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Antibacterial Effect of the Combination of Probiotic Milk and Calliandra Honey against Streptococcus Mutans that Causes Tooth Cavities

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Abstract

Background: *Streptococcus mutans* is a Gram-positive bacterium found in the oral cavity. As a cariogenic bacterium, *Streptococcus mutans* can cause dental caries through its ability to produce an acidic environment that can demineralize tooth structures so that the tooth layer is destroyed.

Objective: To determine the optimal combination of probiotic milk *Lactobacillus paracasei* and calliandra honey which has antibacterial activity in *Streptococcus mutans* bacteria.

Method: This study uses diffusion method on Nutrient Agar media. The study began with an examination of the physical properties of probiotic milk and calliandra honey including color, odor, taste, pH, specific gravity and viscosity. Antibacterial activity was indicated by the diameter of the Zone of inhibition (mm) in the form of clear areas around the well on the media so that containing Streptococcus mutans inoculums 0.25µl/ml.

Result: The combination of probiotic milk *Lactobacillus paracasei* and 50% calliandra honey solution produced the highest activity at a ratio of 8: 2 with Zone of inhibition diameters of 16.40 ± 0.71 mm

Conclusion: The combination of probiotic milk and calliandra honey with 5% concentration and 8: 2 ratio has the highest antibacterial activity against Streptococcus mutans that causes tooth cavities

Keywords: *Streptococcus mutans, antibacterial activity, probiotic milk, Lactobacillus paracasei, calliandra honey.*

Introduction

Streptococcus mutans is a normal flora in the oral cavity that can turn into a pathogen when there is an excessive number colonies[(1)]. Streptococcus mutans is an anaerobic bacterium that is known to produce lactic acid as part of its metabolism and is able to attach to the tooth surface in the presence of sucrose which causes caries in the teeth[(2)]. Streptococcus mutans produces lactic acid through a *homo-fermentation* process, forming colonies that are firmly attached to the tooth surface and more acidogenic than other species so that it can cause demineralization of tooth enamel at a critical pH of 5.5. Continuous tooth enamel demineralization will cause dental caries[(3)].

Several studies show that probiotic bacteria have an influence in the ecology of the oral cavity. There are several strains of probiotic bacteria that have a positive effect in reducing the number of Streptococcus mutans in the saliva of the human oral cavity, namely the acidogenic class of Lactobacillus and Bifidobacterium[(4)].

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The combination of Lactobacillus paracasei and Bifidobacteriumlongum isolates was able to inhibit the growth of Streptococcus mutans[5].

In its development, probiotic bacteria are packaged in a probiotic product with added milk to meet the nutritional needs of these bacteria[6]. Probiotics have proteolytic and lipolytic enzymes that can make milk as a substrate and produce a variety of energy sources [7].

As an antibacterial agent, probiotic milk and honey have different mechanisms, so that a combination of both can affect both activities Antibacterial activity of combination probiotic milk Lactobacillus paracasei and Calliandra honey in inhibiting the growth of Streptococcus mutans are not known, so it needs to be done research to determine the antibacterial activity of the combination of probiotic milk Lactobacillus paracasei ATCC BAA52 and Calliandra Honey (Calliandracalothyrsus) against Streptococcus mutans in various comparisons[8]. Based on this background, this study aims to determine the combination of probiotic milk Lactobacillus paracasei ATCC BAA52 and Calliandra honey (Calliandracalothyrsus) which can provide maximum antibacterial activity against Streptococcus mutans.

