



Sensitivity Test of Bandotan Leaf Extract (*Ageratum conyzoides*) Against *Pseudomonas aeruginosa* Bacteria

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Abstract

The leaves of Bandotan (*Ageratum conyzoides*) are a plant thought to have antibacterial properties. This study aims to determine the sensitivity of Bandotan leaf extract in inhibiting the growth of the bacteria *Pseudomonas aeruginosa*. This study used a stock extract of Bandotan leaves from the Pharmacology Laboratory and a bacterial isolate of *P. aeruginosa* in the Microbiology Laboratory of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, which was identified by Gram staining, indole test, Methyl Red test, and confectionery test. The research method was carried out by planting the re-identified bacterial isolates on Nutrient Broth (NB) media, incubated at 37°C for 24 hours. Then the turbidity composition of the isolates was arranged to match the turbidity in 0.5 McFarland solution. Furthermore, the sensitivity test of the extract on Mueller Hinton Agar (MHA) media was carried out by levelling the bacterial isolates on the surface of the media and attaching a disc containing bandotan leaf extract with a concentration of 25%, 50%, 75% and gentamicin disk as a positive control and distilled water as a negative control. All treatments were incubated at 37°C for 24 hours, and then the inhibition zone was measured using millimeters (mm) callipers. The results showed that concentrations of 25%, 50% and 75%, respectively, had an inhibition zone of 8.16 mm, 9.82 mm, and 16.08 mm, respectively. In contrast, the average inhibition zone for gentamicin was 25, 30 mm and 0 mm distilled water. Therefore, it can be concluded that the Bandotan leaf extract is sensitive to growth inhibition of *P. aeruginosa* bacteria.

Keywords: Bandotan leaves; *Ageratum conyzoides*; *Pseudomonas aeruginosa*; antibacterial

Background

Pseudomonas aeruginosa is a normal floral bacterium that is abundant in nature and is usually located in humid environments, healthy animals and humans. This bacterium is saprophytic, but it can become pathogenic if it is on a host that has abnormal immune system. *P. aeruginosa* causes several infectious diseases, namely dermatitis, otitis externa, folliculitis, eye infections, as well as infections in burns. In addition, *P. aeruginosa* also causes infections of the lower respiratory tract, in the urinary tract and other organs (Milanda *et al.*, 2014). According to the research of Radji (2011) the infection rate of *P. aeruginosa* has exceeded 30% of all bacterial infections.

The treatment of infectious diseases caused by *P. aeruginosa* has been using

antibiotics such as ampicillin, erythromycin, amoxicillin, gentamicin, tetracycline, trimetoprim, tobramycin, cephadroxy, sefuroksim, ceftriaxone, piperacillin, trimetoprim, tobramycin, co-hypedoxazole, nalidical, sulfonamide, penicillin and chloramphenicol. This antibiotic has a bacteriostatic effect and can inhibit protein synthesis by interfering with the formation of amino-tRNA complexes by the ribosome subunit 50S. However, many cases have recently been reported that *P. aeruginosa* is resistant to chloramphenicol, so it needs to be considered for the treatment of bacterial infections (Rukmono and Zuraida, 2013; Hilda and Berliana, 2015).

The presence of *P. aeruginosa* resistance to antibiotics reported by several previous researchers, is considered necessary to find alternative drugs that can

inhibit the growth of these bacteria. One alternative medicine that is suspected to have the potential to inhibit the growth of *P. aeruginosa* bacteria is bandotan leaves (*Ageratum conyzoides*). Traditionally this bandotan plant is used to treat various diseases such as treating ear inflammation, relieving pain, abdominal pain, diarrhea, wound healing and can also function as an antimicrobial / antibacterial with the compounds contained in it. Lestari (2018) reported that bandotan leaves are one of the potential medicinal plants in Indonesia. Bandotan leaves are reported to contain flavonoid chemical compounds, trepenoids, steroids, alkaloids and tannins. Chemical compounds contained in bandotan leaves have antibacterial abilities including flavonoids, alkaloids, steroids and tannins (Munira *et al.*, 2020).

Based on reports that say *P. aeruginosa* bacteria in the environment can cause infections in animals and humans and have been resistant to several antibiotics in their handling, it is considered necessary to conduct sensitivity tests on types of medicinal plants derived from bandotan leaves as an effort to find alternative drugs in inhibiting the growth of *P. aeruginosa* bacteria. This study aims to determine the sensitivity of bandotan leaf extract (*Ageratum Conyzoides*) in inhibiting the growth of *P. aeruginosa* bacteria.

Materials And Methods

Rejuvenation of Sample Bacterial Isolate Stock

The stock of bacteria stored in oblique NA is refreshed by replanting the bacteria into NB and incubated at 37°C for 24 hours. Furthermore, bacteria from NB are carried out simple re-identification by doing Gram staining.

