

# A Review of Bandotan Leaf Extract (*Ageratum conyzoides* L.) in Inhibition Test to the Growth of Bacteria (*Porphyromonas* *gingivalis*) Case of Periodontitis Disease

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## A Review of Bandotan Leaf Extract (*Ageratum conyzoides* L.) in Inhibition Test to the Growth of Bacteria (*Porphyromonas gingivalis*) Case of Periodontitis Disease

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### ABSTRACT

**Introduction:** Periodontitis is defined as an inflammatory disease in the supporting tissues of teeth which is caused by microorganisms of certain groups.

**Objective:** This study aims to know the inhibition power of bandotan leaf extract (*Ageratum conyzoides* L.) against the growth of bacteria causing periodontitis (*Porphyromonas gingivalis*).

**Method:** The type of study used in this research is experimental laboratory. The design of this study used is post test only control group design used the method of dilution and disk-diffusion method. **Results:** The activities of bacteria can only be observed with the method of diffusion to see the inhibition zone. An extract of bandotan leaf (*Ageratum conyzoides* L.) with the concentration of 100% has the largest inhibition zone namely 16,25±0,66 mm and the inhibition zone is larger than control positive namely metrodinazole used as a comparison antibiotic from the extract of bandotan leaf (*Ageratum*

*conyzoides* L.). This research has proven that the extract of bandotan leaf extract (*Ageratum conyzoides* L.) are effective as inhibitory properties against *Porphyromonas gingivalis*.

**Conclusion:** The extract of bandotan leaf (*Ageratum conyzoides* L.) is effective as the inhibition power against *Porphyromonas gingivalis*.

**Keywords:** *Ageratum conyzoides* L, *Porphyromonas gingivalis*, Periodontitis

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### INTRODUCTION

Dental health disease is the sixth highest health problem often complained by Indonesian people.<sup>1</sup>

Periodontal disease is one of the dental and oral diseases that develops very commonly, and affects about 10.5% to 12% of the world population.<sup>2</sup> In Indonesia periodontal disease has a fairly high prevalence suffered by humans almost all over the world and reaches 50% of the adult population.<sup>3</sup>

Periodontal disease is an inflammation that occurs in the supporting tissues of teeth, including alveolar bone and periodontal ligaments. Periodontal disease often found is gum inflammation or gingivitis and periodontitis.<sup>4</sup>

Periodontitis is defined as an inflammatory disease in the supporting tissues of the teeth caused by specific microorganisms of certain groups, which results in progressive damage of the periodontal ligament and alveolar bone by increasing the formation of probing depths, recessions, or both.<sup>2</sup>

A number of experimental evidence has shown that the primary etiological agent of periodontal disease is a generally Gram-negative stem bacterium which includes *P.gingivalis*. The main habitat of *P. gingivalis* is the subgingival sulcus of the human oral cavity. These periodontopathic bacteria are found in 85.75% of subgingival plaque samples from patients with chronic periodontitis. Oral bacteria and especially pathogenic bacteria, such as

*Porphyromonas gingivalis* have great virulence factors, one of which is the ability to penetrate hard intraoral surfaces.<sup>5</sup>

Efforts to overcome these problems, usually use the concept of antimicrobial treatment. Antibiotics are a group of antimicrobial agents, which also consist of antiviral, antifungal, and antiparasitic chemicals. Antibiotics can kill or suppress live bacteria but they cannot eliminate bacterial calculus and residues, which are traditionally considered an important part of periodontal therapy.<sup>6</sup>

When used appropriately, antibiotics provide unquestionable benefits. However, if used or prescribed incorrectly (irrational prescribing) then it can cause extensive losses in terms of health, economy and even for future generations. The emergence of pathogenic germs that are resistant to one antimicrobial resistance or certain types of antibiotics (multiple drug resistance) greatly complicates the treatment process.<sup>7</sup> The effort to find alternative materials that have no side effects is a solution to the above problems.

