Medical Sains : Jurnal Ilmiah Kefarmasian
Vol. 9 No. 2, April - June 2024
https://ojs.stfmuhammadiyahcirebon.ac.id/index.php/iojs

FORMULATION AND ANTIBACTERIAL ACTIVITY TEST OF RAMBUTAN HONEY TOOTHPASTE AGAINST *Streptococcus mutans*

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Submitted: February 15, 2024 Revised: May 10, 2024 Accepted: May 23, 2024

ABSTRACT

Dental caries and periodontal diseases are the most common diseases affecting the oral cavity. This can be prevented by maintaining oral hygiene. Toothpaste plays an important role in the reduction of plaque bacteria and inflammatory conditions. Rambutan honey has potential as a natural ingredient to make toothpaste with antibacterial and antioxidant properties owing to the presence of hydrogen peroxide and catalase enzymes, pH, osmolarity, and flavonoid content. The purpose of this study was to obtain a rambutan honey toothpaste formulation that effectively inhibits *Streptococcus mutans* growth. The laboratory experimental research method was used to combine $CaCO_3$, glycerin, Na CMC, SLS, sodium benzoate, saccharin, menthol, and aquadest as the toothpaste base, which served as the control group. Subsequently, natural ingredients of rambutan honey at concentrations of 20%, 40%, and 60% were added to the toothpaste base to create rambutan honey toothpaste. The formulation of the rambutan honey toothpaste was followed by an antibacterial activity test using the Kirby Bauer method of disc diffusion, where the inhibition zones formed on Mueller Hinton Agar (MHA) medium were calculated. Data were analyzed using one-way and post hoc Tukey's tests ($p<0.05$). The results showed that the formulation of rambutan honey toothpaste with a concentration of 60% had the greatest inhibition with 13.88±0.13 mm with a significant difference in each group ($p = 0.000$), so it can be concluded that rambutan honey toothpaste can inhibit the formation of *Streptococcus mutans*.

Keywords: antibacterial, formulation, rambutan honey, toothpaste, *Streptococcus mutans*

INTRODUCTION

Dental caries and periodontal diseases are the most common diseases affecting the oral cavity (Myers & Curran, 2014). Based on the results of the 2018 Basic Health Research (Riskesdas), the proportion of dental health problems that occur in Indonesia includes dental caries 45.3%, tooth loss 19%, loose teeth 10.4% and teeth filled due to caries 4.1%, while oral health problems that occur in Indonesia include swollen gums (gingivitis) with abscesses 14%, bleeding gums due to trauma when brushing teeth 13.9%, recurrent aphthous stomatitis 8% and canker sores that persist and do not heal for at least 1 month 0.9% (Riskesdas, 2018). This can be prevented by maintaining oral hygiene. Simple and effective oral hygiene can be maintained by brushing the teeth. The type of toothpaste used is one of the factors that plays a role (Yuwono et al., 2012).

Toothpaste plays an important role in the reduction of plaque bacteria, inflammatory conditions, and gum bleeding. Toothpaste contains various main components, including abrasive materials (aluminum hydroxide and calcium carbonate ($CaCO_3$)), fluoride (sodium fluoride), detergents (surfactants), and antimicrobial agents (chlorhexidine) (Lisa et al., 2017).

Honey is a natural compound with antibacterial, anti-inflammatory, and antioxidant properties. Many studies have reported the antibacterial activity of honey due to the osmotic effect of 30% glucose content, hydrogen peroxide content, and enzymes, one of which is the catalase enzyme, which can convert hydrogen peroxidase to cause an antibacterial effect (Prince, 2019). Other factors such as acidic pH, flavonoid content, and lysozyme enzyme content in honey have also been reported to play a role in inhibiting bacterial growth. Research has shown that the antibacterial activity of honey against *Streptococcus mutans* can be observed at a concentration of 6.25% (Yuliati, 2017).

