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Potential of 96% Etanol Extract of Bandotan Leaves (*Ageratum conyzoides* L.) as Larvasida Instar III *Culex quinquefasciatus* Say

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Abstract: The high number of filariasis cases in Indonesia requires efforts to control the population of *Culex quinquefasciatus* larvae. This study aims to analyse the concentration that is effective in killing *Cx. quinquefasciatus* larvae for 48 hours, and to analyse the LC_{50} and LT_{50} values of bandotan leaf extract within 48 hours. Bandotan leaves were collected from Jamblang village, Sleman, Yogyakarta. The leaves were extracted using 96% ethanol, diluted into five concentrations repeated 5 times. Third instar larvae of *Cx. quinquefasciatus* were obtained from the Salatiga Environmental Health Laboratory (BBLKL). Larval mortality was analysed using Kruskal-Wallis test and LC_{50} and LT_{50} probit analysis. The results obtained showed that 96% ethanol extract of bandotan leaves at concentrations of 1% (positive control) and 9% had an effect on larval mortality, with an LC_{50} value obtained of 3.88%. The LT_{50} values at concentrations of 0%, 1%, 3%, 5%, 7%, and 9% were 0 hours; 2.64 hours; 146.90 hours; 102.43 hours; 79, 24 hours; and 53.83 hours. The conclusion of this study is that 96% ethanol extract of bandotan leaves at the concentration used in this study does not have the potential to be used as a larvicide against *Cx. quinquefasciatus*.

Keywords: Bandotan leaves; *Cx. quinquefasciatus*; Larva; mortality; tween 80

Introduction

Filariasis (elephantiasis) is a chronic infectious disease caused by filarial worms transmitted by the mosquito *Cx. quinquefasciatus* (Harviyanto & Windraswara, 2017; Sigit et al., 2022). Some species of filarial worms that attack humans are *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* (Astuti et al., 2023). Data from the Indonesian Health Service states that filariasis cases in Indonesia have increased from 2019-2022, there were 880,531 cases in 2022 (Kemenkes RI, 2022) with filariasis endemic areas in eastern Indonesia (Yunarko et al., 2021). Seeing the high problem of filariasis in Indonesia, it is necessary to control the population of the mosquito *Cx. quinquefasciatus*.

Population control can be done by breaking the life cycle of the *Cx. quinquefasciatus*, one of which is by using larvicides (Oktafian & Siwiendrayanti, 2021). Generally, people often use synthetic larvicides to eradicate mosquito larvae (Huljani & Ahsanunnisa, 2019), one of which is abate (themephos (organic phosphate)). Continuous use of synthetic larvicides can cause side effects such as nausea, vomiting, diarrhoea, abdominal cramps, dizziness and shortness of breath (Satriawan et al., 2019). Therefore, plant-based larvicides are needed to reduce these problems (Glasner et al., 2018). One of the plants that can be used as a vegetable insecticide is bandotan (*Ageratum conyzoides* L.) (Mappasomba et al., 2023).

According to Moore et al. (2018) filariasis cases in the world have increased by 8% every year. Filariasis cases also increased in Indonesia from 2019-2022. In addition, people with filariasis can also experience physical disability due to the inhibition of lymph

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channels by filariasis worms (Caragata et al., 2020). Some countries have been using chemicals to control the mosquito population of *Cx. quinquefasciatus*, as well as in Indonesia (Sinha et al., 2023). According to Randolph et al. (2017) the continuous use of chemicals as mosquito control can make mosquitoes resistant and cause environmental pollution and health problems (Supriatno et al., 2023). Therefore, this study was conducted to reduce the use of chemicals or synthetic larvicides in controlling the population of *Cx. quinquefasciatus* mosquito population.

