THE EFFECT OF RAMBUTAN HONEY TOOTHPASTE ON THE DIAMETER OF THE INHIBITON ZONE FOR THE GROWTH OF *Staphylococcus aureus* (*PENGARUH PASTA GIGI MADU RAMBUTAN TERHADAP DIAMETER ZONA HAMBAT PERTUMBUHAN Staphylococcus aureus*)

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ABSTRACT

Dental plaque is a collection of bacteria embedded in an organic polymer matrix on the tooth surface, which is the main cause of most dental and oral diseases such as caries and periodontal disease. The most important thing to control the accumulation of dental plaque is to brush your teeth with the right technique using toothpaste. The movement when brushing teeth is effective in eliminating plaque, while the antibacterial composition of toothpaste is effective against plaque-forming bacteria. The potential of rambutan honey as an antibacterial agent in toothpaste has been known because honey has high osmolarity, low pH, contains hydrogen peroxide, phenolic acids, and flavonoids. The purpose of this study is to determine the effect of rambutan honey toothpaste in inhibiting the growth of *Staphylococcus aureus*, a bacteria that can form a biofilm and is thought to act as an pathogen of periodontal disease. This research method was an in vitro laboratory experiment with Kirby Bauer diffusion method, to test the inhibitory power of 60% rambutan honey toothpaste, toothpaste base, chloramphenicol as a positive control, and sterile aquadest as a negative control. The zone of inhibition of the growth of *Staphylococcus aureus* formed on Mueller Hinton Agar media was measured using a caliper. Results of this research showed that rambutan honey toothpaste had an inhibitory effect, not different from chloramphenicol ($p=0.081$), and different from aquadest ($p=0.001$), due to the antibacterial activity of honey from high osmolarity, low pH, contains hydrogen peroxide, phenolic acid, and flavonoids. Rambutan honey toothpaste was not different from the toothpaste base ($p=0.096$), related to its low solubility so that the spread of antibacterial was slow. It was concluded that rambutan honey toothpaste could inhibit the growth of *Staphylococcus aureus*, there was no difference with chloramphenicol and there was a difference with aquadest, but there was no difference with toothpaste base.

Keywords: antibacterial agent; honey; *Staphylococcus aureus*; toothpaste.

ABSTRAK

Plak dental adalah kumpulan bakteri tertanam dalam matriks polimer organik pada permukaan gigi, menjadi penyebab utama sebagian besar penyakit gigi dan mulut seperti karies dan penyakit periodontal. Hal paling utama untuk mengontrol akumulasi plak dental adalah dengan menyikat gigi dengan teknik tepat menggunakan pasta gigi. Gerakan saat menyikat gigi efektif dalam mengeliminasi plak, sedangkan kandungan antibakteri pasta gigi efektif terhadap bakteri pembentuk plak. Potensi madu rambutan sebagai agen antibakteri dalam pasta gigi telah diketahui karena madu memiliki osmolaritas tinggi, pH rendah, serta kandungan hidrogen peroksida, asam fenolat, dan flavonoid. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh pasta gigi madu rambutan dalam menghambat pertumbuhan Staphylococcus aureus, yaitu bakteri yang dapat membentuk biofilm dan diduga berperan sebagai patogen dari penyakit periodontal. Metode penelitian ini merupakan eksperimen laboratorium in vitro metode difusi Kirby Bauer, untuk menguji daya hambat pasta gigi madu rambutan 60%, pasta gigi basis, kloramfenikol, serta akuades steril. Zona hambat pertumbuhan Staphylococcus aureus terbentuk pada media Mueller Hinton Agar diukur menggunakan jangka sorong. Hasil penelitian menunjukkan pasta gigi madu rambutan memiliki daya hambat, tidak berbeda dengan kloramfenikol (p=0,081), dan berbeda dengan akuades (p=0,001), karena adanya aktivitas antibakteri madu dari osmolaritas tinggi, pH rendah, kandungan hidrogen peroksida, asam fenolat, dan flavonoid. Pasta gigi madu rambutan tidak berbeda dengan pasta gigi basis (p=0,096), berhubungan dengan kelarutannya yang rendah sehingga penyebaran antibakteri menjadi lambat. Disimpulkan bahwa pasta gigi madu rambutan dapat menghambat pertumbuhan Staphylococcus aureus, tidak ada perbedaan dengan kloramfenikol dan ada perbedaan dengan akuades, tetapi tidak ada perbedaan dengan pasta gigi basis.