The correct alternative material to be the solution to the problem is herbal. Bandotan plants (*Ageratum conyzoides* L.) in Indonesia are classified as weeds so they are often destroyed. But some groups of our people use this plant as traditional medicine to cure various diseases.<sup>8</sup> Some of the uses of this plant are as a drug for new wounds, bleeding wounds, ulcers, eczema, and treating diseases caused by bacterial infections.

Bandotan leaves and flowers (*Ageratum conyzoides* L.) contain saponins, flavonoids, polyphenols, and essential oils. Phenol compounds are generally known as disinfectants used to kill pathogenic microorganisms. Polyphenol compounds have been shown to have antibacterial activity.<sup>9</sup> Phytochemical analysis of *Ageratum conyzoides* L. which has been done by Amadi et al shows that the main compounds in *Ageratum conyzoides* L. plants, namely alkaloids and flavonoids, accumulate on the leaves.<sup>10</sup> The usefulness and efficacy that can be obtained from this plant is very much, but so far there is still little research on Bandotan plants. Therefore this study aims to know the inhibitory test on the extract of bandotan plant (*Ageratum conyzoides* L.) on the growth of bacteria that cause periodontitis (*Porphyromonas gingivalis*).

### MATERIALS AND METHOD

This type of research used in this study is an experimental laboratory research. The research design used is post test only control group design using the dilution method and disk-diffusion method. This research was conducted at the Organic Chemistry Laboratory, Chemical Engineering Department, Ujung Pandang State Polytechnic and the Microbiology Laboratory Faculty of Medicine, Hasanuddin University. The study was conducted in March 2020.

There are three variables in this study, the independent variables are the leaves of the bandotan plant (*Ageratum Conyzoides* L.) starting from a concentration of 15%, 25%, 50%, 75%, 100%. and the dependent variable was the Minimum Inhibitory Concentration (MIC) and the inhibition zone of bacterial growth of *Porphyromonas gingivalis*. Control variables were time,

culture medium, and temperature. The research sample was *Porphyromonas gingivalis* bacteria that have been bred and the leaves of bandotan plant (*Ageratum Conyzoides* L.) that have been extracted by maceration method.

Antibacterial activity testing was done by disk-diffusion method or Kirby-Bauer diffusion method using discs. On the media which was dense then spread the bacteria as much as 0.02 mL, which has been adjusted to the 0.5 McFarland standards evenly using a spreader bar. The first MHA media was divided into seven sections, each of which was placed a disc containing bandotan leaf extract, metronidazole, and negative controls.

The treatment was carried out up to four times. Then incubated at 37°C for 1x24 hours and observed bacterial growth. The length of the inhibition zone formed was measured using calipers in millimeters. The data obtained were analyzed descriptively and displayed in tables and figures. Analysis of the results of research data was carried out with the One Way Anova test using the Statistical Package for the Social Sciences application (SPSS).

### Tools and Materials

Analytical scales, Test tubes, Test tube racks, Glass jars, Rotary evaporators, Petri dishes, Micropipettes, Paper disks, Caliper, Measuring cups, Incubators, Autoclaves, Tweezers, Bunsen, Filter paper, Erlenmeyer flasks, 1 ml spoit, Paper label, Funnel, Handscoon and mask, Vial bottle, GM Towel Bacterial isolates of *Porphyromonas gingivalis*, Bandotan leaves, Metronidazole, Medium Muller Hinton, Agar (MHA), Brain Heart Infusion Broth (BHIB) Media, Methylated spirit, 96% Ethanol, Aluminum foil, Sterile Aquades.

