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# TOXICITY TEST OF BANANA LEAF EXTRACT WITH BRINE SHRIMP LETHALITY TEST METHOD

*Uji Toksisitas Ekstrak Daun Pisang dengan Metode Brine Shrimp Lethality Test*

**Armi Armi[1*], Lina Herida Pinem[1], Yana Setiawan[1], Yennyka Dwi Ayu[1], Susi Hartati[1], La Ode Muhammad Anwar[2], Anisa Rachmita[3]**

[1]Program Studi S1 Keperawatan, STIKes Mitra Keluarga, Bekasi, Indonesia
[2]Program Studi S1 Farmasi, Universitas Medika Suherman, Cikarang, Indonesia
[3]Program Studi S1 Farmasi, STIKes Mitra Keluarga, Bekasi, Indonesia
*Email: ners.armi@gmail.com

***ABSTRAK***

*Metode Brine Shrimp Lethality Test (BSLT) merupakan teknik yang digunakan untuk mengevaluasi potensi toksisitas awal suatu senyawa berdasarkan kemampuan mematikan larva Artemia salina. Penelitian ini bertujuan untuk mengetahui potensi efek sitotoksik dari ekstrak daun pisang ambon (Musa paradisiaca) melalui uji BSLT. Penelitian menggunakan desain eksperimental uji toksisitas secara in vivo Brine Shrimp Lethality Test (BSLT) dengan larva A. salina sebagai subjek, dan dilakukan di Laboratorium Biomedik Universitas Medika Suherman pada periode 16 Agustus hingga 13 September 2024. Uji dilakukan pada beberapa konsentrasi ekstrak yaitu 500, 100, 50, dan 10 ppm, masing-masing dengan tiga kali pengulangan dan kontrol. Observasi dilakukan pada interval waktu 6, 12, 18, dan 24 jam. Analisis data dilakukan menggunakan regresi linear dan metode probit. Hasil menunjukkan bahwa ekstrak daun pisang ambon memiliki nilai LC50 sebesar 272,13 ppm. Kesimpulannya, ekstrak ini menunjukkan aktivitas sitotoksik sedang dan berpotensi untuk dikembangkan lebih lanjut dalam penelitian farmakologis dan toksikologis.*

***Kata kunci**: BSLT, daun pisang ambon, ekstrak, Musa paradisiaca*

**ABSTRACT**

The Brine Shrimp Lethality Test (BSLT) method is a technique used to evaluate the initial toxicity potential of a compound based on the ability to kill Artemia salina larvae. This study aims to determine the potential cytotoxic effect of banana leaf extract (Musa paradisiaca) through the BSLT test. The study used a pre-clinical experimental design for in vivo toxicity testing of the Brine Shrimp Lethality Test (BSLT) with A. salina larvae as subjects, and was conducted at the Biomedical Laboratory of Medika Suherman University in the period August 16 to September 13, 2024. The test was conducted at several concentrations of extracts, namely 500, 100, 50, and 10 ppm, each with three repetitions and a control. Observations were made at time intervals of 6, 12, 18, and 24 hours. Data analysis was performed using linear regression and the probit method. The results showed that the banana leaf extract had an $LC_{50}$ value of 272.13 ppm. In conclusion, this extract showed moderate cytotoxic activity and has the potential to be further developed in pharmacological and toxicological research.

**Keywords:** banana leaf ambon, BSLT, extract, Musa paradisiaca

## INTRODUCTION

Banana trees are easy to grow and are widely found in Indonesia. There are many varieties of banana trees, and the Ambon banana is one of them. One part of the banana tree boasts many health benefits, including the banana leaves. Ambon banana leaves contain anti-ulcer properties, which in pharmacology means wound healing[1]**.** Banana leaves contain many active antibacterial compounds, some of which are beneficial for wound care. Banana leaf extract can increase moisture and accelerate wound healing, reducing the risk of infection[2]**.** Ambon banana leaf extract functions as an antimicrobial[3]**,** and is able to maintain the moisture of diabetic wounds[4].



