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Antibacterial Activity of Jambi Forest Honey on Cariogenic Bacteria *Lactobacillus acidophilus*

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ABSTRACT

Caries is a major dental and oral health problem in Indonesia because of their high prevalence. Based on the 2018 Basic Health Research in Indonesia, the prevalence of caries in Indonesia is 88.8%. Dental caries is a local destructive on dental hard tissue caused by the presence of acids produced from bacterial metabolism in dental plaque. The use of mouthwash has an important role in maintaining dental and oral hygiene, especially in reducing bacteria in plaque. A study showed that gargling with honey can reduce plaque scores in the oral cavity. This study aims to determine the antimicrobial effect of Jambi forest honey on cariogenic bacteria. The caries-causing bacteria used in this study was *Lactobacillus acidophilus*. This research is an in vitro experimental study using the disk diffusion method to obtain the effectivity of Jambi forest honey to inhibit cariogenic bacteria. The inhibition of Jambi forest honey will be compared with antiseptic mouthwash containing 0.2% Chlorhexidine gluconate, antiseptic mouthwash containing 21.6% alcohol, branded honey, and control. In this study, all of the agar media was repeated 6 times in each treatment group. The analysis was carried out using the one-way ANOVA test to see the comparison of inhibition in these five treatment groups. In *Lactobacilus acidophilus*, Jambi forest honey was effective as an antimicrobial compared to control, branded honey, and mouthwash containing alcohol with p-values respectively 0.002; 0.007, and 0.0003. Based on the results of this study, Jambi forest honey was effective to inhibit the growth of cariogenic bacteria *Lactobacilus acidophilus*.

Keywords: Dental caries, cariogenic bacteria, Jambi forest honey

Introduction

Dental caries is one of the preventable oral diseases. Dental caries is responsible to generate tooth pain and tooth loss. Dental caries is a major public health problem that becomes an obstacle in maintaining oral health at all levels of age (Thean et al., 2007). Based on the data of Riskesdas 2018, Indonesia has a high prevalence of dental caries. It reaches 88.8% for all levels of age. This condition makes dental caries one of the health problems that need attention, especially in the susceptible group (Kementerian Kesehatan RI, 2018).

Dental caries is a local destructive on the hard tissues of the teeth. It is caused by the metabolism of carbohydrates that is carried out by cariogenic bacteria. The result of its metabolism is lactid acids, the substrate that harms teeth. Plaque is a soft deposit that coats the tooth surface. The plaque layer is formed due to the attachment between bacteria and the attachment of bacteria to the tooth surface (Yu et al., 2017). One of the cariogenic bacteria that causes caries is *Lactobacillus acidophilus* which is a gram-positive bacterium that produces lactid acid from carbohydrate metabolism (Nurhalisa et al., 2020; Kunarti et al., 2020).

Lactobacillus bacteria are found in large numbers in both superficial and deep caries. Although *Lactobacillus acidophilus* is not a plaque-forming factor, the study shows that

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Lactobacillus bacteria has a dominant number in bacteria culture from dental caries. *Lactobacillus acidophilus* is more commonly found in saliva than plaque (Byur et al., 2004; Badet & Thebaud, 2008).

The use of antimicrobial mouthwash has an important role in maintaining dental and oral hygiene, especially in reducing oral microorganisms. Mouthwash is very useful in reducing the number of microbes in plaque and the oral cavity (Haerian-Ardakani et al., 2015). A study showed that gargling with honey can reduce plaque scores in the oral cavity. In this study, honey was used as a mouthwash with a concentration of 5%, 95% distilled water, and 5% pure honey. This research uses pure Tongra honey. The average plaque score before gargling with honey was 23 and the average plaque score after gargling with honey was 15. This decrease was significant after being tested statistically. The decrease in plaque score is due to the bactericidal and bacteriostatic antimicrobial effects of honey (Alibasyah et al., 2018).

Non-timber forest products (NTFPs) are forest resources that have a comparative advantage and have the most direct contact with forest communities. Several types of NTFPs have high economic value, including rattan, honey, candlenut, agarwood, natural silk, gondorukem, tropical fruits, and others. The potential of NTFPs in a forest area is a business opportunity for the community in managing their forests, in addition to using wood (Tamin et al., 2020). Jambi forest honey is a potential raw material for dental and oral health. One type of honey from the Jambi forest is acacia carpa honey which is produced by *Apis mellifera* bees that take nectar from acacia trees. This study aims to determine the antimicrobial effect of Acacia carpa honey as Jambi forest honey against *Lactobacillus acidophilus* bacteria.

