

Research Article

Antibacterial activity test of South Sulawesi Propolis extract against

Streptococcus mutans

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Abstract: Oral infection such as tooth caries and periodontal disease are the most common bacterial infection in human. The main cause of periodontal disease is microorganisms that colonize and attached to the tooth surface around the gingival margin. *Streptococcus mutans* is the first bacteria that forms colonies and become the initiator of the other bacteria do so. The left bacteria colonies may change to be plaque layer and causes an inflammatory reaction, may lead to tissue damage. Propolis is a natural substance, well known its benefits in dentistry. The type of propolis varies depending on the area or location and type of honey bees that produce it. Propolis is also expected to inhibit *Streptococcus mutans* that cause periodontal disease. The aim of the study is determine the inhibition zone of propolis extract from South Sulawesi on *Streptococcus mutans*. Inhibition test was performed by the agar diffusion method using propolis test materials, provided in a concentration of 2.5%, 5%, and 10% as well as negative control. Inhibition zone was measured after 24 hours incubation. Concentration has effect that causing significant difference in inhibition of the change in concentration of propolis ($p < 0.005$). The use of different solvents in the extraction process also has an influence on the inhibition of propolis or there is a significant difference between the propolis extracted with hexane solution (fat-free propolis) and 70% of propolis solution ($p < 0.005$). The interaction between the type of solvent with a concentration on its effect on the inhibition of propolis also showed a significant difference ($p < 0.005$). South Sulawesi Propolis has inhibitory effect on *Streptococcus mutans* especially those was dissolved in 70% ethanol solution and also influenced by the level of concentration.

Keywords: Propolis, *Streptococcus mutans*, Antibacterial activity.

INTRODUCTION

Periodontal disease is one of the common microbial infections in adults. This disease is an inflammatory disease that originated from bacteria that affect the supporting tissues of the teeth [1]. There are two types of periodontal disease is gingivitis and periodontitis. Gingivitis, the most common forms of periodontal disease, has a high prevalence of 50% - 90% of adults worldwide [2]. While 75% of adult population has at least mild periodontal disease, 20% - 30% exhibits the severe destruction form [3]. According to the morbidity study by 2003 Surkesnas (Indonesia health survey), tooth and oral diseases placed on the first rank of 10 groups of most community-complained diseases.

Periodontal disease induced by colonizing Gram-positive aerob bacteria, *streptococci*, *lactobacilli*, and *actinomyces* to acquired pellicle formed on tooth surface. Two or four days later, colonies of bacteria are

formed, for example *Porphyromonas gingivalis*, *Actinobacillus*, *Prevotella*, and other Gram-negative bacteria. Subsequently, pathogenic bacteria dominate the subgingival plaque, such as *Porphyromonas gingivalis*, *Treponema denticola*, *Tanarella forsythensis*, *Actinobacillus actinomycetem comitans*, *Fusobacterium nucleatum*, and *Eikenella corodens* [4,5,6].

Periodontal disease is an inflammation reaction of host defense against bacterial invasion. In this case, *Streptococcus mutans* plays an important role, as described above, colony of bacteria that initiate the periodontal disease is *Streptococcus mutans*, who dominates the gingival crevice and root surface areas [4,5].

In attempt to manage the tooth and oral disease, most researchers investigate natural remedies

that can be used to maintain tooth and oral health. And, propolis is one of them.

Propolis is a sticky resin substance collected by honeybees from the sap of plants, leaves, and buds, which is mixed with beeswax and saliva on her nest. Bees use propolis to strengthen the walls of the hive and protect it from infection, human use these product to enhance the immune system. There are more than 180 chemicals contained on propolis, and affected by the type of bee, climate, type of plants and trees, and collecting time [7].

In dentistry, propolis was used in post-surgical wound healing, endodontics, dentin hypersensitivity, aphthous ulcers, candidiasis, acute necrotizing ulcerative gingivitis (ANUG), gingivitis, periodontitis and pulpitis [7,8,9].

Streptococcus mutans which has a role in initiating periodontal disease raises the interest for further research of the effects of propolis on *Streptococcus mutans* bacteria, as the cause of periodontal disease.