Research from Dewi (2016) on 70% ethanol extract of bandotan leaves on the mortality of *Aedes* sp. mosquito larvae obtained results at a concentration of 50% can kill *Aedes* sp. instar III larvae as much as 60% of a total of 10 larvae within 48 hours. Research from Indrasari et al. (2015) on the protective power of ethanol extract of bandotan leaves (*A. conyzoides*) against *Aedes aegypti* L. mosquitoes obtained protection power of 81.17% at a concentration of 60% and 96.96% at a concentration of 100%. *Aedes aegypti* itself is a vector of dengue fever in Indonesia (Mokodompit et al., 2019). In addition, mosquito genera that can transmit diseases in Indonesia include Culex, Anopheles, Armigeres and Mansonia (Isfanda & Rahmayanti, 2021; Lesmana, Maryanti et al., 2021).

Some examples of plant-based larvicides that have been used to control *Culex* sp. larvae are mangkokan (*Nothopanax scutellarium*) leaf extract (Ahdiyah & Purwani, 2015) and soursop (*Annona muricata* L.) leaf extract (Melliska, 2022). However, the use of 96% ethanol extract of bandotan leaves to control the population of third instar larvae of *Cx. quinquefasciatus* has never been done. So the purpose of this study was to analyse the concentration of bandotan leaf extract that is most effective to kill instar III larvae of *Cx. quinquefasciatus* for 48 hours, and to analyse the LC_{50} and LT_{50} values of bandotan leaf extract on the mortality of *Cx. quinquefasciatus* for 48 hours. Therefore, this study needs to be conducted as an effort to reduce the use of synthetic larvicides in mosquito population control, especially *Cx. quinquefasciatus* (Rahmawati et al., 2023).

Method

Materials and Tools

The materials used in this study include bandotan leaves (*A. conyzoides*) as much as 9 kg obtained from Jamblang Village, Purwobinangun, Pakem, Sleman, Yogyakarta, *Cx. quinquefasciatus* instar III obtained from Salatiga Environmental Health Laboratory (BBLKL), 96% ethanol solvent, aluminium foil, 8x8 cm filter paper, fish pellets (tetrabites complete), tween 80, distilled water, and 10x50 mm label paper.

The tools used in this research are blender (Philips), digital scales (Oxone), analytical digital scales (Ohauss), dark glass bottle (maceration vessel) size 5 L, spatula 18 cm, glass funnel (Iwaki Pyrex), erlenmeyer (Iwaki Pyrex) 1000 ml, measuring cup (Iwaki Pyrex) 1000 ml, rotary evaporator (Heidolph), water bath, 1,300 ml plastic jar, 250 ml beaker (Iwaki Pyrex), magnetic stirrer (Thermolyne), 3 ml plastic tweezers, gauze, 300 ml plastic mineral water glass, office stationery, and camera.

Research Location

This research was conducted from June to October 2024 at the Ahmad Dahlan University Biology Research Laboratory and the Environmental Health Laboratory Centre, Salatiga. This study used a completely randomised design (CRD) method with the factors tested, namely P1 = (negative control) was not given bandotan leaf extract, P2 = 1% Abate (positive control), P3 = (bandotan leaf extract as much as 3 g), P4 = (bandotan leaf extract as much as 5 g), P5 = (bandotan leaf extract as much as 7 g), P6 = (bandotan leaf extract as much as 9 g) with 5 repetitions of treatment with a total of 20 larvae in each repetition,

Preparation of Simplisia Powder

The stage of making bandotan leaf simplisia powder begins with taking 6 kg of bandotan leaves directly from the local plantation. The leaves are then dried using a wind dryer, until completely dry. After drying, bandotan leaves are pureed using a blender until they become semi-fine powder.

