

# The effectivity of binahong (*anredera cordifolia* (ten.) steenis) leaves extracts for growth inhibition of streptococcus mutans in oral cavity



CrossMark

Gostry A. Dohude,\* Frisca CM. Ginting

## Abstract

**Objective:** This research was conducted to determine the effectiveness of binahong (*anredera cordifolia* (ten.) steenis) leaves extract in inhibiting the streptococcus mutans growth.

**Material and Methods:** The study is true experimental laboratories with posttest-only control group design. The study sample used was Binahong leaves and streptococcus mutans bacteria that were multiplied in Microbiology Laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara. This study used seven treatment groups namely, 75%, 50%, 25%, 12.5%, 6.25% Binahong leaf extract, amoxicillin (positive control) and Dimethyl Sulfoxide (DMSO) (negative control). Testing the treatment group for the Streptococcus mutans was carried out using the Kirby-Bauer disk diffusion method with four repetitions. Extract was done by maceration using 96% ethanol solvent.

**Results:** Indicate that the binahong leaves (*anredera cordifolia* (ten.) steenis) extract can inhibit the growth of streptococcus mutans which can be seen from the clear zone formed on paper discs that have been given binahong leaves extract with a concentration of 75%, 50%, 25%, 12, 5%, 6.25%, along with amoxicillin and dimethylsulfoxide/DMSO. The results also showed a significant difference in diameter of the inhibition zone from each treatment group ( $p < 0.005$ ).

**Conclusion:** Binahong (*anredera cordifolia* (ten.) steenis) leaf extract has antibacterial ability because it can inhibit the growth of Streptococcus mutans in the oral cavity and compared to other concentrations, 75% concentration is more effective in inhibiting the growth of streptococcus mutans in the oral cavity.

**Keywords:** Antibacterial, Binahong Leaves, DMSO, Streptococcus mutans

**Cite this Article:** Dohude GA, Ginting FCM. 2021. The effectivity of binahong (*anredera cordifolia* (ten.) steenis) leaves extracts for growth inhibition of streptococcus mutans in oral cavity. *Journal of Dentomaxillofacial Science* 6(3): 151-155. DOI: [10.15562/jdmfs.v6i3.1040](https://doi.org/10.15562/jdmfs.v6i3.1040)

Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

## Introduction

Currently, the use of medicinal plant products is increasingly chosen by the world community. According to WHO, up to 60% of developed country populations and 80% of developing country populations now use herbal medicines. Indonesia is a developing country that is rich in various natural resources, so that it has the opportunity to utilize the wealth of natural supplies of medicinal plant products. The potential to support the development of medicinal plants in Indonesia is very large, out of 40.000 plant species in the world, of which 28.000 plant species exist, more than 80% of medicinal plants are grown in Indonesia. Potential plants that have many properties that can be used, one of them is binahong.<sup>1</sup>

Binahong plants (*anredera cordifolia* (ten.) steenis) have been widely used on medicinal treatment in various ways. One of the Binahong plants that is very useful is the leaf, because it contains several active chemical compounds that work synergistically as an antibacterial, namely flavonoids, alkaloids, terpenoids, tannins and saponins.<sup>2</sup>

Bacteria in the oral cavity which are normal flora, can turn into pathogenic bacteria and enter the host

body, causing odontogenic infections. Odontogenic infections can occur through several pathways, one of which is the periapical pulpal pathway. The periapical pulpal pathway is the way for bacteria to enter through a network of enamel, dentin, pulp chamber, to the apical teeth. Infection in this pathway most often occurs which usually begins with the appearance of caries. Dental caries is caused by bacterial invasion such as streptococcus mutans.<sup>3</sup>

Streptococcus mutans has an enzyme called glucosyltransferase which can cause glucose polymerization in sucrose by releasing fructose, so it can synthesize high molecular weight glucose molecules consisting of alpha glucose bonds.<sup>1</sup> The formation of alpha is very sticky, so it does not dissolve in water. It is utilized by streptococcus mutans to develop and form dental plaque. The same enzyme continues to add many glucose molecules to each other to form dextran which has a structure very similar to the amylase in the starch. Dextran together with bacteria attach tightly to tooth enamel and lead to the formation of plaque on the teeth. This is the stage of forming a cavity or hole in a tooth called dental caries. The ethyl acetate