Rambutan honey itself has been through preclinical tests on experimental animals, has been proven to be safe and efficacious as an antioxidant. In previous studies, it was found that topical administration of rambutan honey to the oral mucosa of Wistar rats could prevent the formation of free radicals, prevent destructive damage (MDA), and rebuild wound healing by inducing fibroplasia by TGF-β1 (Yuslianti et al., 2015). The potential of rambutan honey as a natural ingredient for making toothpaste with antibacterial and antioxidant content is known, but there has been no research on the formulation and testing of the antibacterial activity of the toothpaste. Based on this, researchers are interested in conducting research on the formulation and antibacterial activity of rambutan honey toothpaste with concentrations of 20%, 40%, and 60% against *Streptococcus mutans* bacteria; therefore, it can be an alternative toothpaste product with antibacterial and antioxidant properties to treat dental and oral diseases without side effects.

RESEARCH METHODS

Equipment and Materials

The tools used consisted of a 50 mL beaker, 100 mL measuring cup, capture glass, parchment paper, stirring rod, porcelain cup, mortar and stamper, digital analytical balance, 1 mL dropper pipette, pH-meter, horn spoon, viscometer, container toothpaste, autoclave, test tube, loops, sterile swab, vortex, sterile petri dish, micropipette, incubator, plastic wrap, diffusion disc (paper disc), caliper.

The ingredients used consisted of rambutan honey, calcium carbonate (CaCO3), glycerin, sodium carboxymethylcellulose (Na CMC), sodium lauryl sulfate (SLS), sodium benzoate, saccharin, menthol and distilled water, *Streptococcus mutans* pure culture, TSA medium (Tryptic Soy Agar), MHA medium (Mueller Hinton Agar), 0.9% physiological NaCl, 70% alcohol, 1% $BaCl_2$ (barium chloride dihydrate), 1% H_2SO_4 (sulfuric acid), sterile distilled water, crystal violet dye, povidone iodine, salt lugol's modified iodine, safranin, paper disc containing chloramphenicol, rambutan honey toothpaste formula, and *Streptococcus mutans* ATCC 35668.

Research Procedure

1. Rambutan Honey Toothpaste Formulation

   The ingredients were weighed according to the formula. Na CMC was dissolved in hot water at a temperature of 80°C to a maximum of 20 times the amount of Na CMC weighed and then allowed to stand for 15 minutes, after which it was stirred to form a homogeneous dough (mass 1). Meanwhile, in a different container, calcium carbonate ($CaCO_3$) was ground and sodium lauryl sulfate (SLS) was added, ground until homogeneous and mixed to mass 1, and ground until homogeneous (mass 2). Rambutan honey was dissolved with glycerin, stirred until homogeneous, and mixed into mass 2 while grinding until homogeneous. Sodium benzoate and saccharin were dissolved in the remaining water and stirred until they were completely dissolved. It was then mixed into mass 2 and ground until it became homogeneous and formed a paste-shaped mass. Next, menthol was added to the pasta mass and ground until it was homogeneous. The paste was then placed in a toothpaste container or tube.

Table I. Rambutan Honey Toothpaste Formulation

Material	Formulas			
	F0	F1	F2	F3
Rambutan Honey	-	20%	40%	60%
$CaCO_3$	50g	40 g	30 g	20 g
Glycerin	15 g	12 g	9 g	6 g
Na CMC	9 g	7.2 g	5.4 g	3.6 g
SLS	5 g	4 g	3 g	2 g
Sodium benzoate	0.20 g	0.16g	0.12g	0.08g
Saccharin	0.40 g	0.32g	0.24g	0.16g
Menthol	0.40 g	0.32g	0.24g	0.16g
Aquades	ad 100 g	ad 100 g	ad 100 g	ad 100 g

2. Testing of Antibacterial Activity of *Streptococcus mutans* ATCC 35668

   Testing the antibacterial activity of rambutan honey toothpaste against *Streptococcus mutans* was carried out by Kirby-Bauer using the disc diffusion method as follows: First, prepare a petri dish containing 20 mL of MHA nutrient medium (Mueller Hinton Agar) and pour it aseptically into a sterile petri dish, then let it stand until solidified. Next, the bacterial suspension was taken using a swab and the swab was inoculated evenly onto the MHA media by means of a spread plate approaching the Bunsen flame; one paper disc was soaked in sterile distilled water as a negative control, one paper disc containing the antibiotic chloramphenicol as a positive control, and 3 paper discs soaked in a test solution of rambutan honey toothpaste with a consistency of 20%, 40%, and 60% above the surface of MHA media. Each paper disc was regularly inoculated at a certain distance to prevent overlapping of the inhibition zones formed, and the plates were incubated at 37°C for 18–24 hours. The MHA medium that had been incubated for 18–24 hours was removed from the incubator, and the results of the clear zone that formed around the paper disc were observed. The results were counted using a vernier measuring instrument with an accuracy of 0.05 mm.