Kata kunci: *agen antibakteri; madu; pasta gigi; Staphylococcus aureus.*

INTRODUCTION

Based on Health Research (Riskesdas) in 2018, 57,6% of Indonesian people have dental and oral health problems, with periodontal disease as the second biggest problem after caries.[1] *Staphylococcus aureus* is thought to play a role as a pathogen of periodontal disease or can even worsen the situation.[2,3] It is a gram-positive facultative anaerobic bacteria, about 0.5-1.5 μm in diameter, cocci shaped in pairs to form grape-like colonies.[4,5] These bacteria are commonly found on the skin and mucous membranes of the anterior nasopharynx of humans, but are often found on the tongue, saliva, mucosal surfaces, and supragingival surfaces of teeth.[2,4] *Staphylococcus aureus* can be pathogenic and cause infection in the oral cavity if oral hygiene is not maintained well or if there are lesions on the mucosal surface.[6,7] These bacteria play a role in periodontal disease because they can form biofilms on dental plaque.[2,3]

Dental plaque is a collection of bacteria embedded in an organic polymer matrix on surface of the tooth.[8,9] To control the accumulation of dental plaque, mechanical cleaning is carried out, namely by brushing teeth using toothpaste.[8–10]

Toothpaste that we used must meet the quality requirements of toothpaste products in accordance with SNI 8861:2020. One of the requirement is that toothpaste has to have antibacterial effect against *Staphylococcus aureus*.[11] The type of toothpaste also needs to be

considered in order to reduce specific bacteria in the mouth, also to prevent caries and periodontal disease.[9,12]

Types of herbal toothpaste continue to be developed. Several studies have shown that herbal toothpastes are more effective than non-herbal toothpastes in reducing plaque index.[8,9] One of the herbal ingredients that have an antibacterial effect and can be used as a toothpaste composition is honey.[13]

Honey is known for its good antibacterial effect since ancient times. The antibacterial activity of honey is influenced by the low level of acidity and water activity, high viscosity and glucose levels, and the presence of H_2O_2 (hydrogen peroxide). Several compounds such as phytochemicals, proteins, glycopeptides, and polyphenols also play a role in the antibacterial effect of honey.[14] Previous research proved that honey has inhibition power against *Staphylococcus aureus* bacteria.[15] One type of honey that has antibacterial effect is rambutan honey.[13]

In one study, rambutan honey had inhibition power against *Staphylococcus aureus* bacteria, as well as against *Streptococcus mutans* bacteria in other studies.[13,16] In addition, rambutan honey can be made into toothpaste formulations with concentrations of 20%, 40%, and 60%, and the patent has been registered with number S00202103399. The composition is the same as toothpaste in general, with the addition of rambutan honey, and does not use fluoride compounds that are generally found in ordinary toothpaste.[17] Toothpaste that does not use fluoride compounds is considered safer because it reduces the potential for excessive entry of these compounds into the body which can later cause side effects, namely fluorosis on the teeth.[18] That rambutan honey toothpaste was tested and have an antibacterial effect against *Streptococcus mutans* bacteria in vitro.[17] The antibacterial effect of honey is obtained from the presence of hydrogen peroxide (H_2O_2), phenolic acids, and flavonoids, high osmolarity, and the low pH of honey.[19]

Until now, there are no studies that showed rambutan honey as one of the compositions of toothpaste can inhibit the growth of *Staphylococcus aureus* bacteria. The authors are interested in conducting research by testing rambutan honey toothpaste with concentration 60% to see the diameter of the inhibition zone for the growth of *Staphylococcus aureus* bacteria, in order to develop an alternative toothpaste product that has an antibacterial effect to treat dental and oral problems such as periodontal disease.