\*Correspondence to:

Gostry A. Dohude, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia  
[gostryaldica@gmail.com](mailto:gostryaldica@gmail.com)

Received: 10 March 2020

Revised: 31 March 2020

Accepted: 11 July 2021

extract of Binahong leaves has inhibitory ability against gram-positive bacteria namely staphylococcus aureus with inhibitory zones at a concentration of 10% by 1.7 mm, 15% by 2.0 mm, and a concentration of 20% by 2.6 mm.<sup>4</sup>

Based on these results, it can be concluded that the higher the concentration of an antibacterial material, the stronger the antibacterial activity. Based on the description above, researchers are interested in conducting research into how the effectiveness of binahong (*anredera cordifolia* (ten.) steenis) leaf extracts against the growth of streptococcus mutans.

## Material and Methods

This study is a true experimental laboratories with posttest-only control group design. The study was conducted at the Laboratory of Traditional Medicine and Laboratory of Microbiology, Faculty of Pharmacy, University of North Sumatra in November until December 2019.

The sample used in this study was the streptococcus mutans which was multiplied in the Microbiology Laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara. The study used seven groups, namely binahong leaf extract with a concentration of 75%, 50%, 25%, 12.5%, 6.25%, amoxicillin (K +) and DMSO (K-) with four repetitions.

The binahong leaves used in this research were obtained from the Keling area, Karo District. Making extracts begins by separating the leaves from contaminants and impurities, such as soil and stems. Then binahong leaves are washed thoroughly. Drying process is carried out 3-5 days until the leaves are completely dried. Then the dried

leaves are blended until it becomes powder.

The extraction process is done by maceration method. Maceration is done by soaking 400 grams of binahong leaf powder in 96% ethanol for 2-3 days with manual stirring at room temperature. The liquid extract of binahong leaves was vaporized with waterbath to obtain 40 grams of the thick extract of binahong leaves. Then the research stock of binahong leaf extract was made with a concentration of 75%, 50%, 25%, 12.5%, and 6.25% by mixing thick extract (100%) and DMSO.

The streptococcus mutans used in this study origins from the stock of Microbiology Laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara. Streptococcus mutans were multiplied for 1x24 hours at 37°C then poured nutrient agar (NA) into a petri dish and then shaken with eight figure movements until homogenous using pourplate technique. Petri dishes are left homogeneous and evenly. Then paper disc that has been dropped with binahong leaf extract concentration of 75%, 50%, 25%, 12.5%, 6.25%, amoxicillin (K +) and DMSO (K-) was brought using sterile tweezers, then placed on the agar surface, a little pressure is applied so that the paper disc is fully embedded. After that, wrap the petri dish in parchment paper and put it in an incubator at  $35 \pm 2^\circ \text{C}$  for 18-24 hours. In the petri dish, clear zone will be formed which is an inhibitory zone of Binahong leaf extract against streptococcus mutans that can be measured using sliding calipers.

## Results

The measurement results of the binahong (*anredera cordifolia* (ten.) steenis) leaf extract inhibition zone diameter in inhibiting the growth of streptococcus

**Table 1** Antibacterial activity of Binahong leaf ethanol extract against streptococcus mutans

Bacteria Isolated	Concentration (% <sup>g/v</sup> )(*)	Repetition				D*(mm)
		P1	P2	P3	P4	
Streptococcus mutans	75	9.7	11.6	11.4	12.6	11.3
	50	9.4	10.4	10.4	11.6	10.4
	25	8.4	8.6	8.9	9.1	8.7
	12.5	8.3	8.0	8.2	8.4	8.2
	6.25	6.0	6.0	7.0	7.3	6.5
	K-**	-	-	-	-	0.00
	K+***	15.6	15.5	15.5	15.7	15.5