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Although banana leaves are often used empirically to bandage wounds or as an external medicine, traditional use does not always reflect long-term or systemic safety. Without toxicity testing, it is unknown whether compounds in the extract can cause skin irritation, liver damage, nephrotoxicity, or other systemic effects if absorbed. Ambon banana leaves contain phenolic compounds[5]. Other compounds from Ambon banana leaves include flavonoids, phenols, and tannins, which are used to heal wounds.[6]Flavonoids in banana leaves have the potential to act as antibiotics, antibacterials, and anticancer compounds[7]**.** Phenol functions as an antibacterial compound by forming complex compounds for wound healing.[8], while tannin is classified as a mixture of polyphenols, which is difficult to separate, because it is not in crystalline form. Tannin is an amorphous, hygroscopic compound, yellowish brown in color, which is polarly soluble in organic[9]**.** In the medical world, some of these compounds are bioactive compounds that must first be tested on laboratory animals. Certain bioactive compounds often have toxic properties; therefore, biotoxicity testing must be carried out to determine the toxicity of a substance[10]. Toxicity testing is a preclinical test conducted in an effort to confirm safety so that the requirements are met and can then proceed to the clinical trial stage[11]. The toxicity test carried out for Ambon banana leaf extract can be in the form of the Brine Shrimp Lethality Test (BSLT).

A substance that is repeatedly exposed to even small amounts can cause long-term toxic effects on the body of living things. BSLT is one of the initial methods for toxicity testing. BSLT is a simple and rapid method, as test results can be obtained within 24–48 hours of exposure. This makes it a very efficient method for initial screening of the toxicity of medicinal plant extracts or pure compounds before proceeding to more complex and expensive tests. The BSLT method is a way to determine the cell toxicity of a compound, from plant extracts through the use of Artemia salina shrimp larvae, a method often used for compounds known to have potential cytotoxic effects[12]. This method is a frequently used method, having a simple procedure, fast and accurate results, and does not require large costs[13]. Information on the benefits of Ambon banana leaves is not yet widely known by the general public; therefore, research is needed to provide information regarding the pharmacological effects of the chemical content. The BSLT test aimed to determine the potential cytotoxic effect of Ambon banana leaf extract (Musa paradisiaca).

## METHODS

This research is a type of experimental qualitative research in order to uncover the truth BSLT test of Ambon banana leaf extract (Musa paradisiaca var. sapientum). The purpose of the experimental research method is to examine the possibility of cause and effect between the independent variable and the dependent variable. The population and sample in this research are 1 gram A. salina Leach larvae. With inclusion criteria of 48-hour-old larvae and no visible anatomical defects, while the exclusion criteria indicate movement activity before treatment. The study was conducted at the Biomedical Laboratory, Suherman Medika University. The study period was from August 16 to September 13, 2024. The BSLT test method was used to determine the toxicity of Ambon banana leaf extract through the use of A. salina Leach larvae, and then the death of A. salina Leach larvae was observed. Ethical review with no. 02/23.11/02951 Ambon banana leaf extract for wound care as an independent variable, while the dependent variable is the Brine Shrimp Lethality Test method.

The BSLT test was conducted based on research conducted by Nofita et al (2020)[14]. Researchers have modified the A. salina Leach storage system in a jar with an aerator to keep it alive. This modification was made to facilitate the collection of A. Salina Leach larvae for testing.

### 1. Penyiapan larva *A. salina* Leach


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Larvae of Artemia salina Leach were prepared by hatching approximately 2.5 mg of eggs in 250 mL of artificial seawater. Artificial seawater was made by dissolving 50 g of sea salt in 2 L of distilled water, followed by filtration. The hatching container was equipped with continuous aeration and illuminated using a 25–40 watt lamp for 48 hours. After 24 hours, the eggs began to hatch, producing nauplii, and at 48 hours, the larvae were considered suitable for toxicity testing. Active larvae were separated from the eggs by carefully pipetting them into clean seawater to ensure that only healthy, motile larvae were used in the test.

**2. Preparation of Stock Solution and Test Concentration Series**

A stock solution was prepared by dissolving 20 mg of the thick extract in 20 mL of artificial seawater to obtain a concentration of 2000 µg/mL. From this stock solution, a series of test concentrations was prepared to yield final concentrations of 1500 µg/mL, 1000 µg/mL, 500 µg/mL, 100 µg/mL, and 0 µg/mL (control). The control solution contained only the solvent without the extract. Each concentration was prepared freshly prior to testing to maintain the stability and accuracy of the solution.