Sample Preparation and Stock Manufacturing of Bandotan Leaf Ethanol Extract (*Ageratum conyzoides*)

Bandotan leaves are taken in the Kajhu area of Baitussalam district, Banda Aceh. As a sample of leaves taken has the criteria of leaves that have grown perfectly with the shape of the leaves have been wide open the making of the extract is carried out

by a process, among others, the bandotan leaves are cleaned and dredged, then weighed as much as ± 5 kg, then mashed using a blender. Samples that have been finely soaked with 96% ethanol 3 times in 24 hours. After that, the extract is filtered so that the filtrate is obtained, then the filtrate is evaporated using a *vacuum rotary evaporator* so that a viscous extract is obtained. The viscous extract is further laid out in the open air so that a dry extract is obtained. Furthermore, for the test, the extract was dissolved with aquades solvent and made in several concentrations namely 25%, 50% and 75%.

Gram Staining

Gram staining is done by making preparations using physiological NaCl on sterile and dry glass objects, then fixed on a spiritus lamp. Next, the preparation is dripped with a violet crystalline dye and let stand 1 minute, remove the dye and then wash it under running water. After which the preparation is dripped with lugol and leave for 1 minute, then washed under running water. Next, fade the dye by dripped 96% alcohol for 10 seconds, then wash it under running water. After that, the preparation is dripped safranin dye wait for 2 minutes, then wash it under running water until the dye disappears. The preparation is dried and then dripped with emersi oil and observed under a microscope with a magnification of 1000x.

Biochemical Tests

Bacteria from Gram staining are carried out biochemical tests which include indol tests, *Methyl red* (MR) tests, and confectionery tests (glucose, lactose, sucrose, mannitol and maltose) to see the metabolic ability of bacteria in the form of chemical reactions caused by each medium used as a property of *P. aeruginosa* bacteria.

Preparation of The Test Bacterial Suspension Based on Mcfarland Standards

The re-identified bacteria were planted in NB media and incubated at 37°C for 24 hours. Furthermore, its turbidity is compared with using the *McFarland* 0.5 standard with a bright light on a black

background. This *McFarland* turbidity standard is made with a 1% BaCl₂ solution of 0.5 mL plus H₂SO₄ 1% as much as 9.5 mL, then homogenized. *McFarland* 0.5 solution is a reference that can be used to adjust to the turbidity of suspension bacteria so that the number of bacteria is within the range given to standardize microbial testing (Noverita *et al.*, 2009; Toy *et al.*, 2015 and Rosmania and Yanti, 2020).

Sensitivity Test Against *Pseudomonas aeruginosa* using MHA Media

This study is a laboratory experimental study using ethanol extract of bandotan leaves and *P. aeruginosa* bacteria. Determination of antibacterial sensitivity is carried out using the *Kirby-Bauer* diffusion method. The materials used include aquades as a negative control, gentamicin disk as a positive control and a test solution of bandotan leaf extract that has been made in several concentrations, namely 25%, 50% and 75%. The test solution was prepared by dissolving ethanol extract of bandotan leaves with aquades concentrations of 25%, 50%, and 75%, respectively. Suspension of *P. aeruginosa* bacteria with a *McFarland* concentration of 0.5 (1x10⁸ CFU/mL) in the swab evenly on the surface of the MHA media, then attached a *paper disk* dripped 20 µL of extract with various concentrations on top of the medium. Furthermore, for negative control, namely aquades, a blank disk was attached and then dripped with aquades 20 µL and positive control, namely gentamicin, was carried out by attaching a gentamicin disk to the MHA media, then the MHA media was incubated at a temperature of 37°C for 24 hours. Each was repeated 3 times in an MHA medium with the same treatment and subsequently observed inhibitory power by measuring the area of the inhibitory zone formed around the *perimeter* of the disk using a caliper (Brooks *et al.*, 2013). Furthermore, the data is analyzed descriptively.

Results

Eight preterm neonates (34 weeks) were removed from the study because of missing data, severe cardiac congenital abnormalities, RDS followed by sepsis, and

meconium aspiration syndrome. Three groups of 142 preterm neonates made up the target population (extremely preterm, very preterm, and moderate preterm). Thirty-three of neonatal mothers (23.2%) had preeclampsia. All demographic data are shown in Table 1.

Results and Discussion

Re-identification of *Pseudomonas aeruginosa*

The results of observations of bacteria with Gram staining show a pink color as a characteristic of Gram-negative bacteria and rod-shaped bacteria. Gram staining results are presented in Figure 1.