Annotation: P1 = inhibition diameter from first repetition;  
 P2 = inhibition diameter from second repetition;  
 P3 = Inhibition diameter from third repetition;  
 P4 = Inhibition diameter from fourth repetition;  
 D\* = Mean of inhibitory zone diameter  
 (\*) = Condensed extract concentration;  
 (\*\*) = Negative control (DMSO);  
 (\*\*\*) = Positive control (Amoxicillin);

**Table 2** Shapiro-Wilk test results

Concentration	Shapiro-Wilk	
	Number of Repetition	P-Value
75%	4	0.682
50%	4	0.659
25%	4	0.855
12.5%	4	0.850
6.25%	4	0.146
K <sup>+</sup>	4	0.272
K <sup>-</sup>	-	-

Annotation:

K<sup>+</sup>: Amoksisilin is the positive control

K<sup>-</sup>: Dimethyl sulphoxide (DMSO) is the negative control

**Table 3** Levene test

Inhibitory	Levene Test	
	Levene Statistic	P-Value
Mean inhibitory	2.139	0.107

**Table 4** Oneway-Anova test

Treatment	Number of Repetition	Means (mm)	Standard Deviation	P-Value ANOVA
75%	4	11.3	1.2038	p = 0.000
50%	4	10.4	0.9000	
25%	4	8.7	0.3109	
12.5%	4	8.2	0.1708	
6.25%	4	6.5	0.6752	
K <sup>+</sup>	4	15.50	0.957	
K <sup>-</sup>	4	0.00	0.000	

mutans are listed in [table 1](#).

After collecting data, the data is tested for normality and homogeneity. The number of samples used is 28 which means less than 50, so we use the Shapiro-Wilk test in the normality test. The data is normal if the p value produced in the Shapiro-Wilk test is greater than 0.005 ( $p > 0.005$ ) and the data is homogeneous if the p value produced in the Levene test is less than 0.005 ( $p < 0.005$ ). In [table 2](#), based on the Shapiro-Wilk test results, the resulting p value is greater than 0.005 which means the data is normal.

In [table 3](#), the Levene test results show a significance value of 0.107, which means greater than 0.005. If the significance value is greater than 0.005, the data is homogeneous. Then the data were analyzed using the Oneway-Anova test to see whether there were significant differences in the diameter of the inhibition zone between the concentrations of 75%, 50%, 12.5%, 6.25% amoxicillin (K<sup>+</sup>) and DMSO (K<sup>-</sup>).

Oneway-Anova test results in [table 4](#) show  $p < 0.005$ , which means that there is inhibition of Binahong leaf extract against Streptococcus mutans. To find out which treatment groups have significant difference, an LSD test was performed. LSD test results can be seen in [table 5](#).

## Discussion

The test was conducted to determine the effectiveness of Binahong leaf extract against the growth of Streptococcus mutans in the oral cavity. This study uses the Kirby-Bauer method with four repetitions. Based on the results of using this paper disc method obtained inhibitory zones. The inhibition zone is the clear area or region that appears around the disc paper. The greater the diameter of the zone, the greater the antibacterial power.<sup>5</sup>

According to Davis and Stout, the criteria for potency of antibacterial are as follows, diameter of inhibition zone 5 mm or less is categorized as weak, diameter of inhibition zone 5-10 mm is categorized as medium, diameter of inhibition zone 10-20 mm is categorized strong and inhibitory zone is 20 mm or more is categorized as very strong.