Material and Method

Materialsand Equipments: The materials are Media Nutrient Agar (Oxoid), Agar de Man Ragosa Sharpe (MRS), Calliandra honey from Yogyakarta, milk, NaCl (Merck), Klindamisin, sterile distilled water and membrane filter with a size of 0.22 µm. The equipments are analytical scales, glassware, petri dishes, micro pipettes (Socorex), öse wire, ependorf, vortex (Type 161700 mixer), incubator (Memmert), vernier caliper, colony counter, shaker, refrigerator, picnometer, viscosity ostwald, viscosity Cup and Bob, autoclave (HL-340 series sterile vertical type steam), pH meter (Schott glass mainz CG 842 type), laminar air flow cabinet, lactodensimeter, and spectrophotometer (Thermo Fisher Scientific 5225 Verona Road).

Agar well diffusion method: Test bacteria aged 18-24 hours are suspended into the agar medium at around 45oC. The bacterial suspension is poured into a sterile petri dish. After the agar becomes solid, holes are made with a diameter of 6-8 mm. Into the hole, a solution of the substance is inserted which will be tested for 20 µL of activity, then incubated at 37oC for 18-24 hours[9].

Results

Table 1: Observation Result of Zone of inhibition diameter of probiotic milk at various concentrations (%) on the growth of mutant Streptococcus.

Probiotic milk concentration	Zone of inhibition diameter (mm)
	Mean ± SD
100%	9,92 ± 0,52
80%	9,50 ± 0,36
70%	9,12 ± 0,10
60%	9,07 ± 0,09
55%	9,32 ± 0,08
50%	-
25%	-
Clindamycin 0,01 ppm	32,33±0,47

From these data, it can be seen that the minimum inhibitory concentration is produced by Lactobacillus paracasei probiotic milk at a concentration of 55.00%, which results in an average diameter Zone of inhibition of 9.3 ± 0.08 mm and at a concentration of less than 55.00% there is no Zone of inhibition. The minimum inhibitory concentration was produced by probiotic milk Lactobacillus paracasei at a concentration of 55% so that for the next combination carried out at the concentration of probiotic milk Lactobacillus paracasei ATCC BAA52 was above 55%.

Table 2: Observation result of Zone of inhibition diameter of calliandra honey at various concentration (%) against Streptococcus mutans

Calliandra Honey Concentration	Zone of inhibition diameter (mm)
	Mean ± SD
50%	24,42±0,42
40%	23,20±0,12
30%	22,28±0,06
25%	21,18±0,12
12,5%	-
6,25%	-
Clindamycin 0,01 ppm	19,12±0,01

From these data, it can be seen that the minimum inhibitory concentration is produced by calliandra honey at a concentration of 25%, which results in an average diameter Zone of inhibition of 21.18 ± 0.12 mm and a concentration less than 25% there is no Zone of inhibition. Based on the table above, the minimum inhibitory concentration is produced by calliandra honey (Calliandracalothyrsus) at a concentration of 25%, so that the next combination is carried out at the concentration of calliandra honey above 25%.

Table 3: ZOI diameter observation of Combination of Probiotic Milk and calliandra Honey to Streptococcus mutans

Combination (Probiotic: Honey)	Zone of Inhibition Diameter (mm)			Mean ± SD
	1st Replication	2nd Replication	3rd Replication	
1 : 9	9,20	10,00	11,00	10,07±0,74
2 : 8	12,35	13,00	13,15	12,83±0,35
3 : 7	11,40	12,35	13,00	12,25±0,66
4 : 6	12,35	13,10	13,10	12,85±0,35
5 : 5	12,35	13,25	14,10	13,23±0,71
6 : 4	13,30	14,00	14,00	13,77±0,33
7 : 3	12,30	12,35	12,35	12,33±0,02
8 : 2**	15,40**	16,80**	17,00**	16,40±0,71**
9 : 1	15,00	15,20	16,10	15,43±0,48
Calliandra Honey	15,40	15,00	14,90	15,10±0,35
Probiotic milk	14,60	13,60	12,00	13,40±1,84
Clindamycin	21,00	21,00	21,00	21,00±0,00

From these data, it can be seen that the maximum ZOI diameter is produced by a combination of L. paracasei probiotic milk and calliandra honey at a ratio of 8: 2 which results in a diameter of the Zone of inhibition greater than the other comparison which is 16.40 ± 0.71 mm. The maximum antibacterial activity is indicated by the combination of Lactobacillus paracasei probiotic milk and 50% calliandra honey at a ratio of 8: 2 which has the largest Zone of inhibition diameter, then one-way anova statistical test (attachment 10) to determine the difference in diameter significance the average zone of inhibition (ZOI) between each group is presented in Table 4.

