Antibacterial activity of ethanol extract of *Beluntas* leaves on *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Enterococcus faecalis*

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ABSTRACT

Introduction: Individuals with poor oral health have a greater risk factor for systemic diseases. Caries, periodontal disease, and root canal infections are a common dental and oral diseases caused by dominance of *Streptococcus mutans, Porphyromonas gingivalis,* and *Enterococcus faecalis bacteria (S. mutans, P. gingivalis, and E. faecalis)*. An alternative way to prevent dental and oral diseases is to use herbal medicine as one of the active ingredients for mouthwash or toothpaste. One of the herbs that can be used is Beluntas leaves (*Pluchea indica (L.) Less* leaves). The objective of study was to analyze the antibacterial activity of ethanol extract of *Pluchea indica (L.) Less* leaves on the growth of *Streptococcus mutans, Porphyromonas gingivalis, and Enterococcus faecalis*. **Methods:** This research was experimental laboratory with post test only control design, using disk diffusion method. There were five concentrations (2.5, 3.5, 4.5, 5.5 and 6.5%, positive controls, and negative controls. Data analysis was performed using One Way Anova and post Hoc test. **Results:** The ethanol extract of *Pluchea indica (L.) Less* leaves has moderate-strong antibacterial activity against *Streptococcus mutans, Porphyromonas gingivalis, and Enterococcus faecalis*. The largest diameter of inhibitory zone in *Enterococcus faecalis* at a concentration of 6.5% followed by *Streptococcus mutans* and *Porphyromonas gingivalis* at the same concentration and the smallest diameter of inhibition zone in *Porphyromonas gingivalis*, followed by Enterococcus faecalis and Streptococcus mutans at 2.5% concentration. **Conclusion:** The ethanol extract of *Pluchea indica (L.) Less* leaves with a concentration of 2.5, 3.5, 4.5, 5.5, and 6.5% has antibacterial activity in inhibiting the growth of *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Enterococcus faecalis*.

Keywords: Antibacterial activity, beluntas leaves, *Enterococcus faecalis*, *Porphyromonas gingivalis*, *Streptococcus mutans*.

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INTRODUCTION

Dental and oral diseases, especially periodontitis can affect general body health.[1] Individuals with poor oral health have a greater risk factor for systemic disorders than individuals with well maintained oral health.[1] Dental and oral diseases such as caries and periodontal disease, are generally caused by pathogenic bacteria such as *S. mutans, P. gingivalis,* and *E. faecalis* in plaque and saliva.[2]

Prevention of dental and oral diseases can be done with plaque control using mouthwash. Mouth washing can help reduce plaque accumulation between teeth that are unreachable during brushing teeth.[3] Antimicrobial ingredients in mouthwashes that are commonly used are chlorhexidine.

Chlorhexidine is a broad-spectrum antimicrobial agent that affects gram positive and gram negative bacteria, fungi and some viruses.[4] However, chlorhexidine has some side effects, such as tooth staining, mucosal erosion, and causes an unpleasant feeling in the oral cavity.[4]

Alternative prevention methods that are being developed are using herbal medicine as one of the active ingredients for mouthwash or toothpaste. One of the herbs that can be used is *Pluchea indica* (L.) Less leaves.

Many people have used *Pluchea indica* (L.) Less leaves to eliminate body odor and bad breath, increase appetite, overcome digestive disorders in children and so on.[5] Dayak Pesaguan tribes have used all parts of the *Pluchea indica* (L.) Less leaves as a medicine for body odor and digestion.[6] In the research conducted by Nahak,[5] ethanol extract of *Pluchea indica* (L.) Less leaves can inhibit the growth of bacteria *S. mutans* with a concentration of 25, 50, 75 and 100%. *Pluchea indica* (L.) Less leaves has strong to moderate antibacterial activity against *E. faecalis and F. nucleatum* bacteria.[7]

Pluchea (L.) Less leaves can also reduce the number of bacterial colonies in *saliva* with a concentration of 2.5, 3.5, 4.5, 5.5 and 6.5%.[8] Objective of this study to analyze the antibacterial activity of ethanol extract of *Pluchea indica* (L.) Less leaves against *Streptococcus mutans, Porphyromonas gingivalis,* and *Enterococcus faecalis* using disk diffusion method.[9]

METHODS

This research was experimental laboratory with posttest only control design, using disk diffusion method.[9] The tools used in this research were sterilization tool, preparation tool, inhibition zone measurement tool. The materials used in this research was Mueller-Hinton Agar (MHA) media, Mueller-Hinton Broth (MHB) media, Brain Heart Infusion Agar (BHIA) media, Brain Heart Infusion Broth (BHIB) media, bacteria, Mc Farland 0.5, *Pluchea indica L.* leaves extract.

