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Antibacterial Activity of Combination between Probiotic Milk and Mango Honey Against Streptococcus Mutans

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Abstract

Background: *Streptococcus mutans* commonly found in oral cavity and can be a pathogenic bacteria that leads to dental caries. Rinsing the oral cavity with antibiotic oral therapy is not suggested as the treatment of dental caries, because it has side effects. It can cause resistance of *Streptococcus mutans* towards antibiotic.

Objective: To analyze the antibacterial activity of honey of mango, prebiotic milk, and the combination of both against *Streptococcus* mutans bacteria

Method: The antibacterial activity test was performed by agar diffusion method with Müeller Hinton agar medium to determine the minimal inhibitory concentration inhibition (MIC). A study had been conducted on the antibacterial activity of the combination of honey of mango and probiotic milk of *Lactobacillus paracasei* ATCC BAA52 on the growth of *Streptococcus mutans*. Fermented milk was made by inoculating *Lactobacillus paracasei* ATCC BAA52 fermented milk, mango honey and their combination at optimum ratio (propotition) into fresh milk at 45°C, then incubated for 24 hours at room temperature

Result: The result of probiotic milk characterization showed that the pH of probiotic milk decreased compared to fresh milk from from pH 6.33 to 3.89. Furthermore, the MIC of each samples against *Streptococcus mutans* were determined

Conclusion: Combination between mango honey (*Mangifera indica*) and probiotic mlik (*Lactobacillus paracasei* ATCC BAA52 can give optimum anti bacteria activities against *Streptococcus mutans*

Keywords: *Antibacterial activity, probiotik milk of Lactobacillus paracasei ATCC BAA52, Mango Honey, Streptococcus mutans.*

Introduction

Streptococcus mutans is a facultative anaerobic bacterium, gram positive cocci bacterium. It is commonly found in human oral cavity and being the most pathogenic bacteria which causes dental caries [(1)]. The characteristics of *S. mutans* are acidogenic, which produces acid, acidoduric, which is capable surviving in an acid environment, and capable to produce a sticky polysaccharide, called dextran. *S. mutans* can adhere to the dental enamel and promote other acidoduric bacteria towards dental enamel, which leads to dental caries [(1).]

Rinsing the oral cavity with liquid containing antibiotic is one of solution to prevent dental caries. Unfortunately, it can not prevent dental caries compeletely because it has side effect that leads to that antibiotic resistance[(2)]. To avoid the antibiotic resistance, scientists nowadays develop extracts and biological active compounds isolated from nature that used for herbal medicine [(3)].

Exploring the probiotics usage is one of the ways to resolve that problem. Probiotic contains hydrogen

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peroxide (H_2O_2), organic acids, and peptide compounds namely bacteriocin which are active as antibacterial[1]. Probiotic milk *Lactobacillus paracasei* has ability to inhibit the growth of pathogenic bacteria *S. mutans* in its host[5]. Consuming probiotics regularly can inhibit the growth *S. mutans* without causing side effects.

In addition to probiotics, currently it has been developed the treatment using natural ingredients that have activity as antimictobial, on of which using honey. Honey is a sweet liquid that derived from plant nectar, which processed by bees and stored in honeycomb cells[4]. The high consentration of sugar in honey, which is 38.5% fuctose, can cause hipertonic condition that promotes bacterial cell plasmolysis. It results inhibiting bacterial growth and promoting bacterial cell death[6]. The carbohydrates in honey are in the form of reducing sugars, which are glucose and fructose, with minimum content is 65% [7].

Both probiotic milk and honey have antibacterial activity with different mechanism[8]. Probiotic milk *Lactobacillus paracasei* contains hydrogen peroxide (H_2O_2), organic acids, and peptide compounds namely bacteriocin which are active as antibacterial[9]. Honey also has ability to inhibit the growth of phatogenic bacteria, such as *E. coli, Listeria monocytogenes,* and *S. aureus,* which is influenced by the concentration of reducing sugars and high concentration of sucroses in honey [6].

Probiotic milk *Lactobacillus paracasei* and honey of mango *Mangifera indica* have a different ingredient, which is active as antibacterial. This study will observe the antibacterial activity of combination of probiotic milk *Lactobacillus paracasei* and honey of mango *Mangifera indica* in various ratio, then it will be found the combination of both ingredients in certain ratio which has maximum antibacterial activity[10]. The purpose of this study is to observe the best ratio of mango honey and probiotic milk to impede the growth of *mutans*.

Materials and Method

Materials: The samples includes, *Lactobacillus paracasei* ATTCC BAA52 honey mangoes and *Streptococcus mutans*. The media was *de Man Ragosa Sharpe Agar* (MRS) (Himedia Lab), Müeller Hinton Agar (OXOID), NaCl (Pa Merch), clindamycin antibiotic (p.g.) as positive control group, and sterile destilled water from the Microbiology Laboratory.