METHODS

This research was purely experimental laboratory research conducted in vitro with a post-test only control group design. It was executed from September to November 2021, after being declared free from ethical review by the Health Research Ethics Committee (KEPK) Faculty of Medicine, General Achmad Yani University, with the number 008/UH3.10/2021. The sample of this research is 32 samples, consisting of 8 repetitions from 4 treatment groups. It is calculated by the Federer's formula and by considering the minimum number of laboratory research samples.[20,21]

Rambutan honey was standardized in Biochemistry Laboratory of Faculty of Medicine, General Achmad Yani University, then used to make rambutan honey toothpaste by the General Achmad Yani University Pharmacy team. To begin the research, *Staphylococcus aureus* ATCC 29213 obtained from Microbiology Laboratory of the Faculty of Medicine, General Achmad Yani University is identified macro and microscopically, then rejuvenated on Tryptic Soy Agar (TSA) medium and incubated for 18-24 hours at 37^0C.[22]

In order to make a bacteria suspension, the bacteria that have been incubated are then taken using a sterile loop and put into a test tube containing 5 ml of physiological NaCl, and has to meet the 0,5 McFarland standard equivalent to a bacterial density of 10^8 CFU/ml.[23]

Meanwhile, 3 test solutions were made, by dissolving 1 g of rambutan honey toothpaste in 10 ml of sterile aquadest as the first test solution, and then dissolving 1 g of toothpaste base in 10 ml of sterile aquadest as the second test solution, and placing the sterile aquadest in another glass dish as the last test solution.

The research conducted using the diffusion method by Kirby Bauer technique, which has simpler process compared to the diffusion well technique.[24] In Mueller Hinton Agar (MHA) medium, the suspension of *Staphylococcus aureus* bacteria is carried out by streaking evenly on the surface of the medium using a sterile swab. Before putting the paper disks, each petri dish is divided into 4 parts and named by each treatment group, that is chloramphenicol (C), aquadest (A), toothpaste base (T), and rambutan honey toothpaste 60% (RH T 60%). Paper disks that were immersed in rambutan honey toothpaste 60% test solution, toothpaste base test solution, aquadest, and also paper disks that already contained chloramphenicol is placed on the medium's surface using tweezers with light pressure so that it can stick, then incubated at 37^0C for 24 hours in incubator (Figure 1).[22] After 24 hours, the clear zone formed around the paper disk is measured using calliper in millimetres (mm) to determine the diameter of the inhibition zone for the growth of *Staphylococcus aureus*.

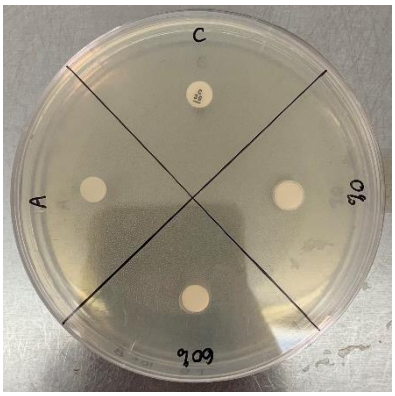

Figure 1. Kirby Bauer diffusion technique of 4 treatment group, which is (A) sterile aquadest, (C) chloramphenicol, (0%) toothpaste base, and (60%) rambutan honey toothpaste.

Statistical analysis of this research was carried out with SPSS software, by conducting normality and homogeneity test first, namely Shapiro-Wilk test and Levene test. Furthermore, the Kruskal-Wallis test was carried out and further analysis was the post hoc Pairwise Comparison test because the data was normally distributed ($p>0,05$), but not homogeneous ($p<0,05$).

RESULT

Using a calliper, the diameter of the clear zone formed around the paper disk from every treatment group of each petri dish is measured. The inhibition zone calculation results can be seen in Table 1.