Table 4: Differences in ZOI Diameter mean value of Combined Zone of Probiotic L.paracasei and Calliandra Honey against S. mutans according to HSD Test

Kel	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1	M	S	K
1:9		2,77*	2,18*	2,78*	3,17*	3,70*	2,27*	6,33*	5,37*	5,03*	3,33*	10,93*
2:8	2,77*		0,58	0,02	0,40	0,93	0,50	3,57*	2,60*	2,26*	0,57	8,17*
3:7	2,18*	0,58		0,60	0,98	1,52*	0,08	4,15*	3,18*	2,85*	1,15	8,75*
4:6	2,78*	0,02	0,60		0,38	0,92	0,52	3,55*	2,58*	2,25*	0,55	8,15*
5:5	3,17*	0,40	0,98	0,38		0,53	0,90	3,17*	2,20*	1,87*	0,17	7,77*
6:4	3,70*	0,93*	1,52*	0,92	0,53		1,43*	2,63*	1,17*	1,33*	0,37	7,23*
7:3	2,27*	0,50*	0,08	0,52	0,90	1,43*		0,97*	3,10*	2,77*	1,07	8,67*
8:2	6,33*	3,57*	4,15*	3,55*	3,17*	2,63*	4,07*		0,97	1,30*	3*	4,60*
9:1	5,37*	2,60*	3,18*	2,58*	2,20*	1,67*	3,10*	0,97		0,33*	2,03*	5,57*
M	5,03*	2,26*	2,85*	2,25*	1,87*	1,33*	2,77*	1,30*	0,33		1,70*	5,90*
S	3,33*	0,57	1,15	0,55	0,17	0,37	1,07	3*	2,03*	1,70*		7,60*
K	10,93*	8,17*	8,75*	8,15*	7,77*	7,23*	8,67*	4,60*	5,57*	5,90*	7,60*	

Description: Yellow - Significant different (p<0,05), White - No significant different (p>0,05), M - 50% calliandra honey, S - 100% probiotic milk, K - Positive control (Clindamycin 0,01ppm)

Based on Table 4, it is known that there are differences in the average diameter of the ZOI according to Tukey-HSD for a ratio of 8: 2 with other ratio except 9: 1. But the optimal ratio is also determined by the diameter of the zone of inhibition (ZOI) which is the largest among

the other ratio. So the optimal ratio is shown by the combination of probiotic milk Lactobacillus paracasei and Calliandra honey in a ratio of 8: 2.

Table 5: ZOI diameters of Probiotic Milk Lactobacillus paracasei and Calliandra honey (Calliandracalothyrsus) combination against Streptococcus mutans

Concentration of Probiotic and Calliandra Honey Combination (8: 2)	ZOI diameter against Streptococcus mutans(mm)
	Mean ±SD
100%	17,13±0,23
50%	12,86±0,15
40%	11,03±0,55
Clindamycin 0,01 ppm	36,00±0,00

From these data, it can be seen that the minimum inhibitory concentration of Streptococcus mutans is produced by a combination of probiotic milk Lactobacillus paracasei and calliandra honey with a ratio of 8: 2 at a concentration of 40.00% which is equal to 11.03 ± 0.55 mm and at concentrations less than 40.00% there is no zone of inhibition that generated.