Implementation Methods

Preparation of 96% Ethanol Extract of Bandotan Leaf

Bandotan leaf extract was made by referring to the method from (Asyuri & Ringoringo, 2018). The leaves taken have the characteristics of dark green leaves, not perforated and fresh. The leaves were taken from Jamblang village, Pakem sub-district, Sleman district, Yogyakarta. The leaves were then stored in plastic rice sacks measuring 45x75 cm, then the leaves were washed with running water until clean. The bandotan leaves used were then weighed using a digital scale as much as 9 kg, then dried in the laboratory room for 5 days (Putri & Yushananta, 2022). The dried bandotan leaves were then weighed using a digital scale to determine the dry weight. The dried leaves were then mashed using a blender until they became simplisia. The fine simplisia was weighed as much as 1,200 g and put into a 5 L dark vessel for maceration. The ratio of simplisia and 96% ethanol solvent was 1:4. The maceration process of bandotan leaf simplisia was carried out for 3 days. Stirring is done every day so that the dry simplisia can be extracted optimally so that chemical compounds can be drawn by the solvent optimally (Susanty & Bachmid, 2016).

Simplisia that has been macerated is then filtered using filter paper with a size of 8 x 8 cm so that the filtrate and maceration are separated. The filtrate obtained is then concentrated using a rotary evaporator until it thickens. The thick extract of babadotan leaves obtained was then diluted using distilled water to produce extracts with concentrations of 0% (negative control), 1% abate (positive control), 3%, 5%, 7%, and 9%.

Preparation of Babadotan Leaf Extract Stock Solution

The bandotan leaf extract used as much as 20 g was put into a 250 ml beaker. Then tween 80 was put into the beaker as much as 2 drops and distilled water was added to the limit line, then homogenised using a magnetic stirrer for 7 minutes. Then dilutions of bandotan leaf extract were made with concentrations of 0%, 1%, 3%, 5%, 7%, and 9%. Dilution of 96% ethanol extract of babadota leaves refers to the method of Unita et al. (2018). The 0% concentration was made by putting 100 ml of distilled water into a 250 ml beaker, without the addition of bandotan leaf extract and used as a negative control.

The 1% concentration was made by putting 1 ml of abate stock solution into a 250 ml goblet, without the addition of bandotan leaf extract, then 99 ml of distilled water was added, and homogenised and used as a positive control. The 3% concentration was made by putting 15 ml of bandotan leaf extract stock solution into a 250 ml goblet, then 85 ml of distilled water was added and homogenised. The 5% concentration was made by putting 25 ml of bandotan leaf extract stock solution into a 250 ml goblet, then 75 ml of distilled water was added and homogenised. The 7% concentration was made by putting 35 ml of bandotan leaf extract stock solution into a 250 ml goblet, then added 65 ml of distilled water and homogenised. The 9% concentration was made by putting 45 ml of bandotan leaf extract stock solution into a 250 ml beaker, then added 55 ml of distilled water and homogenised.

Testing 96% Ethanol Extract of Bandotan Leaf Against Cx. Quinquefasciatus *Instar III Larvae*

Testing the extract against *Cx. quinquefasciatus* instar III refers to the method of (Hasanah et al., 2019). The larvae of *Cx. quinquefasciatus.* The design used in this study was a completely randomised design (CRD) with 6 concentrations of treatment (0%, 1% abate, 3%, 5%, 7%, and 9%) and each concentration was repeated 5 times using the Federer formula (Table 1), so that 30 100 ml glasses of mineral water were needed and the number of instar III larvae used was 600.

Table 1. Research Randomisation Model with Completely Randomised Design (CRD)

Schematic layout of treatment randomization					
P1	P2	P3	P4	P5	P6
P4	P6	P5	P3	P2	P1
P2	P4	P1	P5	P6	P3
P5	P3	P4	P6	P1	P2
P6	P5	P2	P1	P3	P4

Notes:
P1 = Mineral water cups containing food and distilled water for third instar larvae of *Cx. quinquefasciatus* + 10 ml of 0% extract solution.
P2 = Mineral water cups containing food and distilled water for third instar larvae of Cx. quinquefasciatus + 10 ml of 1% abate solution.
P3 = Mineral water cups containing food and distilled water for third instar larvae of Cx. quinquefasciatus + 10 ml of 3% babadotan leaf extract solution.
P4 = Mineral water cups containing food and distilled water for third instar larvae of Cx. quinquefasciatus + 10 ml of 5% babadotan leaf extract solution.
P5 = Mineral water cups containing food and distilled water for third instar larvae of Cx. quinquefasciatus + 10 ml of 7% babadotan leaf extract solution.
P6 = Mineral water cups containing food and distilled water for third instar larvae of Cx. quinquefasciatus + 10 ml of 9% babadotan leaf extract solution.