Equipment: The equipment that used in this study were analytic scales (Sartorius BL10S), incubator (Menmert), Öse (Sengkelit), ring (Silinder), vernier caliper (Jason), vortex (Maxi Mix II Type 37600), micro pipette (Socorex), autoclaf (Huxley HL-340), spectrophotometer (Parkin Elmer Lambda EZ201), ependorf, *shaker, colony counter, centrifuge* (EBA 20), Ostwald viscocity-meter, *Cup and Bob* viscocity meter, pH meter (Schott glass mainz tipe CG 842), *laminar air flow cabinet,* petri dish and the other laboratory glassware.

Results

Table 1: The minimum inhibitory concentration of probiotic Lactobacillus paracasei against Streptococcus mutans in various concetrations (% v/v)

Probiotic Milk Concentration (% v/v)	The diameter of inhibitory zone against Streptococcus mutans (mm)			Mean ± SD
	Replication 1	Replication 2	Replication 3	
100	15,35	16,40	15,75	15,38 ± 0,53
90	14,10	14,95	15,05	14,70 ± 0,52
80	11,75	12,05	11,55	11,78 ± 0,25
70	11,60	11,85	11,05	11,50 ± 0,41
60	11,05	11,75	10,80	11,20 ± 0,49
55	11,00	10,50	10,45	10,65 ± 0,30
Control (+)	26,75	26,70	27,20	26,88 ± 0,28

The results showed the minimum inhibitory concentration of probiotic milk *Lactobacillus paracasei* against *Streptococcus mutans* is 10.65 + 0.30 mm at concentration 55%. It can be interpreted if the concentration of probiotic milk *Lactobacillus paracasei* was less than 55%, so it could not inhibit the growth of *Streptococcus mutans*.

Table 2: The minimum inhibitory concentration of honey of mango in various concentration (% v/v)

Concentration of honey of mango (% v/v)	The diameter of inhibitory zone against Streptococcus mutans (mm)			Mean + SD
	Replication 1	Replication 2	Replication 3	
50	14,80	16,55	17,35	16,23 + 1,30
25	13,50	13,20	12,00	12,90 + 0,79
12,5	-	-	-	-
6,2	-	-	-	-
3,1	-	-	-	-
1,6	-	-	-	-
Control (+)	26,75	26,70	27,20	26,88 + 0,28

The result shows that the minimum inhibitory concentration of honey of mango was 10,40 + 0,96 mm at concentration 17.5% against the growth of *Streptococcus mutans*. It can be interpreted if the concentration of honey of mango solution that was less than 17.5%, could not inhibit the growth of *Streptococcus mutans*.

Table 3: The minimum inhibitory concentration of honey of mango in various concentration (% v/v)

Concentration of honey of mango (% v/v)	The diameter of inhibitory zone against Streptococcus mutans (mm)			Mean + SD
	Replication 1	Replication 2	Replication 3	
22,5	11,95	11,80	12,40	12,13 + 0,24
20	11,50	11,20	10,05	10,91 + 0,77
17,5**	9,50	11,40	10,30	10,40 + 0,96
15	-	-	-	-
Control positive	26,75	26,70	27,20	26,88 ± 0,28

The measurement diameter of inhibitory zone of combination honey of mango and probiotic milk *Lactobacillus paracasei* against tested bacterial in various concentration is displayed in Table 5 and Figure 4. The test of antibacterial activity of the combination honey of mango and probiotic milk *Lactobacillus paracasei* was conducted at ratio 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. The antibacterial activity was determined by the clear zone around hole, that could be measured. To obtain the combination which had maximal antibacterial activity could be done by measuring the diameter of inhibitory zone using Varnier Caliper[9].

Table 4. The antibacterial activity of the combination honey of mango and probiotic milk Lactobacillus paracasei against Streptococcus mutans at vairous ratio (% v/v)

The ratio of (% v/v) honey of mango solution 50% : probiotic milk	Diameter of Inhibitory Zone (mm)			Mean	SD
	Replication 1	Replication 2	Replication 3		
1:9	15,75	14,50	15,40	15,22	0,64
2:8	14,10	16,35	14,20	14,88	1,27
3:7	15,15	15,65	15,30	15,37	0,25
4:6	12,50	12,90	13,50	12,97	0,50
5:5	14,80	14,10	16,10	15,00	1,01
6:4	14,30	16,15	14,40	14,95	1,04
7:3	16,05	15,95	16,35	16,12	0,21
8:2**	17,35**	16,90**	17,10**	17,12**	0,22**
9:1	15,30	16,85	17,50	16,55	1,13
Honey of mango solution 50%	14,80	16,55	17,35	16,23	1,30
Probiotic milk L. paracasei 100%	12,95	14,50	14,15	13,87	0,81
Control positive	18,15	18,10	18,10	18,12	0,03

Discussion

The result of this research shows the positivity. The combination of honey of mango and probiotic milk *L. paracasei* at the ratio 8:2 showed the optimum antibacterial activity against *S. mutans*, with diameter of inhibitory zone as 17,12 + 0,22 mm. Based on statistical analysis using one way ANOVA, the combination honey of mango and probiotic milk *L. paracasei* at the ratio of 8: 2 did not show a significant difference in antibacterial activity compared to the inhibition zone of 50% honey of mango solution. Despite the diameter of inhibition zone of probiotic milk *L. paracasei* is greater than the diameter of inhibition zone of a honey of mango solution. However, when compared to the inhibitory zone of probiotic milk *L. paracasei*, there were significant differences in antibacterial activity [11].

