively (Coutinho 2012).

The decision to use 5% propolis concentration is based on research conducted by Pereira whom showed that the use of mouth rinse with 5% Brazillian propolis concentrate can reduce supragingival plaque and gingivitis as additional therapy in maintaining oral health (Parolia, 2010). Our study used propolis concentration of 10% because a study by Cairo de Maral showed that 10% Brazillian propolis concentrate was effective for treatment of periodontal disease (Fokt et al. 2010)

The results showed that the mouthwash of propolis *Trigona sp.* was effective to lower the number of colonies of anaerobic Gram-negative bacteria at either 5% or 10% concentration (Table 3).

A similar study conducted by Coutinho using the propolis extract from India as an irrigation solution on the periodontal pocket showed there was a decrease in the number of colonies of anaerobic bacteria *Porphyromonas gingivalis* and decreased bleeding at the time of probes after two weeks. That study concluded that subgingival irrigation with

propolis extract as an alternative to periodontal treatment was more effective than scaling and root planing assessed by clinical and microbiological parameters (Agarwal et al. 2012)

The highly variable antibacterial activity of propolis is due to the composition of the propolis used. Study by Koru *et al* showed highly effective antibacterial action against anaerobic pathogens such as *Peptostreptococcus anaerobius, Lactobacillus acidophilus, Actinomyces naeslundii, Prevotella oralis, Prevotella melaninogenica, Porphyromonas gingivalis, Fusobacterium Nucleatum* and *Veillonella parvula*. They concluded that antibacterial properties of propolis are due to the presence of flavonoids and aromatic compounds such as caffeine acid (Sabir 2005).

Flavonoids are one of the widespread natural phenol compounds in plants which are synthesized in small amounts and can be found in almost all parts of the plant (Anonim 2009). The antibacterial mechanism is based on inhibition of bacterial RNA polymerization. In addition, there is functional and structural damage of the bacterial cytoplasmic membrane which causes the loss of potassium ions resulting in cell autolysis (Hasan et al. 2012).

Our study also showed that there was a significant difference between the treatment group and the control group. In the group of 10% propolis extracts showed a decrease in the number of higher anaerobic Gram-negative bacteria compared to the 5% propolis extract group, which was 20.466. Our result was similar with the result from in vitro study in China by Agarwal *et al* whom showed that 10% propolis solution was effective in inhibiting growth of anaerobic Gram-negative bacteria such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* that caused by periodontal disease.

Greater concentration of propolis showed greater antibacterial activity which means the antibacterial properties of propolis might have dose-dependent effect; perhaps due to the flavonoids.

Our study showed that 5% and 10% solution mouthwash containing extract of *Trigona sp.* propolis were effective to lower the number of colonies of anaerobic, periodontogenic, Gram-negative bacteria due to antibacterial properties of the propolis content of flavonoids. Usage of mouthwash containing extract of propolis can be recommended as an alternative therapy of periodontal disease.

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