**3. Toxicity Test**

The toxicity test was conducted by transferring 6 mL of each test concentration into separate test tubes, followed by the addition of ten Artemia salina larvae aged 48 hours into each tube. All concentrations, including the control group, were tested in triplicate. The test tubes were then incubated under constant illumination and aeration. Larval mortality was observed at 6, 12, 18, and 24 hours. Larvae were considered dead if no movement was observed after gentle stimulation. After 24 hours, the number of dead larvae in each test group was recorded. The median lethal concentration ($LC_{50}$) was calculated using probit analysis based on the linear regression equation:

$$y = a+bx$$

This analysis was used to determine the extract's toxicity level against Artemia salina larvae.

## RESULT

The results of the toxicity test of banana leaf extract with shrimp larvae using the LC50 calculation can be reviewed in Table 1:

**Table 1. LC50 Calculation Results**

| Concentration (ppm) | Concentration Log | Number of Shrimp Larvae | Repetition | | | Average | % Death | Probit Value | LC50 (ppm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | I | II | III | | | | |
| 1000 | 3 | 10 | 10 | 10 | 10 | 10 | 100 | 7,326 | 272.134796 |
| 500 | 2.698970004 | 10 | 2 | 3 | 10 | 5 | 50 | 5 | |
| 100 | 2 | 10 | 2 | 0 | 0 | 0.67 | 6.67 | 3,501 | |
| 50 | 1.698970004 | 10 | 0 | 0 | 1 | 0.33 | 3.33 | 3,162 | |

In table 1 there is a percentage of mortality of A. salina larvae with a concentration log showing that a concentration of 1000 ppm with a mortality percentage of 100%, a concentration of 500 ppm with a mortality percentage of 50%, a concentration of 100 ppm with a mortality percentage of 6.67%, and a concentration of 50 ppm with a mortality percentage of 3.33%.

The log concentration graph with probit shows the results of the banana leaf extract toxicity test on a. salina on the log concentration. The linear regression graph of the equation above is y = 3.0455x – 2.4553 with an R2 value of 0.9048. The LC50 results obtained a value of 50 on X, the Y value in the following equation is 149.8197. Figure 1



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and Table 1 show that the value of the extract concentration is greater and the mortality value on A. salina is also greater.