The result shows that there was a decrease in the diameter of the inhibition zone along with the decreasing concentration of honey of mango. It can be seen from the diameter of the inhibition zone produced by 50% and 25% honey of mango of 13.03 + 0.15 mm and 13.03 + 0.57 respectively. While the concentration honey of mango below 25% did not produce antibacterial activity. The test results showed that the MIC of mango honey solution to *S. mutans* was at a concentration of 17.5% with a inhibition zone diameter of 10.40 + 0.96 mm.

After that, the determination of MIC in probiotic milk *L. paracasei* was carried out at various concentrations, namely at concentrations of 100%, 90%, 80%, 70%, 60%, 55%, 50%, 45%, 25%, 12.5%, 6.2%, 3.1% and 1.6%. In the result was found that the MIC of probiotic milk *L. paracasei* against *S. mutans* was at a concentration of 55%, with a inhibition zone diameter of 10.65 + 0.30 mm. By knowing the MIC of probiotic milk *L. paracasei* at a concentration of 55%, it proved that probiotic milk *L. paracasei* has a smaller antibacterial activity compared to honey of mango which has MIC at a concentration of 17.5% [12].

The result showed the combination honey of mango and probiotic milk *L. paracasei* at the selected ratio was 25% with inhibition zone diameter 13.03 + 0.57 mm against *S. mutans*. When compared to MIC each sample of honey of mango and probiotic milk *L. paracasei*, it can be said that the combination of honey of mango and probiotic milk *L. paracasei* at the selected ratio has a minimum inhibitory concentration greater than honey of mango [13]. But it is lower than the concentration minimum inhibition of probiotic milk *L. paracasei*. Then the optimum combination characterization was carried out, which included organoleptic (color, taste odor), pH, viscosity and specific gravity [14]. Based on the results of the selected combination characterization it has a pH of 3.89 + 0.00, the viscosity is 5.33 ± 0.390 cps and the specific gravity is 1.096 ± 0.000 g/mL.

In the combination of honey of mango and probiotic milk *L. paracasei* at the optimum ratio 8:2, there was a synergistic effect even though the concentration of probiotic milk was less than honey of mango [14]. It caused by honey of mango containing more sources which can be used as an energy for probiotic bacteria *L. paracasei*. In a combination honey of mango and probiotic milk *L.paracasei*, honeyof mango can play a role in two things, namely as an energy source for probiotic milk bacteria *L. paracasei* or can inhibit the growth of probiotic milk bacteria *L. paracasei* [15]. This study examnies the effect honey of mango on the growth of probiotic bacteria *paracasei*, proved that mango honey did not inhibit the growth of probiotic bacteria *L. paracasei*.

The analysis of antibacterial activity in this study using difusion method, because of its advantages. This method is quite simple, does not require long time and preparation, and can also be used to see the senstivity of antibacterial samples at certain concentratiom of various types of tested bacteria [16]. The standard solution used in this study was clindamycin with a concentration of 0.01 ppm which was previously optimized for the antibacterial activity of clindamycin with various concentrations of *S. mutans* [3]. A concentration of 0.01 ppm was chosen because at concentrations above 0.01 ppm clindamycin produced a diameter of the inhibition zone that was too large which could lead to difficulty of measuring the diameter of the sample inhibition zone. Clindamycin is chosen as a standard solution or positive control because it is an effective antibacterial used to cure tooth damage due to the growth of Streptococci bacteria [17].

Conclusion

Based on the results of this study, it can be concluded that the Minimum Inhibitory Concentration (MIC) of probiotic milk *Lactobacillus paracasei* ATCC BAA52 on *Streptococcus mutans* was 55%, with inhibition zone diameter of 10.65 + 0.30 mm. Minimum Inhibitory Concentration (MIC) of honey of mango solution against *Streptococcus mutans* was 17.5%, with inhibition zone diameter 10.40 + 0.96 mm.

Minimal Inhibition Concentration (MIC) combination of honey of mango (*Mangifera indica*) and probiotic milk *Lactobacillus paracasei* ATCC BAA52 at a ratio of 8: 2 to *Streptococcus mutans* by 25% with inhibition zone diameter 13.03 + 0.57 mm.

Ethical Clearance: This research process did not involve any participant in the survey, but instead using agar diffusion method in laboratory in accordance with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficenct, and justice.

Conflict of Interest: There is no report about any conflict related with this author's research.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5