The sample of this research was *Streptococcus mutans* ATCC 25175, *Porphyromonas gingivalis ATCC* 33277 *and Enterococcus faecalis* ATCC 29212.There are five concentration of *Pluchea indica L.* leaves extract (2.5, 3.5, 4.5, 5.5 and 6.5%, positive controls (Chlorhexidine 0.2%), and negative controls (aquadest) with 4 times repetition. The results of the research data were processed by ANOVA and *Post Hoc* test.

RESULTS

The assessment of the antibacterial activity of ethanol extract of *Pluchea indica* (L.) Less leaves towards *S. mutans, P. gingivalis,* and *E. faecalis* growth was performed with the Kirby-Bauer agar diffusion method.[9] The measurement results of the ethanol extract of *Pluchea indica* (L.) Less leaves antibacterial inhibitory zone towards *S. mutans, P. gingivalis,* and *E. faecalis* in 5 concentration and positive controls (Chlorhexidine 0.2%), and negative controls (aquadest) tested were shown in Table 1.

Based on the results shown in Table 1, there were differences in the inhibitory zone of each sample. The biggest inhibitory area of ethanol extract of *Pluchea indica* (L.) Less leaves was found in *E. faecalis* bacteria at a concentration of 6.5% followed by *S. mutans* and *P. gingivalis* at the same concentration and the smallest inhibitory area was found in *P. gingivalis* bacteria, followed by *E. faecalis* and *S. mutans* at a concentration of 2.5%.

Based on comparison test between variables, it was found that there were no significant differences ($p > 0.05$) between concentrations of 5.5% and positive control in the *S. mutans*, between the concentrations of 2.5 and 3.5%, between 4.5 and 5.5% in the *P. gingivalis*,

and between the concentrations of 4.5 and 5.5%, also between 4.5% and 5.5% on positive controls in the *E. faecalis*, the rest variables had significant values ($p < 0.05$).

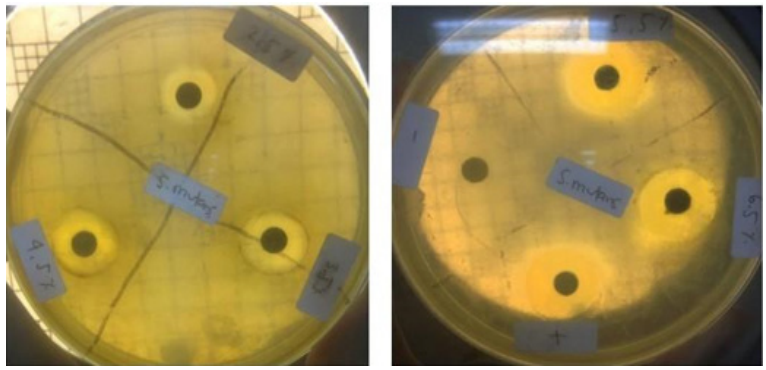

Figure 1. Antibacterial inhibitory zone of ethanol extract of *Pluchea indica (L.) Less*. leaves towards Streptococcus mutans

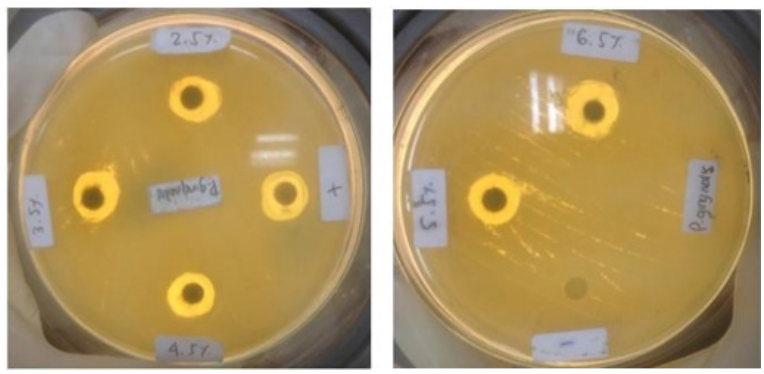

Figure 2. Antibacterial inhibitory zone of ethanol extract of *Pluchea indica (L.) Less* leaves towards *Porphyromonas gingivalis*