The results showed that 75% concentration had an average inhibition of 11.3 mm, 50% concentration had an average inhibition of 10.4 mm, a concentration of 25% had an average inhibition of 8.7 mm, the concentration 12.5% has an average inhibition of 8.2 mm and 6.25% concentration has an average inhibition of 6.5 mm. The results of the study showed that the higher the concentration of the extract, the greater the zone of inhibition formed, which meant that it had a lot of antibacterial active ingredients.<sup>6</sup>

Inhibition zone diameters formed at concentrations of 75% and 50% are classified as strong that can be correlated to SPSS data analysis at concentrations of 75% and 50%, which found no significant differences. Although both are classified as strong, but the diameter of the inhibition zone formed at a concentration of 75% is greater which is 11.3 mm than the diameter formed at a concentration of 50% which is 10.4 mm. The value produced at a concentration of 50% is very close to the limits of the strength criteria of antibacterial power by Davis and Stout. It can be concluded that, from several concentrations of Binahong leaf extract which have been examined for the growth of streptococcus mutans, 75% concentration of Binahong leaf extract is more effective in inhibiting the growth of streptococcus mutans.

Phytochemical screening tests that have been carried out show that binahong (*Anredera cordifolia* (Ten.) Steenis) leaf extracts positively contain

**Table 5** Post hoc with LSD test of Binahong leaf extract inhibition zone diameter in inhibiting streptococcus mutans growth

No	Variable	N	Mean Difference	P Value
1.	75%	4	0.8750	0.066
	50%	4		
2.	75%	4	2.5750	0.000
	25%	4		
3.	75%	4	3.1000	0.000
	12.5%	4		
4.	75%	4	4.7500	0.000
	6.25%	4		
5.	75%	4	-4.2500	0.000
	K+	4		
6.	75%	4	5.3250	0.000
	K-	4		
7.	50%	4	1.7000	0.001
	25%	4		
8.	50%	4	2.2250	0.000
	12.5%	4		
9.	50%	4	3.8750	0.000
	6.25%	4		
10.	50%	4	-5.1250	0.000
	K+	4		
11.	50%	4	4.4500	0.000
	K-	4		
12.	25%	4	0.5250	0.258
	12.5%	4		
13.	25%	4	2.1750	0.000
	6.25%	4		
14.	25%	4	-6.8250	0.000
	K+	4		
15.	25%	4	2.7500	0.000
	K-	4		
16.	12.5%	4	1.6500	0.001
	6.25%	4		
17.	12.5%	4	-7.3500	0.000
	K+	4		
18.	12.5%	4	2.2250	0.000
	K-	4		
19.	6.25%	4	-9.9000	0.000
	K+	4		
20.	6.25%	4	0.5750	0.216
	K-	4		
21.	K+	4	9.5750	0.000
	K-	4		

alkaloids, flavonoids, saponins, tannins and steroids/triterpenoids. The secondary metabolite compounds inhibit the growth of streptococcus mutans by damaging the cell walls by flavonoids and phenols. Flavonoids inhibit the growth of streptococcus mutans by reacting with the streptococcus mutans protein which results in protein denaturation. Protein coagulation in the cell wall of streptococcus mutans results in bacterial cell membrane malfunction and increased osmotic pressure in the cell, causing the lysis of streptococcus mutans bacterial cells and die. Whereas phenol compounds damage bacterial cell walls by breaking the bonds of peptidoglycan. Unstable bacterial cell walls cause the function of selective permeability, active transport, and control of protein composition disrupted, so that bacterial cells become lost in shape and lysis.<sup>7</sup>

Saponin compounds have antibacterial action mechanisms that interfere with the permeability of bacterial cell membranes. Impaired membrane permeability causes it to be non-selective, so that all substances can enter and exit cells easily. This condition will damage the membrane and cause the release of various important components from the bacterial cell, namely proteins, nucleic acids, and nucleotides.<sup>8-10</sup>

After the cell membrane has been damaged by the activity of flavonoid compounds, phenols, and saponins, subsequently tannin penetrate into the cell nucleus to attack streptococcus mutans. Tannin compounds have a mechanism of coagulating and denaturing proteins. Tannins bind to proteins to form H<sup>+</sup> ions, causing the pH to become acidic so that protein is denatured. Acidic conditions also activate enzymes in bacteria and cause metabolism to be disrupted, so cells will be damaged or even die. Bacterial cells also cannot be formed again because tannins inhibit the DNA.<sup>11</sup>