MATERIAL AND METHODS

This study is an experimental laboratory research with posttest only control group design, and conducted in two location, which is the Center of Research Activities UNHAS and Mathematics Microbiology Laboratory UNHAS. In this study, we extract the propolis and then the inhibition test against *Streptococcus mutans* was done.

The propolis used in this study isolated from *Trigona* sp. Honey bee which are found in South Sulawesi. The propolis was extracted using maceration technique or simple maceration technique. There was two solutions used as solvent, ethanol and hexane (fat-free). First, the propolis was heated in the oven with 40°C for 3 days. A total of 800 grams of propolis dissolved in 2 L ethanol 70%, and the same mass of propolis also diluted with hexane solution. To enhance the dissolution, propolis was stirred. The propolis in the ethanol and hexane solution was left for 48 hours, and stirred every 24 hours. Subsequently, filtration was done and the filter is left for certain period so the unnecessary substances precipitated, not soluble in the ethanol or hexane.

The propolis extract that prepared before was diluted. The concentration used was 2,5%, 5% and 10%. A total of 2 g propolis extract was diluted in 2 mL of distilled water to obtain a concentration of 10% propolis. Subsequently, 10 mL 10% propolis was transferred to another test tube and 10 mL of distilled water was added to obtain the concentration of 5% propolis. To obtain the 2,5% propolis, 10 mL of distilled water was added to 10 mL 5% propolis.

The next step was preparation of mueller hinton agar (MHA) medium. A total of 4,75 gr Mueller Hinton agar diluted with 125 distilled water, heated and stirred homogenously. The agar was sterilized in an autoclave for 15 minutes, 121°C.

Agar diffusion method was used in the inhibition test. This method is commonly used in the inhibitory test. The propolis levels used in the samples was 2,5%, 5%, 10% and negative control. The inhibition test performed was as follows, the prepared MHA medium was heated and melted, allowed to cool. Distilled water was added to pure isolates of *Streptococcus mutans* to facilitate the bacterial retrieval using a syringe. Bacteria was added to the Erlenmeyer flask containing MHA medium, then the bacteria agar was stirred and mixed. Paper disc was placed on three petri dish, each dish contain 4 paper disc, propolis with different concentrations were applied on it. Except on one paper disc that were used as negative control. About 25 mL of mixed bacteria medium was added to every petri dish. The petri dishes was incubate for 24 hours at 37°C. The inhibition zone was measured with a millimeter ruler.

The data was analyzed using two-way ANOVA test to determine the inhibition differences at various concentrations and type of solvents used during the extraction. Subsequently, Least Significant Difference (LSD) test was done to determine the inhibition differences at each concentration and compare it with another.

RESULT AND DISCUSSION

The inhibition zone found in this study depicted in Figure 1, the mean value was showed in Table 1. The effect of various propolis concentrations to the width of inhibition zones and different solvents during propolis extraction was analyzed with two-way ANOVA test, and the result summarized in Table 2.

Table 1, the mean value of propolis inhibition zones against *Streptococcus mutans* bacteria, at different concentration and type of solvents used during the extraction periods, showed different inhibition strength. The ethanol 70%-diluted propolis showed higher inhibition strength than fat-free propolis or hexane-diluted propolis.

Table 2 showed that propolis concentration induce an effect or, in other words, different propolis concentration exerts significantly different inhibition strengths ($p < 0.005$). The type of solvents used in the extraction process also influenced the propolis inhibition strength or there was a significant difference between hexane-diluted (fat-free propolis) and ethanol 70%-diluted propolis ($p < 0.005$). Likewise, an interaction between type of solvent and the propolis concentration also affecting the inhibition strength of propolis significantly ($p < 0.005$).

This result supported by a study showed that propolis is a good antibacterial agent. Antibacterial activity of propolis varies depending on the sample, dose or concentration and solvent used to extract all of the samples tested [9,10,11]. An explanation for the probable mechanism of action of propolis is a fact that one or all elements contained significantly inhibits the bacterial mobility and enzyme activity and affects the cytoplasmic membrane, which change the bacterial membrane's ionpermeability[7,11].