Material and Methods

Ethical approval for this study was obtained from the Health Research Ethics Committee of the Health Polytechnic of Jambi with number LB.02.06/2/09/2022. This research method is in vitro experimental study. This research is conducted to determine the consequences of an intervention given by the researcher to the subject. This study used post-test control only with the disk diffusion technique. In this study, we will examine the effectiveness of acasia carpa honey in inhibiting the growth of *Lactobacillus acidophillus* as a cariogenic bacteria. The research was carried out at the Health Analyst Laboratory of the Jambi Health Polytechnic. The research was conducted from February to October 2022.

The samples of this study were *Lactobacillus acidophilus* bacteria. The study consisted of five groups. The number of repetitions calculated by the Federer formula shows of five repetitions for each group. This study was done with six repetitions. The five groups in this research consist of:

1. The group was treated with branded mouthwash containing 26.1% alcohol
2. The group was treated with branded honey containing 100% pure honey
3. The group that was given acacia carpa honey
4. The group was treated with branded mouthwash containing 0.2% Chlorhexidine Gluconate.

We used 100% acasia carpa honey as a testing material. The group with that material will compare with mouthwash branded contain 26.1% alcohol, mouthwash branded contain of 0.2% Chlorhexidine gluconate, branded honey, and blank paper disk. We were using the colony of *Lactobacillus acidophilus* bacteria isolates from the laboratory of health analyst Jambi health polytechnic. We used the streak plate method for *Lactobacillus acidophilus* culture and the disk diffusion method for the sensitivity test of *Lactobacillus acidophilus*. A sterile one is touched to the bacterial colonies and then spread on the surface medium. Then put disk containing the material to be tested on it, incubated at 37^0C for 24 hours. Each blank disk was dipped in material for ten minutes. Results are read by measuring the zones that inhibit bacteria using Caliper in units mm.

The diameter of the inhibition in medium bacterial of each treatment will be measured. Furthermore, the normality test was carried out on the data, the results of the normality test showed that the data distribution was not normal so the Kruskal Wallis test was carried out to see

the difference in the average diameter of the inhibition zone in the five treatment groups. In addition to the Kruskal Wallis test, the Mann Whitney test was also carried out to compare the average bacterial inhibition zone for each treatment group.

Results and Discussion

Univariate analysis

Univariate analysis was carried out on data of diameter inhibition zone in each agar media. The results of univariate analysis on *Lactobacillus acidophilus* bacteria are shown in Table 1:

Table 1. Average of diameter inhibition zone

Groups	Inhibition Zone (mm)	Std. Deviation	Min-Max
Acasia Honey	13,5	2,81	12 - 19
Branded Honey	10,83	0,75	10 - 12
Positive Control[$]	10,33	0,51	10 - 11
Positive Control [$$]	28,50	1,51	26 - 30
Negative Control	0	0	0-0

[$]Mouthwash branded contains Alkohol 26,1%
[$$] Mouthwash branded contain Chlorhexidine Gluconate 0.2%

Table 1. shows the diameter of the inhibition zone for *Lactobacillus acidophilus* bacteria in each treatment group. Mouthwash containing 0.2% Chlorhexidine Gluconate had the widest diameter of the inhibition zone, followed by acacia carpa honey. The diameter of the inhibition zone for the negative control branded honey treatment, and mouthwash containing 26.1% alcohol treatment was smaller than akasia carpa treatment. The figure of sensitivity media is shown in Figure 1:

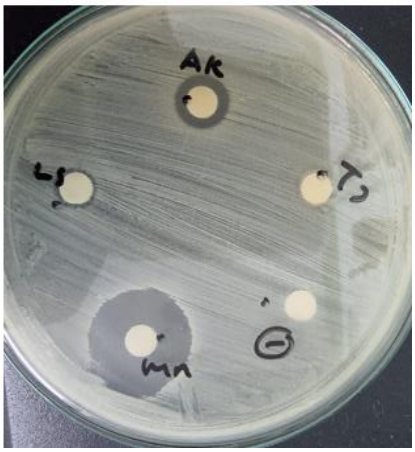

Figure 1. Sensitivity media

Normality test

A normality test was performed before analyzing bivariate data for numerical data. A normality test was carried out on the average inhibition zone of each bacterium. The normality test uses the Shapiro-Wilk test because the number of samples is less than 50. The results of the analysis of the normality test (Shapiro-Wilk) data distribution (variables) can be seen in Table 2.

Table 2. The result of the normality test

Bacteria	P -Value	Resume
Lactobacillus acidophilus	0,001	The data did not distributed normally

Table 2. shows the results of the analysis of the normality test (shapiro-wilk) in the zone of inhibition of each bacterium. The results of the analysis on *Lactobacillus acidophilus* bacteria showed a P-value of 0.001 (<0.05) so the data was declared not normally distributed.