### RESULTS

**Table 1:** Results of Measurement of *Porphyromonas gingivalis* Bacterial Inhibition Zone Diameters

Type of Intervention	Concentration (%)	Inhibition (mm)				Average
		I	II	III	IV	
Leaves Extract Bandotan plants ( <i>Ageratum conyzoides</i> L.)	15	7,7	8,7	9,8	8,2	8,60±0,89
	25	9,5	9,8	10,9	12,6	10,70±1,40
	50	13,3	12,2	13,9	13,1	13,12±0,70
	75	14,3	14,2	14,9	15,3	14,67±0,51
	100	16,7	16,7	15,3	16,3	16,25±0,66
Control (+) Metronidazole		13,2	13,8	13,7	15,3	14,00±0,90
Control (-) Aquades		6,2	6,2	6,2	6,3	6,20±0,08

From Table 1. it can be seen descriptively that the extract of the bandotan plant leaf (*Ageratum conyzoides* L.) concentration of 15% has the lowest average inhibition zone of 8.60 ± 0.89 mm when compared to the entire concentration of the bandotan plant leaf extract (*Ageratum*

*conyzoides* L.). The inhibition zone at 100% concentration of bandotan leaves plant extract (*Ageratum conyzoides* L.) had the largest inhibition zone of 16.25 ± 0.66 mm.

**Table 2:** Test Results of Inhibition Zone Statistics of *Porphyromonas gingivalis* Bacteria

Type of Intervention	Concentration	N	Normality test*	Comparison test**
Leaves Extract	15	4	0.796	
Plants	25	4	0.467	
Bandotan	50	4	0.846	
( <i>Ageratum conyzoides L.</i> )	75	4	0.467	
	100	4	0.117	0.000
Control (+)Metronidazole		4	0.284	
Control (-) Aquades		4	0.683	

\*. The mean difference is significant at the 0.05 level.

Based on Shaphiro-Wilk statistical test results to know the normality value obtained  $p > 0.05$  which means the data was normally distributed thus the test was continued with the parametric test namely OnewayAnova (Table 2).

Based on the One-way Anova statistical test it was found that the significance value was 0,000 ( $p < 0.05$ ) which means that there are significant differences between treatment groups.

**Table 3:** Results of Post Hoc LSD (Least Significant Difference) Inhibition Zone of Porphyromonasgingivalis Bacteria (15%, 25% and 50%)

TreatmentGroup (I)	Comparison(J)	Mean Difference (I-J)	p-value
Concentration 15%	25%	-2.10	0.002
	50%	-4.52	0.000
	75%	-6.07	0.000
	100%	-7.65	0.000
	control+	-5.40	0.000
	control-	2.40	0.001
Concentration 25%	15%	2.10	0.002
	50%	-2.42	0.000
	75%	-3.97	0.000
	100%	-5.55	0.000
	control+	-3.30	0.000
	control-	4.50	0.000
Concentration 50%	15%	4.52	0.000
	25%	2.42	0.000
	75%	-1.55	0.015
	100%	-3.12	0.000
	control+	-0.87	0.150
	control-	6.92	0.000

\*. The mean difference is significant at the 0.05 level.

At a concentration of 15% the extracts of bandotan leaves plant (*Ageratum conyzoides L.*) when compared with the extracts of bandotan leaves plant (*Ageratum conyzoides L.*) the concentrations of 25%, 50%, 75%, 100%, positive and negative controls had  $p < 0, 05$  which means there are significant differences or have different effects.

At a concentration of 25% the extracts of bandotan leaves plant (*Ageratum conyzoides L.*) when compared with the extracts of bandotan leaves plant (*Ageratum conyzoides L.*) concentration of 15%, 50%, 75%, 100%, positive and

negative controls had a value of  $p < 0, 05$  which means there are significant differences or have different effects.

At a concentration of 50% the extracts of bandotan leaves plant (*Ageratum conyzoides L.*) when compared with the extracts of bandotan leaves plant (*Ageratum conyzoides L.*)

75% concentration had a  $p = 0.015 > 0.05$  and on a positive control that had a  $p \text{ value} = 0.150 > 0.05$  which means that all three have no significant difference or have the same effect. When compared to 15%, 25%, 100%, and negative controls with the value of  $p < 0.05$  which means there are significant differences or have different effects.