Each mineral water plastic cup was labelled according to the concentration of bandotan (*A. conyzoides*) leaf extract that had been made. A total of 20 third instar larvae of *Cx. quinquefasciatus* third instar were put into each mineral water plastic cup containing feed, water media, and bandotan leaf extract solution. After that, the aqua plastic cups were closed using gauze. Mortality observations of *Cx. quinquefasciatus* larvae were observed for 48 hours at the 0th, 4th, 8th, 12th, 16th, 20th, 24th, 28th, 32nd, 36th, 40th, 44th, and 48th hours after the application of bandotan leaf extract.

Mortality Observation of Cx. quinquefasciatus for 48 Hours

Mortality observation of *Cx. quinquefasciatus* mosquito larvae were observed by observing the morphology of the larvae, characterised by the larvae not moving when touched and the body condition bent with the larvae at the bottom of the extract. The number of *Cx. quinquefasciatus* larvae was recorded and the data was processed using regression test and probit analysis to determine the Lethal Concentration (LC_{50}) and Lethal Time (LT_{50}) values.

Data Analysis

Percentage Mortality of Third Instar Larvae of Cx. quinquefasciatus

Observations of third instar larvae of *Cx. quinquefasciatus* were conducted for 48 hours after the application of ethanol extract of bandotan leaves. Observations were made by counting the number of deaths of instar III larvae that died every 4 hours, then the mortality data was expressed in units of percent. Mortality indicates the number of deaths caused by the bandotan leaf extract used and is expressed in per cent.

T-tests between the Mortality of Larvae of Cx. quinquefasciatus in Each Treatment

The data obtained were tested for significant differences between the mortality of *Cx. quinquefasciatus* larvae in each treatment. The real difference test carried out was using the Kruskal-Wallish test and obtained a value (sig>0.05) which means that there is a significant difference in the number of third instar larval deaths of *Cx. quinquefasciatus* in each treatment.

Regression Test between the Mortality of Cx. quinquefasciaus Larvae with Bandotan Leaf Extract Treatment

The data obtained were then subjected to linear regression tests to determine the relationship between the mortality of third instar larvae of *Cx. quinquefasciatus* with bandotan leaf extract treatment. The results obtained from the simple linear regression test results obtained an R value of 0.21, which means that the correlation of the independent variable and the dependent variable has a distant relationship and the level of influence of the extract on larval mortality is low, because the value obtained is not close to 1.

Probit Analysis

LC_{50} analysis was conducted to see the concentration that was able to kill 50% of the larvae of *Cx. quinquefasciatus* larvae after treatment. LT_{50} analysis was conducted to see the time taken to kill 50% of larval mortality at what time.

Result and Discussion

Effect of Ethanol Extract of Bandotan (A. conyzoides) Leaves on Mortality of Instar III Larvae of Cx. quinquefasciatus

Mortality of third instar larvae of *Cx. quinquefasciatus* was highest after 48 hours of extract administration at 15 in treatment P6, while in P1 there was no mortality of third instar larvae of *Cx. quinquefasciatus* (Table 2).