It also in line with a research by Sabir (2005) who examined the ability of flavonoids from *Trigona sp* propolis against *Streptococcus mutans* and found that periods of time affecting the inhibition zoneformation, although concentration of flavonoid >0,1% are required for a period of 24 hours [12].

Because the two-way ANOVA test showed differences, we conducted further analysis using Least Significant Difference (LSD) test to determine the differences in each concentration and compare it with another. The LSD result are summarized in Table 3.

Table 3 shows that the negative control have significant inhibition strength against all of the propolis concentrations. Likewise, another concentration also exert a significant inhibitor effect, except the 5% and 10% propolis which is non-significant ($p>0.005$).

A study using experimental rat also found that propolis can inhibit the growth of *Streptococcus mutans*. In the study, extraction of propolis content, ethanol and hexane, was done then applied topically on the rat's tooth surface, twice a day for 5 weeks [13].

The same step also described in a study of 41 volunteers who use propolis as a mouthwash, it was found that the number of *Streptococcus mutans* in the saliva of 81% of the 41 volunteer was decreased after a week [14]. Inhibition effect of propolis against the growth of *S. mutans* bacteria also investigated *in vitro*, using *S. mutans* bacteria isolated from the saliva [15,16]. The same study also conducted clinically and it was found that propolis was able to reduce the number of *S. mutans* and *Lactobacili* [17].

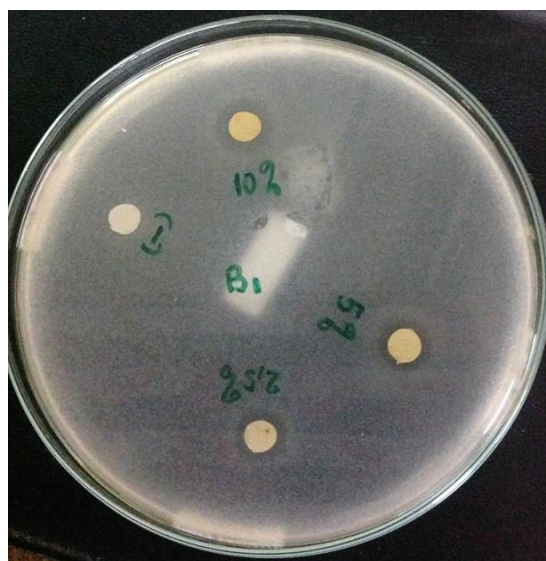


Figure 1. Inhibition zone of propolis against *Streptococcus mutans* bacteria after 24 hour incubation

Table-1: The mean value of propolis inhibition zones against *Streptococcus mutans* bacteria

Mean Diameter Inhibition Zone (mm)				
S	Mean KN	Konsentrasi		
		Mean 2,5 %	Mean 5 %	Mean 10 %
FFR	-	7.0000	7.6667	8.0000
PE	-	9.3333	10.0000	10.3333

S = solvent; FFR= Fat Free Propolis; PE= Propolis Ethanol 70%

Table-2: Results of two-way ANOVA test on the interaction between the concentration and type of solvent

	Df	MS	F	Sig.
Concentration	3	115.153	921.222	.000
S	1	18.375	147.000	.000
Concentration S	3	2.042	16.333	.000

df= degree of freedom; MS= Mean Square; S = solvent

Table-3: Test results Least Significant Difference (LSD) of the difference of each concentration

C	NC	2.5%	5%	10%
NC	-	.000	.000	.000
2.5%	.000	-	.005	.000
5%	.000	.005	-	.122
10%	.000	.000	.122	-

Keterangan :C= concentration; NC: Negative control

CONCLUSION

South Sulawesi Propolis has an inhibitory effect on bacteria *Streptococcus mutans*, especially ethanol 70%-diluted propolis. In this case, the type of solvent used at extraction affecting the inhibitory effect of propolis. In addition, the inhibitory effectiveness of propolis also affected by the concentration. The greater the concentration, the inhibitory effect exerted also higher. There is an interaction between the type of solvents used and the propolis concentrations.

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