Table 1. Inhibition zone from every treatment group against *Staphylococcus aureus* bacteria

Test Results	Inhibition Zone Diameter (mm)			
	C	A	T	60% RH T
1	26,20	0,00	10,36	11,63
2	25,79	0,00	10,30	10,96
3	27,28	0,00	10,99	11,18
4	25,19	0,00	10,87	11,09
5	27,42	0,00	10,03	11,35
6	26,58	0,00	10,46	11,70
7	27,01	0,00	10,50	11,62
8	25,87	0,00	9,92	11,33

From Table 1, it can be seen that in all treatment groups, a clear inhibition zone was formed, except the aquadest group, which meant that chloramphenicol, toothpaste base, and 60% rambutan honey toothpaste group had antibacterial effect against the growth of *Staphylococcus aureus* bacteria.

Kruskal-Wallis statistical analysis was carried out from the mean result of the inhibition zone of each treatment group, and the inhibition power classification can also be determined (Table 2).

Table 2. Mean of the inhibition zone

Treatment Group	Mean ± SD of Inhibition Zone (mm)	Inhibition Power Classification	p-Value
Chloramphenicol	26,42 ± 0,79	Strong	
Aquadest	0,00 ± 0,00	No inhibition power	
Toothpaste Base	10,43 ± 0,37	Weak	0,000
60% Rambutan Honey Tootpaste	11,36 ± 0,27	Weak	

Based on the inhibition power classification data in Table 2, 60% rambutan honey toothpaste and the toothpaste base has weak inhibition power, while chloramphenicol has strong inhibition power. Meanwhile, the Kruskal-Wallis statistical analysis showed that there were differences in the treatment group (p-value=0,000). To find out which treatment groups were different, a post hoc Pairwise Comparison test was carried out (Table 3).

Table 3. Pairwise comparison test

Group	p-Value	Interpretation
Chloramphenicol—aquadest	0,000	There is significant difference
Chloramphenicol—T	0,001	There is significant difference
Chloramphenicol—60% RH T	0,081	There is no significant difference
Aquadest—T	0,081	There is no significant difference
Aquadest—60% RH T	0,001	There is significant difference
T—60% RH T	0,096	There is no significant difference

Table 3 shows that rambutan honey toothpaste was not significantly different from chloramphenicol and significantly different from aquadest, but not significantly different with toothpaste base.

DISCUSSION

The inhibition zones formed in each treatment group had various diameters. Varied diameters can occur due to several things, one of which is incubation temperature that is less that optimal in each petri dish, where incubation is at least carried out at a temperature of 35^0C. In this study, incubation was carried out at a temperature of 37^0C with the placement of 4 petri dish in stacks, which could cause the temperature of petri dish in the middle of the stacks to decrease, even less than 35^0C, so that the diameter of the inhibition zone became larger. The thickness of the agar medium at the time of the inhibition test also affected, with an effective thickness of about 4 mm, if it was not thick enough, the antibacterial compound would spread quickly and vice versa. However, in this study, the thickness of the MHA medium used for the inhibition test was not calculated, so it is not known whether this affects it or not.[25–28]

From this study it was found that there are large inhibition zone around the positive control group paper discs. The positive control used in this study was chloramphenicol because it was proven to be active inhibiting the growth of *Staphylococcus aureus* bacteria compared to other antibiotics. Chloramphenicol itself has a strong inhibition power because it is a bacteriostatic antibiotic with mechanism of work is to destroy the synthesis process of bacterial proteins by inhibiting peptidyl transferase, and has a broad spectrum so that it can inhibit gram negative and positive bacteria, both aerobic and anaerobic bacteria.[29,30] It was supported by previous studies that indicate chloramphenicol has a strong inhibition towards *Staphylococcus aureus* bacteria because the diameter of the inhibition zone that formed was 29 mm.[31]