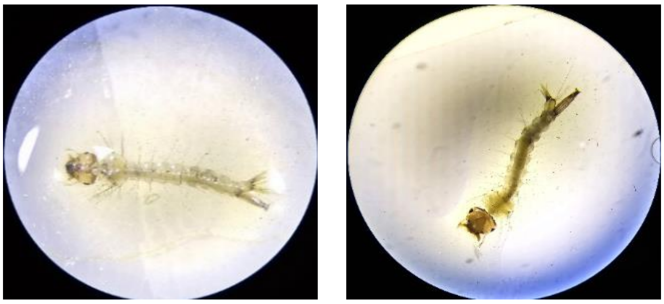
Table 2. Mortality of Third Instar Larvae of *Cx. quinquefasciatus* Treated with Babadotan (*A. conyzoides*) Leaf Extract for 48 Hours

Treatment	Larva abundance (individual)	Larval mortality in each replicate (individual)					Average larval mortality
		I	II	III	IV	V	
P1	20	0	0	0	0	0	0 %
P2	20	20	20	20	20	20	100 %
P3	20	3	4	5	5	6	23 %
P4	20	9	5	7	9	6	36 %
P5	20	8	11	9	12	9	49 %
P6	20	12	11	15	13	12	63 %

Notes:
P1 = 0% Concentration (aquades) (negative control)
P2 = 1% concentration (abate) (positive control)
P3 = 3% concentration
P4 = 5% concentration
P5 = 7% concentration
P6 = 9% concentration

The results obtained in this study are different from the research (Hasanah et al., 2019) which used ceremai leaf extract. The 1% ceremai leaf extract obtained the highest percentage of larval mortality at 100%, while in this study the bandotan leaf extract at 9% concentration obtained the highest percentage of mortality at 63%. The death of third instar larvae of *Cx. quinquefasciatus* is caused by secondary metabolites found in bandotan (*A. conyzoides*) leaves, namely alkaloid compounds (Susanty & Bachmid, 2016). Alkaloid compounds can act as stomach poisons for larvae, because alkaloid compounds consumed by larvae and entering the digestive organs can inhibit the impulse delivery system to the muscles and affect the nervous system so that the larvae gradually die (Chowanski et al., 2016).

The presence of alkaloid compounds as gastric toxins in this study is evidenced by the observation of dead larvae experiencing changes in abdominal colour on the ventral part, from clear white to blackish grey (Price & Garcia, 2024). Where the ventral part of the larva is the organ of food digestion (Putri et al., 2018). According to Febri et al. (2024) the larvae of *Cx. quinquefasciatus* larvae are white, mobile, and active (Figure 1a), while *Cx. quinquefasciatus* larvae are immobile, found at the bottom of the water, and the digestive tract and head are blackish brown in colour (Figure 1b).



A B

Figure 1. Third instar larvae of *Cx. quinquefasciatus*; (A) live and (B) dead larvae. 10x microscope magnification. (Source: Personal Documentation, 2024)

Death of third instar larvae of *Cx. quinquefasciatus* in this study showed changes in body colour in live and dead larvae after treatment. Discolouration in third instar larvae of *Cx. quinquefasciatus* is called melanisation (Mustapa et al., 2023). This refers to Oktadiana et al. (2020) which states that the melanisation process is one of the insect's defence mechanisms against foreign compounds that enter the insect's body and can inhibit the activity of enzymes contained in the insect's body (Moniharapon et al., 2023). According to Sutikno et al. (2023), larvae that experience melanisation or change their body colour to dark will show symptoms of slowed body movement, reduced appetite, and enlarged head (Davis et al., 2021). Symptoms such as slowed movement and reduced appetite occurred in third instar larvae of *Cx. quinquefasciatus* in this study according to Sinha et al. (2023). The melanisation process of third instar larvae of *Cx. quinquefasciatus* in this study is a sign that larval death is caused by toxins from secondary metabolite compounds in bandotan (*A. conyzoides*) leaf extract consumed by the larvae (Krisna et al., 2022).

Flavonoid compounds in bandotan leaf extract can be toxic to larvae, according to Maisel et al. (2023), stating that flavonoid compounds can attack the respiratory system of *Cx. quinquefasciatus* through the siphon located on the posterior abdomen then enters the larval body which causes the nerves in the siphon to be damaged and weakened, this also causes the larvae to be unable to breathe and die (Torto & Tchouassi, 2024). The extract used as a vegetable larvicide test material, added 2 drops of tween 80 which aims to homogenise the extract with distilled water so that the extract does not clot and the compounds in the extract can function as larvicides (Kusuma & Mahardi, 2021). According to Merry et al. (2024) using quercetin solution to measure the flavonoid content of bandotan leaf extract using a UV-Vis spectrophotometer with a wavelength of 415 nm to measure the absorbance value of each concentration (Table 3). The absorbance values obtained were plotted on the quercetin standard curve (Figure 2).