**Table 4:** Results of Post Hoc LSD (Least Significant Difference) Inhibition Zone of Porphyromonasgingivalis Bacteria (75% and 100%)

Treatment Group (I)	Comparison (J)	Mean Difference (I-J)	p-value
Concentration 75%	15%	6.07	0.000
	25%	3.97	0.000
	50%	1.55	0.015
	100%	-1.57	0.014
	control+	0.67	0.262
	control-	8.47	0.000
Concentration 100%	15%	7.65	0.000
	25%	5.55	0.000
	50%	3.12	0.000
	75%	1.57	0.014
	control+	2.25	0.001
	control-	10.05	0.000

\*. The mean difference is significant at the 0.05 level.

At a concentration of 75% the extracts of bandotan leaves plant (*Ageratum conyzoides* L.) when compared to the extracts of bandotan leaves plant (*Ageratum conyzoides* L.) concentration of 50% had a value of  $p = 0.015 > 0.05$ , 100% concentration had a value of  $p = 0.014 > 0.05$ , and positive control had a value of  $p = 0.262 > 0.05$  which means that all four have no significant difference or have the same effect. When compared with 15%, 25%, and negative controls with  $p < 0.05$  which means there are significant differences or have different effects.

At a concentration of 100% the extracts of bandotan leaves plant (*Ageratum conyzoides* L.) when compared with the extracts of bandotan leaves plant (*Ageratum conyzoides* L.) 75% concentration had a p value =  $0.014 > 0.05$ , which means that there are no significant differences or have the same significant effect. When compared to 15%, 25%, 50%, positive and negative controls had a p value  $< 0.05$  which means there are significant differences or have different effects.

**Table 5:** Results of Post Hoc LSD (Least Significant Difference) Inhibition Zone of *Porphyromonas gingivalis* Bacteria (Positive Control and Negative Control)

Treatment Group (I)	Comparison (J)	Mean Difference (I-J)	p-value
Control (+) Metronidazole	15%	5.40	0.000
	25%	3.30	0.000
	50%	0.87	0.150
	75%	-0.67	0.262
	100%	-2.25	0.001
	control-	7.80	0.000
Control (-) Aquades	15%	-2.40	0.001
	25%	-4.50	0.000
	50%	-6.92	0.000
	75%	-8.47	0.000
	100%	-10.05	0.000
	control+	-7.80	0.000

\*. The mean difference is significant at the 0.05 level.

In the positive control of metronidazole when compared with the extract of bandotan leaves plant (*Ageratum conyzoides* L.) the concentration of 50% had a value of  $p = 0.015 > 0.05$ , a concentration of 75% had a value of  $p = 0.262 > 0.05$ , which means that there are no significant differences or have the same effect. When compared with 15%, 25%, 100% and negative controls that had a  $p < 0.05$  which means there are significant differences or have different effects.

In negative control aquades when compared with concentrations of 15%, 25%, 50%, 75%, 100%, positive control and negative control had a  $p < 0.05$  which means there are significant differences or have different effects. Based on the results of the Post Hoc LSD test the inhibition zone between treatment groups on the average *Porphyromonas gingivalis* bacteria showed a significant value ( $p < 0.05$ ).

## DISCUSSION

In the study of inhibition zone test using the extracts of bandotan leaves plant (*Ageratum conyzoides* L.) on the growth of *Porphyromonas gingivalis*, it was seen that the inhibition zone formed increased in proportion to the increasing concentration of the extract. *Ageratum conyzoides* L. has many useful phytochemical compounds. It contains saponins, flavonoids, polyphenols, and essential oils.<sup>11,12,13</sup>

Phenol compounds are generally known as disinfectants used to kill pathogenic microorganisms. Flavonoids are the largest phenol compounds in nature and have been known to have biological activities as antioxidants, antimelanogenesis and antimutagenesis. Polyphenol compounds have been shown to have antibacterial activity. In addition, the leaves also contain essential oils and there are also coumarin.<sup>14,15</sup>