The results also showed that the 60% rambutan honey toothpaste group had an effect on the diameter of the inhibition zone for the growth of *Staphylococcus aureus* bacteria, there was no significant difference with the positive control group (chloramphenicol), and there was a significant difference with the negative control group (sterile aquadest). This is because rambutan honey has antibacterial activity. The antibacterial activity comes from the formation of hydrogen peroxide (H_2O_2) which can damage the bacterial cells, more precisely

the functional groups of biomolecules.[14,32] High osmolarity can also inhibit the proliferation of bacteria or provide a direct bactericidal effect by leaving less water for bacterial growth, so that bacteria become dehydrated and die.[19] In addition, honey also has a low pH which provides acidic conditions to inhibit bacterial growth. The phenolic acids and flavonoids in honey also act as non-peroxide compounds with antibacterial and antioxidant effects that can penetrate into bacterial cells to interfere with their enzyme metabolic activity, and if the levels are high they will agglomerate proteins so that the cells become lysed.[14,19,33] Results of this study is supported by previous studies, which showed that honey had an antibacterial effect on the growth of *Staphylococcus aureus* bacteria, and in other studies, toothpaste with the composition of rambutan honey had an antibacterial effect.[15,17]

In the study towards *Streptococcus mutans* bacteria, 60% rambutan honey toothpaste formed greater zone of inhibition when compared to this study which tested towards *Staphylococcus aureus* bacteria. The previous study had an average diameter of 13.77 mm, while in this study it was 11.36 mm.[17] Bacterial differences between *Streptococcus mutans* and *Staphylococcus aureus* can be the cause. Although both bacteria belong to gram-positive bacteria, different types of bacteria produce different responses to antibacterial agents.[4,17,34] Under certain conditions, a fibrin layer can form on the surface of *Staphylococcus aureus* bacteria which can reduce the efficacy of antibacterial compounds.[34] In addition, *Staphylococcus aureus* can neutralize the bactericidal effect of H_2O_2, which is one of the content that plays a role in antibacterial activity of honey, by producing the enzyme catalase to break down H_2O_2 into water (H_2O) and oxygen (O_2).[14,32,35,36] So that the catalase enzyme from *Staphylococcus aureus* can reduce the antibacterial efficacy of honey, which is in accordance with other studies, which proves a decrease in the antibacterial activity of honey after the addition of catalase enzyme. This can be seen from the minimum mean inhibitory concentration (MIC) which was greater in the honey sample with the addition of catalase than without the addition of catalase.[37,38] *Streptococcus mutans* does not have the ability to form a fibrin layer or to produce catalase enzyme.[34,36]

From the average diameter of the inhibition zone, it can be concluded that the 60% rambutan honey toothpaste group has a weak inhibition power classification. This classification refers to Greenwood (1995), divided into 4 classification, that is strong (>20 mm), moderate (16-20 mm), weak (10-15 mm), and no inhibition power (<10 mm).[39] Rambutan honey toothpaste has a weak inhibition power because it was affected by the type of honey that was used. In another study, it was found that rambutan honey formed a 10,9 mm diameter of the inhibition zone towards the growth of *Staphylococcus aureus* bacteria, and in another study it formed a 7,95 mm diameter of the inhibition zone towards *Streptococcus mutans* bacteria, indicating that rambutan honey itself has a weak inhibition power classification.[13,16] In addition, the antibacterial effect of honey can also be less than optimal because honey is contaminated with other microorganisms such as yeast, molds, and bacterial spores that can come from pollen, bee digestive tract, air or wind, water, insects or other animals, humans, or processing equipment, and may have existed since the time it was still in the nectar, the ripening process, or storage.[40,41] In a previous study, 60% rambutan honey toothpaste was also an antibacterial agent with a weak inhibition power classification.[17]

Honey itself is a natural ingredient or herbal ingredients, so honey toothpaste is included in herbal toothpaste. Several studies have shown that herbal toothpastes are more effective than non-herbal toothpastes in reducing plaque index.[8,9,13] This is certainly an advantage when compared to toothpaste base, because with a decrease in plaque index, the risk of periodontal disease and caries can also be reduced.[9] Supported by this study and the previous studies, an inhibition zone was formed against the growth of plaque-forming bacteria, that is *Staphylococcus aureus* and *Streptococcus mutans* bacteria.[2,17,42]