Table 3. Absorbance Value of Standard Flavonoid Compound Quercetin

Concentration (μg/ml)	Absorbance
20	0.11
40	0.28
60	0.40
80	0.62
100	0.73

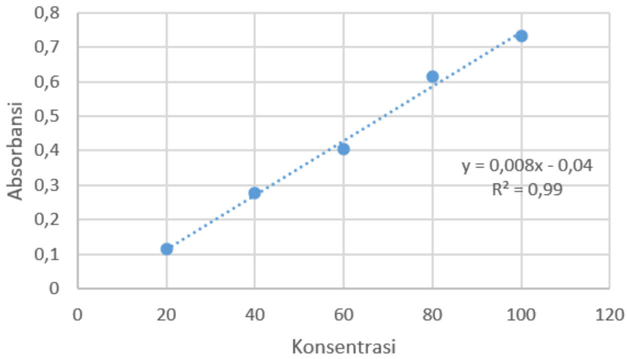


Figure 2. Standard curve of flavonoid compound quercetin

The results of measuring the absorbance of quercetin standard obtained with a maximum wavelength of 415 nm, and obtained the value of the linear regression equation between the absorbance of quercetin with concentration y = 0.008x - 0.04 with a value of R2 = 0.99. The linear regression equation of quercetin can be used to determine the total concentration of flavonoid compounds in bandotan leaf extract. The total flavonoid content obtained is 10.01 mg/ml, so it is in accordance with research Putri et al. (2021) which states that the dominant secondary metabolite compound in bandotan leaf extract is a titerpenoid compound, then the data obtained was analysed using statistical analysis.

Mortality data of third instar larvae of *Cx. quinquefasciatus* was then analysed using the Kruskal-Wallis nonparametric test. The results obtained from the Kruskal-wallis test showed a value (sig>0.05) which means that there is a significant difference in the number of deaths of instar III larvae of *Cx. quinquefasciatus* in each treatment. This means that different concentrations of bandotan leaf extract have different killing power on the number (Supriatno et al., 2024). According to Tatontos et al. (2024) the higher the concentration of the extract, the greater the number of larvae that die. Furthermore, the mortality data of third instar larvae of *Cx. quinquefasciatus* was tested using a simple linear regression test to see the effect of bandotan extract concentration on the mortality of *Cx. quinquefasciatus* (Table 4). Regression values were plotted on a graph (Figure 3).

Table 4. Linear Regression Test Result

Model summary			
R	R Square	Customised R Square	Standard Error of Estimation
0.21	0.05	0.01	6.47