Discussion

From these results, it was found that probiotic milk Lactobacillus paracasei and calliandra hone had antibacterial activity against Streptococcus mutans at a minimum inhibitory concentration (MIC) of 55% and 25%. Calliandra honey has smaller MIC than probiotic Lactobacillus paracasei milk which is 25%, so it can be assumed that Calliandra honey is more potent in inhibiting the growth of Streptococcus mutans than Lactobacillus paracasei probiotic milk[10].

From these results, it can be seen that with a decrease in concentration from 100% to 60% of Lactobacillus paracasei probiotic milk, there was a decrease in the diameter of the inhibition zone, although at 55% concentration of probiotic milk Lactobacillus paracasei increased. The results also showed that Lactobacillus paracasei probiotic milk was able to inhibit the growth of Streptococcus mutans at the lowest concentration of 55% with inhibitory zone diameters of 9.32 ± 0.08 mm, because at concentrations below 50% no inhibition zones were produced.

Based on the research that has been done, the ZOI diameter of probiotic milk Lactobacillus paracasei at a concentration of 100% is 9.92 ± 0.52 mm, while at a concentration of 50%; 25% and 12.5% do not produce inhibition zones. So that the test is continued at a concentration of 80%; 70%; 60% and 55% are produced in succession of inhibitory zone diameters of 9.50 ± 0.36 mm; 9,12 ± 0,10 mm; 9.07 ± 0.09 mm and 9.32 ± 0.08 mm.

Furthermore, the antibacterial activity of a combination of probiotic milk Lactobacillus paracasei and calliandra honey was carried out to the growth of Streptococcus mutans. In this study, testing was done with agar diffusion method because in the process it is simple, inexpensive and able to test various types of microorganisms and antibacterial agents with easy results of interpretation techniques[11].

According to Ghabanchi in his research, antibacterial activity was shown by the presence of an inhibition zone (mm) in the form of a clear area around the well. The inhibition zone shows that Streptococcus mutans are not resistant to the test solution[12]. As a positive control used in testing this antibacterial activity is clindamycin, because the antibiotic is known to be sensitive to gram-positive, facultative anaerobes and has been shown to inhibit the growth of oral bacteria, especially Streptococcus mutans[10].

Based on the research by Bhushan and Chithra (2010), it was said that some probiotic bacteria act as bacteriocin or like inhibitors specifically preventing the growth of cariogenic bacteria, having the ability to protect teeth and affect the growth of supragingival plaques. Adhesion reduction can be an effective way to reduce cariogenic bacteria such as Streptococcus mutans[13].

The effect of osmotic pressure from honey is related to saturated solutions of sugar with water content usually only around 15-21% of its weight. Solids in honey, 84% is a mixture of monosaccharides namely fructose and glucose[14]. The strong interactions between sugar molecules produce water molecules that are not enough for microorganisms. Microorganisms will lose water from this osmosis process and will become dehydrated, so it can kill these microorganisms. Gluconic acid is the most dominant acid[15]. This acid is the result of enzymatic changes in glucose by the glucose oxidase enzyme, which bees secreted from the hypopharyngeal gland becomes a balance between gluconic acid and gluconolactone[16].

Conclusions

Based on the results of the study, it can be concluded that the MIC of probiotic milk Lactobacillus paracasei ATCC BAA52 against Streptococcus mutans was 55%. While the MIC of calliandra (Calliandracalothyrsus) honey against Streptococcus mutans is 25%. The combination of probiotic milk Lactobacillus paracasei ATCC BAA52 and Calliandra honey (Calliandracalothyrsus) can provide maximum activity against Streptococcus mutans in a ratio of 8: 2. The combination MIC of probiotic milk Lactobacillus paracasei ATCC BAA52 and Calliandra honey (Calliandracalothyrsus) against Streptococcus mutanswas 40%.

Ethical Clearance: The research process did not involve with human, instead it is only laboratory research of parasites that is in accordant 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-maleficence, and justice.

Conflict of Interest: The author has not received any conflict of interest so far. It is safe from the conflict

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