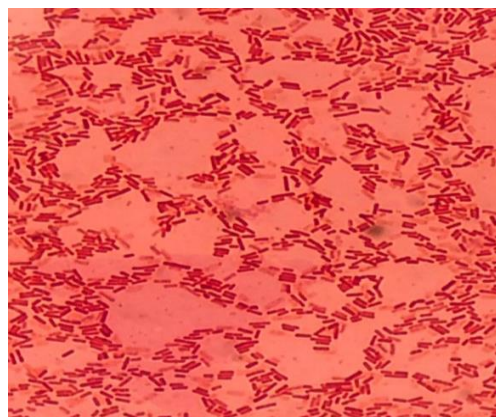


Figure 1. Reidentification by Gram staining of *P. aeruginosa* bacteria

The result of Gram staining is known that the bacteria are gram-negative rod-shaped and pink in color. Gram-negative bacteria are pink because they absorb the dye safranin, this is because gram-negative bacteria have a thin peptidoglycan layer, and have high permeability, so that the complex of violet crystal dyes that have entered the cell wall during the initial step in the female process can be extracted after staining with alcohol (Brooks *et al.*, 2005).

Biochemical test observations show that the Indol Test (-), Mr Test (-) and glucose (+), sucrose (-), lactose (-) and mannitol (-), this is due to the fact that *P. aeruginosa* bacteria have the characteristics of being motile, non-fermentative as well as the ability of bacteria to utilize sugars for oxidase metabolism with oxygen which acts as an electron acceptor terminal. Biochemical test instruments are presented in Table 1.

Table 1. Results of biochemical tests on *P. aeruginosa* bacteria

Bacterial name	Indol test	Methyl Red (MR) Test	Confectionery Test			
			Glucose	Sucrose	Lactose	Mannitol
<i>P. aeruginosa</i>	(-)	(-)	(+)	(-)	(-)	(+/-)

Based on Figure 1 and Table 1, it can be seen that the stock bacteria used in this study are gram-negative bacteria from the species *P. aeruginosa*. This bacterium uses glucose in forming acids, does not utilize lactose and maltose, decomposes nitrites into nitrates which are then broken down into gaseous nitrogen, these bacteria are also unable to react to indole and methyl red (Bengi *et al.*, 2017).

The results of biochemical test observations on sample bacteria showed a negative indol reaction because there was no formation of a red ring on the media after being given Kovacs reagent, this is because the bacteria do not have the enzyme tryptophanase which can degrade amino acids tryptophan which will produce indol products (Rapi *et al.*, 2017; Rahayu and Gumilar, 2017). On the methyl red test showed a negative result, characterized by a red methyl indicator turning yellow. This happens because *P. aeruginosa* bacteria cannot ferment glucose and produce acidic products so that the pH of the growth medium becomes lower (Rapi *et al.*, 2017). Furthermore, in the confectionery test, only glucose media undergo acid formation which is characterized by a change in the color of the media from blue to yellow, meaning that these bacteria form acid from glucose fermentation (Fardiaz, 2002).

The Results Of The Sensitivity Test of Bandotan Leaf Extract (*Ageratum conyzoides*) Against The Bacteria *Pseudomonas aeruginosa* *in vitro*

The results of the sensitivity test of bandotan leaf extract in inhibiting the growth of *P. aeruginosa* bacteria with concentrations of 25%, 50% and 75% respectively can be seen in Figure 2.

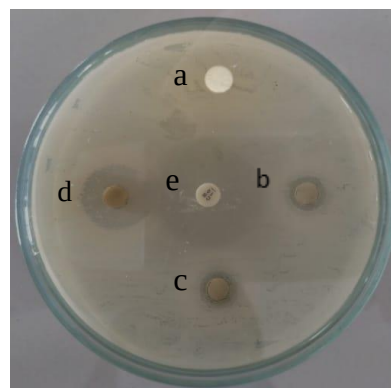


Figure 2. Inhibition zone of bandotan leaf extract (*Ageratum conyzoides*) against the growth of *P. aeruginosa* bacteria, concentration a) aquades, b) 25%, c) 50%, d) 75% and e) gentamicin

Antimicrobial inhibitory activity tested positive based on the resulting clear zone around the disc paper. The diameter of the bacterial growth inhibition zone is measured in mm units (Brooks *et al.*, 2013). The results of the inhibition zone measurement of all disks at the concentration of bandotan leaf ethanol extract as well as positive and negative controls that had been incubated at a temperature of 37 °C for 24 hours were presented in Table 2.