Bivariate analysis

Bivariate analysis was carried out using the Kruskal-Wallis test because the data distribution was not normal. The results of bivariate analysis on the inhibition zone of *Lactobacillus acidophilus* bacteria are shown in Table 3:

Table 3. The result of Bivariate analysis with Kruskal-Wallis test

Groups	Mean	P-Value
Acasia Honey	13,5	
Branded Honey	10,83	
Positif Control[$]	10,33	0,001
Positif Control [$$]	28,50	
Negatif Control	0	

[$]Mouthwash branded contains Alkohol 26,1%
[$$] Mouthwash branded contain Chlorhexidine Gluconate 0.2%

The results of the bivariate analysis on the data of *Lactobacillus acidophilus* bacteria showed a p-value of 0.001 (<0.05) so it was stated that there were significant differences between treatment groups in the inhibition zone of *Lactobacillus acidophilus* bacteria.

Furthermore, bivariate analysis was carried out by testing the acacia carpa honey group against other groups one by one using the Mann-Whitney test. The results of the Mann-Whitney test analysis on the *Lactobacillus acidophilus* test are shown in Table 4:

The results of the bivariate test by testing one-on-one between the acacia carpa honey group and other groups on the *Lactobacillus acidophilus* bacteria showed that:

1. Compared with the control, namely paper disks that did not contain anything, acacia carpa honey was effective in inhibiting the growth of *Lactobacillus acidophilus* bacteria with p value 0.002 (<0.05)
2. Acacia carpa honey is compared with mouthwash containing 26.1% alcohol, then acacia carpa honey is more effective in inhibiting the growth of *Lactobacillus acidophilus* bacteria with p value 0.003 (<0.05).
3. When compared to packaged honey, acacia carpa honey was more effective in inhibiting the growth of *Lactobacillus acidophilus* bacteria with p value 0.007 (<0.05).
4. Acacia carpa honey was compared with mouthwash containing 0.2% Chlorhexidine Gluconate, then the mouthwash is more effective in inhibiting the growth of *Lactobacillus acidophilus* bacteria.

Dental caries is the destruction of dental tissue caused by the biofilm layer as the location for the fermentation of carbohydrates into lactic acid produced by cariogenic bacteria in the oral cavity. Bacteria that have an important role in the occurrence of caries are Streptococcus mutans and *Lactobacillus acidophilus* bacteria. Streptococcus mutans bacteria have a role to initiate early in the formation of caries, then *Lactobacillus acidophilus* bacteria have a role to continue the caries process. *Lactobacillus acidophilus* is a non-motile bacteria and produces lactic acid as the main product of the carbohydrate fermentation process. These bacteria are rarely found before the onset of caries. This bacterium is suspected of being the bacteria that initiates the caries process, especially when it reaches dentinal caries (Nurhalisa et al., 2020).

When compared with control, brand honey, and trademark mouthwash containing 26.1% alcohol, acacia carpa honey was more effective in inhibiting the growth of *Lactobacillus acidophilus* bacteria. However, when compared to branded mouthwashes containing 0.2% chlorhexidine

gluconate, this drug was more effective in inhibiting the growth of *Lactobacillus acidophilus* bacteria than Acacia carpa honey.

These results are following research conducted by Al Dany, where the study showed that there was a decrease in the number of *Lctobacillus acidophilus* bacteria after consuming honey (Atwa et al., 2014).

Honey is antimicrobial because it is composed of several sugar molecules such as glucose and fructose as well as several minerals and vitamins. Below is the general content of pure honey consisting of water (17.0%), fructose (38.5%), glucose (31.5%), maltose (7.2%), carbohydrates (4.2%), sucrose (1.5%), enzymes, minerals, vitamins (0.5%), energy calories/100 grams/294.0%. According to Puspitasari, honey's ability to act as an antibacterial is thought to be due to its high osmolarity, hydrogen peroxide content, low pH, and low water activity. In addition, honey also has antibacterial activity, especially on Gram (+) bacteria, namely S. aureus and B. cereus (Siregar, 2022).

Conclusion

There was a significant difference in the average inhibition zone against *Lactobacillus acidophilus* in the five treatment groups. If the acacia carpa honey group was compared with other groups, then acacia carpa honey was more effective in inhibiting the growth of *Lactobacillus acidophilus* bacteria compared to mouthwash containing 26.1% alcohol, brand honey, and controls. When compared to mouthwash containing 0.2% chlorhexidine, this branded mouthwash was more effective in inhibiting the growth of *Lactobacillus acidophilus* bacteria than acacia carpa honey.

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