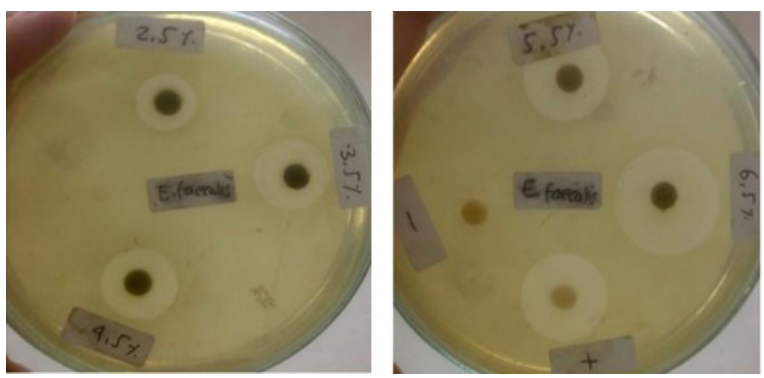

Figure 3. Antibacterial inhibitory zone of ethanol extract of *Pluchea indica (L.) Less* leaves towards *Enterococcus faecalis*

Table 1. The result of th he inhibitory zone measurement of ethanol extract of Pluchea indica (L.) Less leaves

Concentration %	*Streptococcus mutans*	*Porphyromonas gingivalis*	*Enterococcus faecalis*
	Mean (mm)	Mean (mm)	Mean (mm)
Negative Control	0.0±0.00	0.0±0.00	0.0±0.00
2.5	7.5±0.7	5.3±0.4	5.5±0.6
3.5	8.7±0.4	5.5±0.2	7.0±0.1
4.5	9.9±0.4	6.5±0.2	10.5±0.2
5.5	11.3±0.4	6.9±0.3	11.2±0.2
6.5	12.2±0.4	8.8±0.1	12.6±0.3
Positive Control	11.4±0.8	10.6±0.5	10.9±0.5

Notes: Four times repetitions. The data is presented in mean ± SEM. Disc diameter approximately 5 mm. data were processed by ANOVA and Post Hoc test.

Table 2. ANOVA result of the diameter of Streptococcus mutans inhibitory zone (mm)

	Sum of squares	df	Mean square	F	Sig.
Between Groups	64.751	5	12.950	66.534	0.000
Within Groups	3.503	18	0.195		
Total	68.254	23			

Table 3. ANOVA result of the diameter of *Porphyromonas gingivalis* inhibitory zone (mm)

	Sum of squares	df	Mean square	F	Sig.
Between Groups	86.132	5	17.226	268.034	0.000
Within Groups	1.157	18	0.064		
Total	87.289	23			

Table 4. ANOVA result of the diameter of *Enterococcus faecalis* inhibitory zone (mm)

	Sum of squares	df	Mean square	F	Sig.
Between Groups	150.053	5	30.011	234.494	0.000
Within Groups	2.304	18	0.128		
Total	152.357	23			

Table 5. Multiple comparisons of the inhibitory zone diameter of ethanol extract of of *Pluchea indica (L.) Less* leaves towards *Streptococcus mutans* (mm) with Tukey HSD