Data Analysis

The number of sample groups in this study consisted of 6 treatment groups, namely 1 group of toothpaste base preparations, 3 groups of rambutan honey toothpaste with concentrations of 20%, 40%, and 60%, chloramphenicol as a positive control and sterile distilled water as a negative control. The results obtained in the study were processed using univariate analysis, which was tested using one-way ANOVA parametric statistical analysis followed by post hoc analysis of the Tukey test if the results of the data normality test using the Shapiro Wilk test were normally distributed.

RESULTS AND DISCUSSION

Rambutan honey can be formulated into toothpaste with varying CaCO3, glycerin, Na CMC, and SLS contents to concentrations of 20%, 40%, and 60%. The results of can be seen in Table I.

The mean, median, standard deviation, standard error, upper-lower limit, and P value of the pH of the rambutan honey toothpaste for all groups are shown in Table II.

Table II. Value of Mean, Median, Standard Deviation, Standard Error, Upper-Lower Limit, and p-Value on The pH of Rambutan Honey Toothpaste for All Groups

Group	Inhibition Zone Diameter (mm)						
	N	Average	Median±SD	SE	95% CI Upper Limit	95% CI Lower Limit	p-Value
Rambutan honey 0%	3	8.65	8.74 ± 0.28	0.16	9.36	7,94	0.000*
Rambutan honey 20%	3	7,72	7.76 ± 0.63	0.03	7.88	7.56	
Rambutan honey 40%	3	7,19	7.20 ± 0.05	0.03	7,33	7.05	
Rambutan honey 60%	3	6.98	6.98 ± 0.05	0.03	6,84	7,12	

*p <0.05 there is a significant difference with the one-way ANOVA test

The output results in Table II show a p-value = 0.000. There was a significant difference between the concentrations of 20%, 40%, and 60% rambutan honey in toothpaste in terms of the pH value obtained. These results are assumed to be due to differences in the formulation of rambutan honey toothpaste preparations, storage methods, and storage time. The concentration of rambutan honey affects the viscosity of the toothpaste preparation, as shown in Table III.

Table III. Value of Mean, Median, Standard Deviation, Standard Error, Upper-Lower Limit, and p-Value on Viscosity of Rambutan Honey Toothpaste for All Groups

Group	Inhibition Zone Diameter (mm)						
	N	Average	Median ± SD	SE	95% CI Upper Limit	95% CI Lower Limit	p-Value
Rambutan honey 0%	3	389966,67	392600 ± 45107.68	26042.93	502020,37	277912.96	0.000*
Rambutan honey 20%	3	211700	181900 ± 76862.14	44376,38	20763,83	402636,16	
Rambutan honey 40%	3	184733,33	181800 ± 8013.32	4626,49	204639.52	164827,13	
Rambutan honey 60%	3	151066,67	150500 ± 8663.90	5002,11	172589.01	129544,32	