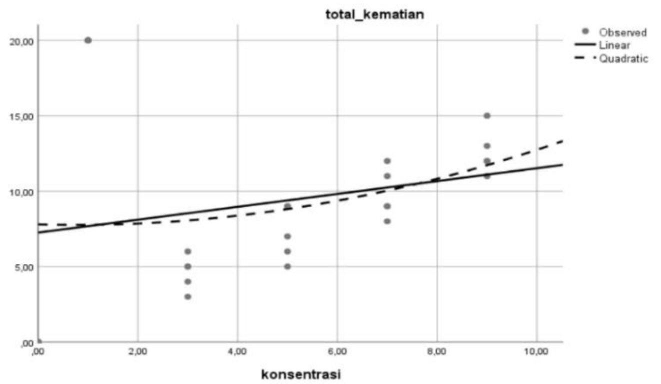


Figure 3. Mortality curve of third instar larvae of *Cx. quinquefasciatus*

The results obtained from the simple linear regression test results obtained an R value of 0.21, which means that the correlation of the independent variable and the dependent variable has a distant relationship and the level of influence of the extract on larval mortality is low, because the value obtained is not close to 1 (Trisnawati & Azizah, 2019). The R square value of 0.04 means that the effect of giving bandotan leaf extract in killing test larvae is 4%. The results of this study differ from research from Hasanah et al. (2019) which used ceremai leaf extract to obtain an R value of 0.909 and for an R square value of 90.94% on the death of *Cx. quinquefasciatus* larvae. This is due to differences in the content of secondary metabolite compounds in the two leaves. According to Ernawati (2024) the secondary metabolite compound that dominates in ceremei leaf extract is saponin by 45% of the content in 400 grams of dry simplisia, while in bandotan leaf extract according to Putri et al. (2021) the dominant secondary metabolite compound in bandotan leaf extract is a triterpenoid compound of 25% in 500 grams of dry simplisia. Triterpenoid compounds are one of the compounds that act as antibacterials and these compounds can cause nutritional deficiencies so that bacterial growth will be inhibited and cause bacterial death (Unita & Voon, 2018).

This is in accordance with this study because bandotan leaf extract is able to kill the test larvae, but in a long period of time. According to Adnyani et al. (2016) stated that the death of test larvae can be influenced by the concentration level used and the content of secondary metabolite compounds contained in plant extracts. The low toxicity of bandotan leaf extract against instar III larvae of *Cx. quinquefasciatus* is also evidenced by the length of time the test larvae die as indicated by the LT50 value (Suripto et al., 2023).

LC_{50} Value of Bandotan Leaf Extract (A. conyzoides) on Mortality of Instar III Larvae of Cx. quinquefasciatus

The results of mortality data of third instar larvae of *Cx. quinquefasciatus* were analysed for probit values using excel, obtained a regression equation y = 0.15x + 1.94 with r2 = 0.05 (Figure 4) and the LC_{50} probit value equation data of bandotan leaf extract against instar III larvae of *Cx. quinquefasciatus* by entering the ay value of 5 in the equation y = 0.15x + 1.94 (Table 5), the LC_{50} value is the antilog of the x value so that a value of 31.50 is obtained and the antilog of the x value is 3.88%.

Table 5. Equation for Probit Analysis of LC_{50} of Bandotan Leaf Extract against Third Instar Larvae of *Cx. quinquefasciatus*