*Ageratum conyzoides* L. is known to have a good antibacterial effect. Antibacterial activity was demonstrated by the AC-1 component isolated from the leaves of *Ageratum conyzoides* L. In Mitra P's study, the AC-1 component of the bandotan plant showed an antibacterial effect on 4 types of gram-negative bacteria and 4 types of gram-positive bacteria. Based on research by Odeleye, et al., Ethanol extract of *Ageratum conyzoides* L. has potential antibacterial effects for use in medication.<sup>11,16,17</sup>

According to research conducted by Kamboj, antibacterial activity of aqueous extract were tested against three Gram-positive bacteria and seven Gram-negative bacteria and evaluated by the Piter paper disc diffusion method. Results showed a significant control of the growth of *A. viscolactis*, *K. aerogenes*, *B. cereus* and *S. pyogenes*. In an investigation for in vitro anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity found that MIC range of 55.4-71.0 mcg/ml were recorded for ethanol and water extracts of AC. The concentrations were too high to be considered active and AC were found to be ineffective in vitro in the study; therefore, suggest the immediate stoppage of their traditional use against MRSA-associated diseases in Lagos, Nigeria.<sup>18,19</sup>

According to research conducted by Budiman, the results show that both *Ageratum conyzoides* L. and *Piper Betle* L. extracts have antibacterial activity against *Staphylococcus aureus* with MIC value of 2 % and 5 %, respectively. Then, the gel containing 4 % sodium CMC showed the best physical stability, either containing *Ageratum conyzoides* L. or *Piper Betle* L. extract. The gel dosage forms of both extracts did not show any difference in organoleptic properties, pH and viscosity after 28 days storage. The gel dosage forms of *Ageratum conyzoides* L. and *Piper Betle* L. extracts have antibacterial activity with inhibition zone of 20.3 mm  $\pm$  1.3mm and 15.21  $\pm$  1.3 mm, respectively. The antibacterial activity of *Ageratum conyzoides* L. extract was higher compared to that of *Piper Betle* L. extract in the gel dosage form.<sup>20,21</sup>

According to research conducted by Garg, the results obtained from this study indicated that *A. conyzoides* shows potential antibacterials to againts five human pathogenic bacteria i.e gram + (*Bacillus subtilis*,

*Staphylococcus aureus*) and gram - (*Escherichia coli*, *Klebsiellapneumoniae* and *Pseudomonas aeruginosa*).<sup>22,23,24</sup> Thus, bacterial activity can only be observed in diffusion method to see inhibition zones. Bandotan leaves plant extract (*Ageratum conyzoides* L.) at a concentration of 100% had the greatest inhibition zone of 16.25  $\pm$  0.66 mm and the inhibition zone was greater than the positive control of metrodinazole which was used as a comparative antibiotic from the extracts of bandotan leaves plant (*Ageratum conyzoides* L.). This research has proven that the extracts of bandotan leaves plant (*Ageratum conyzoides* L.) are effective as an inhibitory power against *Porphyromonas gingivalis*. Thus the research hypothesis was accepted.

## CONCLUSION

1. The extracts of Bandotan leaves plant (*Ageratum conyzoides* L.) are effective as an inhibitory against *Porphyromonas gingivalis*.
2. The extracts of Bandotan leaves plant (*Ageratum conyzoides* L.) at a concentration of 15% has the smallest average inhibition zone as much as 8.60  $\pm$  0.89 mm when compared to the entire concentration of bandotan leaves plant extract (*Ageratum conyzoides* L.). The inhibition zone at the concentration of 100% of the extracts of bandotan leaves plant (*Ageratum conyzoides* L.) has the biggest inhibition zone of 16.25  $\pm$  0.66 mm.
3. Minimal Inhibitory Concentration (MIC) of each of the extracts of bandotan leaves plant (*Ageratum conyzoides* L.) cannot be determined because the color of the extract is too concentrated thus it cannot be clearly seen the difference between clear and turbid tubes.

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