*p <0.05 there is a significant difference with the one-way ANOVA test

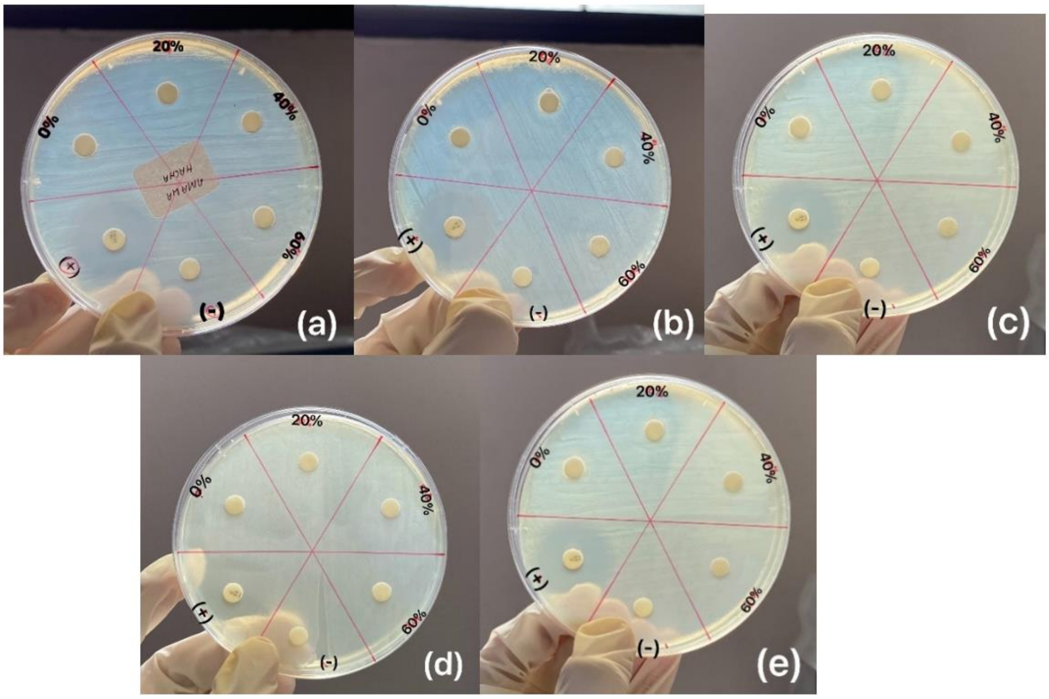
The output results in Table III have a p-value = 0.000. There was a significant difference in the viscosity value obtained between the concentrations of 20%, 40%, and 60% rambutan honey in toothpaste. These results are assumed to be due to differences in the formulation of rambutan honey toothpaste preparations, storage methods, and storage time. The results of determining the inhibition zone on rambutan honey toothpaste at concentrations of 20%, 40%, and 60% on the growth of *Streptococcus mutans* using the Kirby-Bauer method with the disc diffusion method are shown in Table IV.

Table IV. The Mean, Standard Deviation, and Min-Max Diameters of The Inhibition Zones for All Groups

Group	Inhibition Zone Diameter (mm)						
	N	Average	Median±SD	SE	95% Cl Upper Limit	95% Cl Lower Limit	p-Value
Rambutan Honey 0%	5	12,21	12.25±0.31	0.13	12.59	11.82	0.000*
Rambutan Honey 20%	5	12.87	12.80±0.43	0.19	13,41	12,32	
Rambutan Honey 40%	5	13,14	13.05±0.35	0.15	13.57	12.70	
Rambutan Honey 60%	5	13.77	13.80±0.13	0.06	13.93	13.60	
Control of chloramphenicol	5	20.99	20.75±0.78	0.34	21.95	20.02	
Negative control	5	0.00	0.00±0.00	0.00	0.00	0.00	

*p <0.05 there is a significant difference with the one way ANOVA test

In Table IV it can be seen that the results of the inhibition zones that formed on all paper discs except for paper discs which were soaked in sterile distilled water did not show the formation of an inhibition zone around the bacterial culture. This shows that the toothpaste base and toothpaste containing rambutan honey at concentrations of 20%, 40%, and 60% had an antibacterial effect on *Streptococcus mutans*, with a significant difference between groups (p=0.000). Variation in inhibition zones of rambutan honey at concentrations of 20%, 40%, and 60% compared with the positive control on paper discs is shown in Figure 1.



Description: Disk diffusion test for (−) negative control, (+) positive control, and rambutan honey concentrations of 0%, 20%, 40%, and 60% in the first plate (a), second (b), third (c), fourth (d), and fifth (e).