(I) Treatment	(J) Treatment	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
Concentration of 2.5%	Concentration of 3.5%	-1.24825*	0.31196	0.001	-1.9037	-0.5928
	Concentration of 4.5%	-2.42750*	0.31196	0.000	-3.0829	-1.7721
	Concentration of 5.5%	-3.84500*	0.31196	0.000	-4.5004	-3.1896
	Concentration of 6.5%	-4.69000*	0.31196	0.000	-5.3454	-4.0346
	Positive control	-3.90250*	0.31196	0.000	-4.5579	-3.2471
Concentration of 3.5%	Concentration of 2.5%	1.24825*	0.31196	0.001	0.5928	1.9037
	Concentration of 4.5%	-1.17925*	0.31196	0.001	-1.8347	-0.5238
	Concentration of 5.5%	-2.59675*	0.31196	0.000	-3.2522	-1.9413
	Concentration of 6.5%	-3.44175*	0.31196	0.000	-4.0972	-2.7863
	Positive control	-2.65425*	0.31196	0.000	-3.3097	-1.9988
Concentration of 4.5%	Concentration of 2.5%	2.42750*	0.31196	0.000	1.7721	3.0829
	Concentration of 3.5%	1.17925*	0.31196	0.001	0.5238	1.8347
	Concentration of 5.5%	-1.41750*	0.31196	0.000	-2.0729	-0.7621
	Concentration of 6.5%	-2.26250*	0.31196	0.000	-2.9179	-1.6071
	Positive control	-1.47500*	0.31196	0.000	-2.1304	-0.8196
Concentration of 5.5%	Concentration of 2.5%	3.84500*	0.31196	0.000	3.1896	4.5004
	Concentration of 3.5%	2.59675*	0.31196	0.000	1.9413	3.2522
	Concentration of 4.5%	1.41750*	0.31196	0.000	0.7621	2.0729
	Concentration of 6.5%	-0.84500*	0.31196	0.014	-1.5004	-0.1896
	Positive control	-0.05750	0.31196	0.856	-0.7129	0.5979
Concentration of 6.5%	Concentration of 2.5%	4.69000*	0.31196	0.000	4.0346	5.3454
	Concentration of 3.5%	3.44175*	0.31196	0.000	2.7863	4.0972
	Concentration of 4.5%	2.26250*	0.31196	0.000	1.6071	2.9179
	Concentration of 5.5%	0.84500*	0.31196	0.014	0.1896	1.5004
	Positive control	0.78750*	0.31196	0.021	0.1321	1.4429
Positive control	Concentration of 2.5%	3.90250*	0.31196	0.000	3.2471	4.5579
	Concentration of 3.5%	2.65425*	0.31196	0.000	1.9988	3.3097
	Concentration of 4.5%	1.47500*	0.31196	0.000	0.8196	2.1304
	Concentration of 5.5%	0.05750	0.31196	0.856	-0.5979	0.7129
	Concentration of 6.5%	-0.78750*	0.31196	0.021	-1.4429	-0.1321

Table 5. Multiple comparisons of the inhibitory zone diameter of ethanol extract of of *Pluchea indica* (L.) Less leaves towards *Porphyromonas gingivalis* (mm) with Tukey HSD

(I) Treatment	(J) Treatment	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
Concentration of 2.5%	Concentration of 3.5%	-0.22000	0.17926	0.818	-0.7897	0.3497
	Concentration of 4.5%	-1.21250*	0.17926	0.000	-1.7822	-0.6428
	Concentration of 5.5%	-1.60750*	0.17926	0.000	-2.1772	-1.0378
	Concentration of 6.5%	-3.49500*	0.17926	0.000	-4.0647	-2.9253
	Positive control	-5.37000*	0.17926	0.000	-5.9397	-4.8003
Concentration of 3.5%	Concentration of 2.5%	0.22000	0.17926	0.818	-0.3497	0.7897
	Concentration of 4.5%	-0.99250*	0.17926	0.000	-1.5622	-0.4228
	Concentration of 5.5%	-1.38750*	0.17926	0.000	-1.9572	-0.8178
	Concentration of 6.5%	-3.27500*	0.17926	0.000	-3.8447	-2.7053
	Positive control	-5.15000*	0.17926	0.000	-5.7197	-4.5803
Concentration of 4.5%	Concentration of 2.5%	1.21250*	0.17926	0.000	0.6428	1.7822
	Concentration of 3.5%	0.99250*	0.17926	0.000	0.4228	1.5622
	Concentration of 5.5%	-0.39500	0.17926	0.283	-0.9647	0.1747
	Concentration of 6.5%	-2.28250*	0.17926	0.000	-2.8522	-1.7128
	Positive control	-4.15750*	0.17926	0.000	-4.7272	-3.5878
Concentration of 5.5%	Concentration of 2.5%	1.60750*	0.17926	0.000	1.0378	2.1772
	Concentration of 3.5%	1.38750*	0.17926	0.000	0.8178	1.9572
	Concentration of 4.5%	0.39500	0.17926	0.283	-0.1747	0.9647
	Concentration of 6.5%	-1.88750*	0.17926	0.000	-2.4572	-1.3178
	Positive control	-3.76250*	0.17926	0.000	-4.3322	-3.1928
Concentration of 6.5%	Concentration of 2.5%	3.49500*	0.17926	0.000	2.9253	4.0647
	Concentration of 3.5%	3.27500*	0.17926	0.000	2.7053	3.8447
	Concentration of 4.5%	2.28250*	0.17926	0.000	1.7128	2.8522
	Concentration of 5.5%	1.88750*	0.17926	0.000	1.3178	2.4572
	Positive control	-1.87500*	0.17926	0.000	-2.4447	-1.3053
Positive control	Concentration of 2.5%	5.37000*	0.17926	0.000	4.8003	5.9397
	Concentration of 3.5%	5.15000*	0.17926	0.000	4.5803	5.7197
	Concentration of 4.5%	4.15750*	0.17926	0.000	3.5878	4.7272
	Concentration of 5.5%	3.76250*	0.17926	0.000	3.1928	4.3322
	Concentration of 6.5%	1.87500*	0.17926	0.000	1.3053	2.4447