Probit equation	y=ax + b 5 = 0.15x + 1.94
X	31.50
LC50 (antilog x)	3.88%

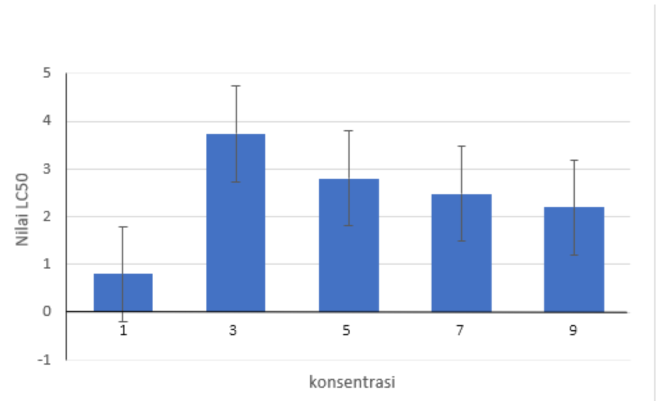


Figure 4. LC_{50} probit analysis equation graph

The LC_{50} value is the concentration of an extract that can cause the death of instar III larvae of *Cx. quinquefasciatus* by 50% (Hasanah et al., 2019). The result obtained from the LC_{50} value of 3.88% means that bandotan leaf extract at that concentration should be able to kill 50% of the third instar larvae population tested. However, the results of this study are not in accordance with the LC_{50} results obtained. According to Putri et al. (2021) the compounds in bandotan leaves that really dominate are triterpenoids. Triterpenoids are compounds that act as antibacterials. According to Pay et al. (2022) the triterpenoid compound in bandotan leaves is found at a value of 35% in 400gr dry simplification, so this dominant compound can cause a decrease in the toxic properties of bandotan leaf extract against instar III larvae of *Cx. quinquefasciatus*. This is also reinforced in research from Putri et al. (2021) which reports that triterpenoid compounds will work actively to damage the membrane layer and break down development in *Streptococcus pyogenes* bacteria, but do not make the bacteria die. This is in accordance with the study because at a concentration of 3% it was unable to cause the death of test larvae by 50%. The low toxicity of bandotan leaf extract on the mortality of *Cx. quinquefasciatus* is evidenced by the length of time for larval death as indicated by the LT_{50} value (Dalming et al., 2022).

LT_{50} Value of Bandotan Leaf Extract (A. conyzoides) on Mortality of Instar III Larvae of Cx. quinquefasciatus

The results of the LT_{50} value from probit analysis showed that the concentration capable of killing 50% of the larvae was 34.409 hours as shown in (Table 6). The LT_{50} value is the time required by a certain concentration of substance or extract to kill 50% of the test animal population.

Table 6. Probit Analysis of LT_{50} of Bandotan Leaf Extract on Mortality of Third Instar Larvae of *Cx. quinquefasciatus*

	0%	1%	3%	5%	7%	9%
Probit equation	-	y= 7.39x -6.96	y= 3.46x -2.88	y= 2.57x – 2.37	y= 2.7541x -2.8174	y= 3.3921x + 3.1739
LT_{50}	-	2.64 hours	146.90 hours	10.43 hours	79.24 hours	53.83 hours

Based on the results obtained in (Table 6) the LT_{50} value above shows that bandotan leaf extract at a concentration of 3% has a low LT_{50} with a value of 146.90 hours or ± 6 days. The LT_{50} value has a greater value compared to other concentrations of bandotan leaf extract because based on observations during the study showed that the first death of instar III larvae of *Cx. quinquefasciatus* at a concentration of 3% was longer at the 28th hour than the other concentrations (Hilaliyah, 2021). Meanwhile, the first death of the third instar larvae of *Cx. quinquefasciatus* at concentrations of 5%, 7%, and 9% occurred at the 16th and 20th hours after administration of the extract at each concentration, respectively. The LT_{50} values obtained in this study at concentrations of 3% and 5% exceeded the life limit of the third instar larval phase of *Cx. quinquefasciatus*, while at concentrations of 7%, 9% and 1% abate the LT_{50} value obtained did not exceed the life limit of the third instar larval phase of *Cx. quinquefasciatus*. This is reinforced by research from (Kauffman et al., 2017) which states that the growth phase of *Cx. quinquefasciatus* will develop into pupae within 72 - 96 hours or 3 - 4 days, so that these conditions cause larval feeding activity to stop and puparia will form and then turn into pupae on water media.

Conclusion

The 96% ethanol extract of bandotan leaves at concentrations of 0%, 3%, 5%, and 7% had no effect on the mortality of third instar larvae of *Cx. quinquefasciatus*, but the 9% concentration had an effect on the mortality of the test larvae. The LC_{50} value obtained was 3.88%, the LT_{50} value of third instar larval mortality of *Cx. quinquefasciatus* were 0 hours; 2.64 hours; 146.90 hours; 102.43 hours; 79.24 hours; and 53.83 hours. So it can be concluded that 96% ethanol extract of bandotan leaves at the concentration used in this study has no potential as a larvicide of *Cx. quinquefasciatus*. In the future, 96% ethanol extract of bandotan leaves can be tested with higher concentrations and testing of secondary metabolite compounds as a whole in bandotan leaves to see the LC_{50} and LT_{50} values, so that the larvicidal potential of this bandotan plant can be known.

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SNY and ILIP write the manuscript; ILIP designing the research and overseeing the entire process; SNY collecting the data. SNY and ILIP analyzing data.

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Conflict of Interest
All authors declare no conflict of interest.

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