Figure 1. Variation of inhibition zones rambutan honey at a concentration of 20%, 40% and 60% on paper discs

The results obtained in Table I show that F2 (rambutan honey toothpaste with a concentration of 40%) and F3 (rambutan honey toothpaste with a concentration of 60%) underwent a change in shape with the presence of precipitate from rambutan honey, which could occur because of an adsorption process. The rambutan honey bonded homogeneously to the toothpaste preparation, eventually forming a thin layer or film on its surface. This reaction arises because of the physical properties of the toothpaste semisolid preparations that have pressure. In addition, the storage temperature also affects adsorption; the higher the temperature, the lower the rate of adsorption of rambutan honey (Evahelda et al., 2017; Weston, 2019; Mariyam, 2014).

The results obtained in Figure 1 show that the inhibition zone diameter of rambutan honey toothpaste at concentrations of 20%, 40%, and 60% showed an increase in the diameter of the inhibition zone with increasing concentrations of rambutan honey active ingredients in each repetition. It can be concluded that the higher the concentration of rambutan honey, the more active substances with antibacterial properties are present in the toothpaste. As observed for the positive control, the inhibition zone was not clear. It is possible that this activity is only bacteriostatic. Variations in the diameter of the inhibition zone in the paper disc diffusion test procedure in this study can be caused by several factors, including: (1) application of the test solution to the paper disc, (2) preparation of the test solution, (3) thickness of the agar medium, (4) inoculum concentration, (5) temperature exposure, (6) chemical composition of the media and pH, and (7) storage time considerations (Nadilla, 2014; Syofyan & Nofita, 2020).

Based on the results of inhibition zones, the formulated rambutan honey toothpaste preparations are homogeneous because the preparations remain homogeneous in one period, at least during the period between mixing and stirring the active ingredients in the toothpaste according to the desired concentration or dosage, as well as the precipitation that occurs in the toothpaste during storage can be easily redispersed (Evahelda et al., 2017).

The results of the one-way ANOVA showed a p-value <0.05, indicating that there were significant differences in preparations F0, F1, F2, and F3. This can be influenced by the level of viscosity of the preparation; a low viscosity value causes the preparation to become diluted so that the active compounds in toothpaste can easily exit from the toothpaste base to the media properly so that the antibacterial power formed will be maximized. The formation of an inhibition zone around a paper disc soaked in rambutan honey toothpaste can be caused by the hydrogen peroxide content in honey, which effectively kills gram-positive and gram-negative bacteria due to its oxidizing ability and the formation of hydroxyl free radicals, which are more toxic than peroxide, thus facilitating cell damage (Samaranayake, 2018). In addition, the presence of low osmotic pressure in honey causes bacteria to become dehydrated so that they can kill bacteria. Honey's acidic Power of Hydrogen (pH) can also lyse bacterial cell walls by inhibiting their metabolic processes. The flavonoid content in honey also acts as an antibacterial through the process of phenol penetration into bacterial cells and causes precipitation and denaturation of proteins making up the protoplasm, thereby inhibiting bacterial metabolism and growth (Rezvani et al., 2017). The antibacterial effect of honey consists of two mechanisms: direct and indirect antimicrobial action. The direct microbial action of honey can be divided into two types: peroxidative and non-peroxidative. Peroxidative antibacterial activity of honey indicates that honey contains hydrogen peroxide produced by the enzyme glucose oxidase, which effectively kills gram-positive and gram-negative bacteria. Non-peroxidative antibacterial in honey indicates that honey has several factors that can inhibit bacterial growth through its acidic pH content, osmotic pressure due to low water activity, flavonoid content, and the presence of lysozyme enzyme in honey is also reported to play a role in inhibiting bacterial growth (Nadilla, 2014; Syofyan & Nofita, 2020).

CONCLUSION

In conclusion, this study demonstrated that rambutan honey has significant potential as a natural antibacterial agent in toothpaste formulations. The experimental approach successfully formulated toothpaste with varying concentrations of rambutan honey, showing that 60% concentration had the greatest inhibitory effect against *Streptococcus mutans*. These findings highlight the possibility of utilizing rambutan honey as an effective component of toothpaste for oral hygiene and disease prevention. Further research should focus on optimizing the stability of rambutan honey toothpaste formulations for practical use.

ACKNOWLEDGMENT

Thank you to Faculty of Dentistry Universitas Jenderal Achmad Yani Cimahi Indonesia.

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