Table 6. Multiple comparisons of the inhibitory zone diameter of ethanol extract of of *Pluchea indica (L.) Less* leaves towards *Enterococcus faecalis* (mm) with Tukey HSD

(I) Treatment	(J) Treatment	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
Concentration of 2.5%	Concentration of 3.5%	-1.48000*	0.25296	0.000	-2.2839	-0.6761
	Concentration of 4.5%	-4.93000*	0.25296	0.000	-5.7339	-4.1261
	Concentration of 5.5%	-5.71500*	0.25296	0.000	-6.5189	-4.9111
	Concentration of 6.5%	-7.06750*	0.25296	0.000	-7.8714	-6.2636
	Positive control	-5.41750*	0.25296	0.000	-6.2214	-4.6136
Concentration of 3.5%	Concentration of 2.5%	1.48000*	0.25296	0.000	0.6761	2.2839
	Concentration of 4.5%	-3.45000*	0.25296	0.000	-4.2539	-2.6461
	Concentration of 5.5%	-4.23500*	0.25296	0.000	-5.0389	-3.4311
	Concentration of 6.5%	-5.58750*	0.25296	0.000	-6.3914	-4.7836
	Positive control	-3.93750*	0.25296	0.000	-4.7414	-3.1336

(I) Treatment	(J) Treatment	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
Concentration of 4.5%	Concentration of 2.5%	4.93000*	0.25296	0.000	4.1261	5.7339
	Concentration of 3.5%	3.45000*	0.25296	0.000	2.6461	4.2539
	Concentration of 5.5%	-0.78500	0.25296	0.058	-1.5889	0.0189
	Concentration of 6.5%	-2.13750*	0.25296	0.000	-2.9414	-1.3336
	Positive control	-0.48750	0.25296	0.418	-1.2914	0.3164
Concentration of 5.5%	Concentration of 2.5%	5.71500*	0.25296	0.000	4.9111	6.5189
	Concentration of 3.5%	4.23500*	0.25296	0.000	3.4311	5.0389
	Concentration of 4.5%	0.78500	0.25296	0.058	-0.0189	1.5889
	Concentration of 6.5%	-1.35250*	0.25296	0.001	-2.1564	-0.5486
	Positive control	0.29750	0.25296	0.842	-0.5064	1.1014
Concentration of 6.5%	Concentration of 2.5%	7.06750*	0.25296	0.000	6.2636	7.8714
	Concentration of 3.5%	5.58750*	0.25296	0.000	4.7836	6.3914
	Concentration of 4.5%	2.13750*	0.25296	0.000	1.3336	2.9414
	Concentration of 5.5%	1.35250*	0.25296	0.001	0.5486	2.1564
	Positive control	1.65000*	0.25296	0.000	0.8461	2.4539
Positive control	Concentration of 2.5%	5.41750*	0.25296	0.000	4.6136	6.2214
	Concentration of 3.5%	3.93750*	0.25296	0.000	3.1336	4.7414
	Concentration of 4.5%	0.48750	0.25296	0.418	-0.3164	1.2914
	Concentration of 5.5%	-0.29750	0.25296	0.842	-1.1014	0.5064
	Concentration of 6.5%	-1.65000*	0.25296	0.000	-2.4539	-0.8461

***. The mean difference was significant at the 0.05 level.**

DISCUSSION

The inhibitory zone diameters of each sample interpreted based on Davis & Stout (1971) criteria, where the diameter of the inhibitory zone divided into three categories: 1) weak, if the diameter of the inhibitory zone of less than 10 mm, 2) moderate, if the diameter of the inhibitory zone 10-20 mm and 3) Strong, if the diameter of the inhibitory zone of more than 20 mm.[10] According to Table 1, the result showed that the ethanol extract *Pluchea indica (L.) Less* leaves had moderate-strong antibacterial activity towards *S. mutans*, *P. gingivalis* dan *E. faecalis* bacteria.