Table 2. Results of *in vitro* test of bandotan leaf ethanol extract (*Ageratum conyzoides*) against *P. aeruginosa* bacteria *in vitro*

Block zone diameter (mm)		
Dilution		X $\pm$ SD
a.	Control (-) aquades	0 $\pm$ 0
b.	Concentration 25%	8.16 $\pm$ 0.29
c.	Concentration 50%	9.82 $\pm$ 0.54
d.	Concentration 75%	16.08 $\pm$ 0.13
e.	Control (+) gentamicin	25.30 $\pm$ 0.43

Bacterial inhibition zones formed according to Davis and Stout (1971) cited Lingga *et al.* (2015) is divided into several criteria including ≤ 5 mm has weak inhibitory activity, 5-10 mm medium, 10-20 mm strong, and ≥ 20 mm very strong. From Table 2, it can be explained that the criteria for the inhibitory activity of bandotan leaf extract formed at a concentration of 25% is 8.16 mm and a concentration of 50% of 9.82 mm is considered to have moderate

inhibitory activity because the inhibitory zone formed is between 5-10 mm. Furthermore, at a concentration of 75% the results of the bacterial inhibition zone with a diameter of 16.08 mm are considered to have a strong inhibitory activity because the inhibitory zone formed is in the range of 10-20 mm, while for gentamicin as a positive control, it has an inhibitory zone diameter of 25.30 mm which means it has a very strong inhibitory activity because the inhibition zone is ≥ 20 mm.

In Table 2, aquades as a negative control showed no antimicrobial effect on the growth of *P. aeruginosa* bacteria. The formed inhibition zone is 0 mm, which is indicated by the invisibility of the bright zone around the disk that has been dripped by aquades. Negative control functioned to show that the solvent of the test extract used did not have the effect of inhibiting the growth of bacteria so that the antibacterial activity produced was purely derived from the extract tested.

In the positive control, namely the antibiotic gentamicin, it appeared that there was an inhibitory zone with a diameter of 25.30 mm (very strong). The extent of the inhibition zone formed on the gentamicin disk is due to gentamicin being included in the class of aminoglycoside antibiotic drugs, where the single active substance is able to work by killing while preventing the growth of bacteria (Kristiani *et al.*, 2015). The mechanism of action of this gentamicin antibiotic is in inhibiting protein synthesis in bacteria by binding to ribosomes to cells which causes incorrect readings of amino acids in bacteria (Pratiwi, 2008). The use of gentamicin as a positive control because it is considered still able to inhibit the growth of *P. aeruginosa* bacteria, with a sensitivity of 54.5% (Anggraini *et al.*, 2018). Research of Rachman *et al.* (2016) gentamicin has a sensitivity of 66.7% to *P. aeruginosa* bacteria. While in the study Sulviana *et al.* (2017) gentamycin has a high sensitivity property to *P. aeruginosa* bacteria, which is 90.09%.

The results of observations of the area of the inhibitory zone in the treatment based on different concentrations of bandotan leaf extract showed differences in

the area of the inhibitory zone. The extracts used in this test are included in the viscous extract. Where the higher the concentration of extract used, the higher the viscosity and the inhibition zone will decrease. According to Priyatmoko (2008) the higher the viscosity of an extract, the diffusion process of an antibacterial substance into the media will be lower so that it will affect the result of the diameter of the inhibitory zone. However, Bonang and Koeswardono (1982) found that the width of the obstacle area around the disk disk depends on the absorption of the extract inward order and the sensitivity of bacteria to the extract used does not mean ignoring the concentration of the extract. Thus, the difference in the high and low concentration of the extract given also greatly affects the magnitude of the inhibition zone. It can be seen that the higher the concentration given, the greater the inhibition zone that can inhibit the growth of bacteria (Suciari *et al.*, 2017).

This is in accordance with the statement of Brooks *et al.* (2005) that the effectiveness of an antimicrobial substance is influenced by a given concentration. The increasing concentration of extracts results in a high content of active ingredients that function as antimicrobials so that the ability to inhibit microbial growth is also greater.

Overall bandotan leaf extract has sensitivity in inhibiting the growth of *P. aeruginosa* bacteria at concentrations of 25%, 50% and 75%. This is because the secondary metabolite compounds contained in the extract play a role in inhibiting the growth of bacteria. Phytochemical tests conducted by Melisa and Muchtaridi (2017) bandotan leaf extract have secondary metabolic compounds, so that active compounds such as alkaloids, flavonoids, saponins, and tannins, these compounds are able to act as antibacterials.

Alkaloids as antibacterials can inhibit the constituent components of peptidoglycans in bacterial cells, which can cause cell death (Wulandari *et al.*, 2019). Nurhayati and Setiawan (2018) said that flavonoid compounds act as antibacterials by damaging bacterial cell walls. Saponins can lyse bacterial cell membranes so that they can reduce the surface tension of the

bacterial cell wall and its mechanism of action (Zahro and Agustini, 2013). Tannins can inhibit *the enzymes reverse transcriptase* and DNA topoisomerase so that they cannot form bacterial cells (Nuria and Faizatun, 2009).

Conclusion

Based on the results of the study, it can be concluded that bandotan leaf extract (*Ageratum conyzoides*) with a concentration of 25%, 50%, and 75% shows the presence of antibacterial sensitivity in inhibiting the growth of *P. aeruginosa* bacteria *in vitro* judging from the presence of inhibition zones formed.

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