In addition there are terpenoids and alkaloids. The terpenoid compounds action mechanism is by damaging the outer membrane, inner membrane and can also interact with membrane proteins with the help of alkaloids by inhibiting the formation of enzymes (esterase, DNA-, RNA-polymerase), inhibiting cell respiration.<sup>12</sup>

## Conclusion

The best treatment group on the effectiveness of binahong (*Anredera cordifolia* (Ten.) Steenis) extract as antibacterial against streptococcus mutans are at a concentration of 75% with an average inhibition diameter of 11.3 mm. Amoxicillin which is used as a positive control

produces an average inhibition diameter of 15.5 mm. The average inhibition diameter owned by amoxicillin is quite strong. The best treatment of binahong leaf extract (*anredera cordifolia* (Ten.) Steenis) when compared to positive control treatments, still cannot exceed the antibacterial activity possessed by amoxicillin but can be an alternative treatment.

### Acknowledgment

Thank you for the support Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara.

### Conflict of Interest

The authors report no conflict of interest.

### References

- Rukmana, Rahmat H. Farm Bigbook-Cultivation & post-harvest of superior medicinal plants. ed 1. Lily Publisher; Yogyakarta: 2006. p. 2- 3, 16, 26- 7, 40- 2. (In Indonesia)
- Rahmawati F, Bintari SH. Study of the antibacterial activity of binahong leaf extract (*anredera cordifolia*) on the growth of *bacillus cereus* and *salmonella enteritidis*. *Unnes J Life* 2014;3: 105. (In Indonesia)
- Zenia AU, Purwanti N, Wahyudi IA. Effect of lime peel extract (*Citrus aurantifolia* swingle) concentration of 10% on the activity of the enzyme glucosyltransferase *strep-tococcus mutans*. *Maj Ked Gi* 2013;20: 130. (In Indonesia)
- Ainurrochmah A, Ratnasari E, Lisdiana L. The effectiveness of Binahong leaf extract (*Anredera cordifolia*) against the growth inhibition of *shigella flexneri* bacteria by using the Sumuran method. *LenteraBio* 2013;2: 236. (In Indonesia)
- Sanders ER. Aseptic laboratory techniques: Plating methods. *J Vis Exp* 2012;63: 1- 3.
- Dwitiyanti, Harahap Y, Elya B, et al. Impact of solvent on the characteristics of standardized Binahong leaf (*anredera cordifolia* (Ten.) steenis). *Pharmacog J* 2019;11: 1464- 1468.
- Tiwari P, Bimlesh K, Mandeep K, et al. Phytochemical screening and extraction: A review. *Int Pharmaceutic Sci* 2011;1: 98- 100.
- Ying LY, Hernawan I, Hendarti HT. Inhibition of binahong leaf extract (*anredera cordifolia* (Ten.) Steenis) against polybacteria in recurrent aphthous stomatitis (SAR). *Oral Med Dent J* 2011;3: 19. (In Indonesia)
- Suhendra CP, Widarta IWR, Wiadnyani. The effect of ethanol concentration on the antioxidant activity of grass roots rhizome extract (*imperata cylindrica* (L) beav) in extraction using ultrasonic waves. *J Ilmu Teknologi Pangan* 2019;28: 30- 31. (In Indonesia)
- Wardhani LK, Sulistyani N. Antibacterial activity test of ethyl acetate extract of binahong leaves (*anredera cordifolia* (L.) steenis) against *shigella flexneri* along with thin layer chromatography profile. *J Ilmiah Kefarmasian* 2012;2: 3. (In Indonesia)
- Hanafiah OA, Hanadiah DS, Bayu ES, et al. Quantity differences of secondary metabolites (saponins, tannins and flavonoids) from binahong plant extract (*anredera cordifolia* (Ten.) steenis) treated and untreated with cplchicines that play a role in wound healing. *World J Dent* 2017;8: 296- 299.
- Rahmawati F, Bintang M, Artika IM. Antibacterial activity and phytochemical analysis of geranium *homeanum turez* leaves. *Curr. Biochem* 2017;4: 19- 21.



This work is licensed under a Creative Commons Attribution