Strong antibacterial activity is only found at concentrations of 5.5% and 6.5% against *S. mutans* and *E. Faecalis* bacteria, and the other concentrations have moderate activity. This finding was equivalent to previous studies by Syafira, that showed ethanol extract of *Pluchea indica (L.) Less* could reduce the number of bacteria in saliva, where at a concentration of 5.5% and 6.5% the results were equivalent to positive control, chlorhexidine gluconate 0.2%, which there is no colonies of bacteria grown.[8]

Inhibitory zone of ethanol extract of *Pluchea indica (L.) Less* leaves increased according to the increase in concentration of extracts tested on *S. mutans* and *E. faecalis*. This finding was caused by the amount of the active compounds contained in the *luchea indica (L.) Less* leaves extract. The higher concentration of the extract, the broader its inhibitory zone towards the growth of the bacterias.[11,12] Antibacterial ability of ethanol extract of *Pluchea indica* (L.) *Less* related to the active compounds which is flavonoids, phenolics, tannins, alkaloids, lignin glycosides, and triterpenoids that are bacteriostatic and bactericidal.[5,13] Flavonoids work as antibacterials in several ways, such as, inhibiting bacterial cell wall synthesis, membrane disruption, inhibit bacterial protein synthesis, and inhibition of nucleic acid synthesis.[14] The mechanism of phenol was by denaturing the bacterial cell protein.[13,15] Tanin has an antibacterial effect by forming a complex with proline which is a type of protein in the cell wall of bacteria, which causes protein leakage and damage to bacterial cell walls.[8,13]

Alkaloid compounds have inhibitory mechanisms by interfering the constituent

components of peptidoglycan in bacterial cells, so the cell wall layer is not formed intact and causes cell death. In addition, in the alkaloid compound there is a nitrogen-containing base group that will act and affect bacterial DNA. This reaction results in changes in the structure and arrangement of amino acids, which will cause damage and encourage bacterial cell lysis.[15]

However the results from *P. gingivalis* bacteria showed there were no significant differences at the concentrations of 2.5% and 3.5% and at concentrations of 4.5% and 5.5%. There are multiple contributing factors that affect the inhibitory zone, in which are the amount of bacteria being used, bacterial growth speed, bacterial sensitivity, type of bacteria, toxicity of the test material, temperature and the interaction between medium and micro-environment in vitro.[11,16,17]

P. *gingivalis* also has a smallest inhibitory zone diameter compared to *S. mutans* and *E. faecalis*. This may occur because of the composition of the cell wall *P. gingivalis*. The Gram negative bacteria's cell wall was more complex than Gram positive so it is difficult for the active compounds of *Beluntas* leaves to penetrate the cell wall. Gram negative and positive bacteria have differences in cell wall components.[11,18]

Gram negative bacteria has endotoxins in the form of lipopolysaccharide complexes on their cell walls, and double membranes in which the plasma membrane is surrounded by a permeable outer membrane and the middle part of the membrane is peptidoglycan.[11,18] In the previous study by Suhartono also showed that there were differences activity of ethanol extract of *Pluchea indica* (L.) *Less* in inhibiting the growth of *Escherichia coli* bacteria, which also Gram-negative bacteria.[19]

In this study, there were differences in activity strength between ethanol extract of beluntas leaves and amoxicillin, where the active compounds of *Pluchea indica (L.) Less* leaves was difficult to penetrate into the cell wall of gram negative bacteria compared to amoxicillin.[19] This research is also in accordance with research by Wibowo, where the extract of *Plumeria acuminata* Ait has the lower ability to inhibit the growth of *P. gingivalis* compared to *S. mutans and E. faecalis*.[20]

CONCLUSIONS

The ethanol extract of *Pluchea indica* (L.) *Less* with a concentration of 2.5, 3.5, 4.5, 5.5, and 6.5% has antibacterial activity in inhibiting the growth of *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Enterococcus faecalis*.

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