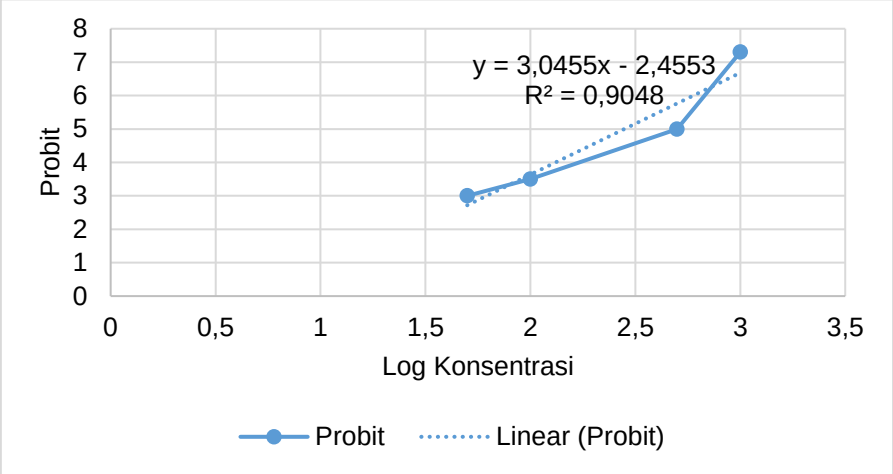


**Figure 1. Results of linear regression Log concentration Vs Probit**

## DISCUSSION

The BSLT test results showed that Ambon banana leaf extract had an LC50 value of 272 ppm, which is classified as moderate toxicity. This finding indicates the presence of bioactive compounds in the extract that have the potential for further development, especially as an antimicrobial agent or topical wound healing agent. Despite its promising potential, further in vivo toxicity testing is still needed to ensure its safety, especially for systemic or long-term use. Based on observational data, the test solution at a concentration of 1000 ppm caused the death of all larvae (mean mortality = 10). At a concentration of 100 ppm, the average mortality decreased to 7.3; then to 2.3 at a concentration of 10 ppm; and only 0.3 larvae died at a concentration of 1 ppm. This pattern indicates a clear dose-response relationship, where increasing extract concentration is proportional to the increase in the level of larval mortality. These results support the accuracy of the BSLT method in assessing the toxic potential of a compound practically and efficiently. This is in accordance with the research of Aris et al (2022) that the greater the extract concentration value, the higher the mortality of Artemia salina shrimp larvae[15].

The LC50 score of the red miana leaf ethanol extract, 323.225 µg/mL, indicates that the extract has a low level of toxicity. This is in line with the principle that the lower the LC50 value, the higher the level of toxicity of a substance[10]. Toxicity testing using the Brine Shrimp Lethality Test (BSLT) method using Artemia salina shrimp larvae is known to be very sensitive, because these larvae have a thin skin structure and simple cells.[16]The LC50 value is an indicator of the concentration of a compound that can cause the death of 50% of the test larval population. In this study, the average mortality of A. salina larvae was recorded at 10 at a concentration of 1,000 ppm, 7.3 at 100 ppm, 2.3 at 10 ppm, and 0.3 at 1 ppm. These results further confirm that the ethanol extract of red miana leaves is low toxic and still within safe limits[17].

The research of Fitri et al (2021) showed that the ethanol extract of Arabica coffee rind and Dayak onion has the potential for toxicity to Artemia salina Leach shrimp larvae. The highest toxicity potential was shown by the Arabica coffee rind extract, with the lowest LC50 value of 51.7639 ppm, which means it can cause death of up to 50% in test animals[18]. Both extracts (Arabica coffee rind and Dayak onion) are known to cause mortality of A. salina larvae up to 98%. Another study that also used the Brine Shrimp Lethality Test (BSLT) method showed that the ethanol extract of Kepok banana rind (Musa paradisiaca L.) has cytotoxic activity with an LC50 value of 393.5500 µg/mL and is included in the toxic category[19].



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The results of this study showed a log concentration with probit showing the results of the toxicity test of banana leaf extract against A. salina on the log concentration. The linear regression graph of the equation above is y = 3.0455x - 2.4553. The LC50 results obtained a value of 50 on X, the Y value in the following equation is 149.8197. This research shows that the greater the extract concentration value, the greater the mortality value in A. salina.

The LC50 value was obtained from the results of a probit analysis of banana leaf extract. In this research, the analysis indicates a relationship between the percentage of Artemia Salina Leach larvae mortality and the concentration of ethanol extract of Arabica coffee fruit peel and Dayak onion ethanol extract. Graph of the relationship between the log concentration of ethanol extract of Arabica coffee fruit peel and the probit value[18]. Based on research by Armi (2023), it was stated that Ambon banana leaf extract at a concentration of 15% has antibacterial activity with an inhibition zone of 2.33 mm. Ambon banana leaf extract can be useful for repairing tissue structure in wounds and accelerating the re-epithelialization process of epidermal tissue[20]. This is in line with research by Anwar et al. (2024), who stated that Ambon banana leaf extract ointment was proven to be effective in accelerating the healing of diabetic ulcers at an optimal concentration of 10% for topical application[21]. The research's strengths include the results of a toxicity test at moderate concentrations using a fast, precise, and efficient method. Limitations encountered during the study included the inaccuracy in calculating larval mortality and the limited food supply. The implications of the toxicity test results suggest that banana leaf extract ointment can be administered to humans.

## CONCLUSION

Ambon banana leaf extract (Musa paradisiaca var. sapientum) has a cytotoxic value on shrimp larvae (Artemia salina Leach) with an LC50 value of <1000 ppm. Based on the probit analysis method, the LC50 score was 272.13 ppm, which is classified as moderate toxicity. It is hoped that in future research, observations of larval mortality should be carried out by two or more people to minimize the risk of error.

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