In addition to the 60% rambutan honey toothpaste group, the toothpaste base group also had an antibacterial effect against *Staphylococcus aureus*. Rambutan honey toothpaste and toothpaste base should indeed be able to inhibit the growth of *Staphylococcus aureus* bacteria, in accordance with the quality requirements of toothpaste products SNI 8861:2020.[11] The inhibition zone formed is due to the composition of $CaCO_3$, which has nanoparticles that can enter through the bacterial cell membrane and interact on the inside of the cell to stop the bacteria from maintaining their life.[43] Sodium lauryl sulfate (SLS) that is used, can also act as an antibiofilm agent, which has been shown to be effective against *Staphylococcus aureus* biofilms, and has antimicrobial activity, with mechanism of action is by denaturing proteins and lysing the microorganism cells.[44,45] Sodium benzoate are also commonly used to inhibit or stop the growth of pathogenic microorganisms, due to the accumulation of protons and anions in the cells, which then interfere with the normal metabolism of these microorganisms.[46] Antibacterial activity of sodium benzoate against *Staphylococcus aureus* bacteria has been proven in previous studies.[47] Another composition that can act as an antimicrobial is menthol, because of its lipophilic characteristics that allow it to migrate and then interact with the phospholipid membrane, resulting in structural damage, even causing leakage of intracellular material, and the microbial agent becomes unstable.[48]

The average diameter of the inhibition zone around the 60% rambutan honey toothpaste paper disks was greater because there was no rambutan honey content as an additional antibacterial in the toothpaste baset. This is in accordance with previous studies, where the higher the antibacterial concentration, the larger the inhibition zone that formed. However, according to the results of statistical tests, there was no significant difference between this two treatment groups. This is related to the solubility of the paste, where the concentration of 60% has a lower solubility than the toothpaste base, causing a slowdown in the spread of the active ingredient of the antibacterial compound into the agar medium.[25,28]

Rambutan honey herbal toothpaste has good prospects in the future, especially in the field of dentistry. In addition to their effectiveness, herbal ingredients are also used more often because of the back to nature lifestyle trend that is being implemented by the community and developed by industries both abroad and domestically.[49] Another advantage is that it is easy to obtain herbal ingredients on the market, and is relatively inexpensive and has minimal side effects.[50] In an effort to increase the prospects of the rambutan honey toothpaste used in this study in dentistry, researchers did not use fluoride compounds as one of its compositions to minimize side effects that can occur, because fluoride compounds themselves have the potential to enter the body in excess and can cause fluorosis of the teeth.[18,51]

Herbal toothpastes that use sodium lauryl sulfate (SLS) can cause side effects, because they can cause irritation to the oral mucosa.[51,52] The content of herbal ingredients can also cause negative effects, such as the content of hydrogen peroxide in honey, which has been shown to reduce the surface hardness of tooth enamel. But there are no studies that shows rambutan honey toothpaste has the same effect.[53]

The limitation of this study is that it is not known what specific chemical substances and their amounts or levels can actively play a role in the antibacterial effect of rambutan honey so that it is better than other honeys. In addition, limited information regarding the interaction between rambutan honey and the composition of the toothpaste base that has the possibility to reduce the inhibition of the 60% rambutan honey toothpaste group or cause negative effects, is also a limitation of this study.

CONCLUSION

The conclusion that can be drawn from this research is that rambutan honey toothpaste has an effect on inhibiting the growth of *Staphylococcus aureus* bacteria in vitro. Also, there was no significant difference between the 60% rambutan honey toothpaste and the positive control (chloramphenicol) and there was a significant difference with the negative control (sterile aquadest), but there was no significant difference with the toothpaste base.

CONFLICT OF INTEREST

We declare that there is no conflict of interest in the scientific articles we write.

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