

LC₅₀ Toxicity Activity Test Of Ethanol Extract Of Cardamom Leaves (Amomum cardamomum L.) On Shrimp Larvae (Artemia salina L) Using Brine Shrimp Lethality Test (BSLT) Method

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ABSTRACT

There are many negative effects on the use of chemical-based medicine, therefore many researchers are interested in finding natural ingredients that have the potential to replace chemical treatment. One of the plants from the Zingiberaceae family that is used as a medicinal ingredient is cardamom plant (Amomum cardamomum L). Many people often use cardamom for treatment such as aromatic ingredients, carminative (reducing gas in the stomach), cough medicine, halitosis and so on. Cardamom contains phytochemical compounds such as alkaloids, flavonoids, tannins, and saponins. The purpose of this study was to analyze the level of bioactivity of cardamom leaf ethanol extract to Artemia salina L shrimp larvae by BSLT method and determine the LC₅₀ value. This study used quantitative methods that were experimental and used 6 treatment concentrations, namely 25 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm, 1000 ppm and 1 negative control with each concentration replicated 5 times. Artemia salina L larvae were used as many as 350 heads with 10 heads each for each concentration. The death of larvae is calculated after 24 hours of treatment. The calculation result of LC₅₀ from cardamom leaf ethanol extract (Amomum cardamomum L) with data analysis using SPSS version 25 is 151,671 ppm. This shows that cardamom leaf ethanol extract (Amomum cardamomum L) is toxic due to LC₅₀.

Keywords: Cardamom Leaf (Amomum cardamomum L), BSLT (Brine Shrimp Lethality Test), LC₅₀ value.

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INTRODUCTION

In essence, a pharmaceutical industry must create and create drugs that are in accordance with their intended use. Drugs to be distributed must meet the requirements listed in the distribution permit document and do not pose a risk of harm to users due to lack of safety, low quality, or ineffectiveness of a drug. The dangers of side effects of a drug in humans can be identified by studying the effects that are considered detrimental, such as cumulative, carcinogenic, teratogenic, mutagenic to toxic effects of a drug (Tresnafuty, et al., 2022).

The use of chemical drugs for a long period of time can also cause new diseases because many organs of the body are damaged by the chemical drugs (Jenta & Suharti 2019). The many negative side effects of using chemical-based treatments, therefore many researchers are interested in finding natural ingredients that have the potential to replace chemical treatments. Currently, many studies are being conducted on plants to determine the content of plants and their benefits and toxicity.

One of the plants from the zingiberaceae family that is used as a medicinal ingredient is the cardamom plant (Amomum cardamomum L.). It is known that medicinal plants that can produce essential oils and are included in the top 9 large spice commodities in the world are cardamom. Cardamom contains phytochemical compounds such as alkaloids, flavonoids, tannins, polyphenols, saponins and so on. In addition, cardamom plants also contain fat, protein, calcium oxalate and kersit acid (Arista, et al., 2023).

So far, people know that plants from the zingiberacea family have many benefits, but not many people know the toxic effects of plants from the zingiberaceae family, one of which is the cardamom plant. This is supported by research conducted by (Fitri, et al., 2015) entitled "Bioactivity of kecombrang stems (*Etlingera elatior* (jack) R.M.Sm.) against termites *Coptotermes curvignathus*, sp" with the results showing that the chloroform fraction has high toxicity because with a minimum concentration of 4% it can kill 100% of the test termites on the sixth day.

The method often used to determine the toxic activity of an extract or natural compound is the Brine Shrimp Lethality Test (BSLT) method using *Artemia salina* L sea shrimp larvae. Based on the description of the background, this study will conduct "LC₅₀ toxicity activity test of ethanol extract of cardamom leaves (*Amomum cardamomum* L.) against shrimp larvae (*Artemia salina* L) using the Brine Shrimp Lethality Test (BSLT) method".

MATERIALS AND METHODS

Population and Sample

The animals used in this experiment were 48-hour-old shrimp larvae (*Artemia salina* L). The *Artemia salina* L larvae used were 10 for each extract concentration. In this study, 6 concentrations of ethanol extract of cardamom leaves (*Amomum cardamomum* L) and one negative control were made. Each concentration and negative control were carried out 5 times. So, the total sample required was 350 *Artemia salina* L larvae. The test animals used were obtained from an online store (Lazada), with the name FISHLOOK store (<https://s.lazada.co.id/s.okaTf>). The sample for this study was Cardamom leaves (*Amomum cardamomum* L.) obtained from Sikhorilafau village, Aramo District, South Nias Regency, North Sumatra Province.

Statistical analysis

The toxicity effect was obtained from observations by calculating the percentage of death (mortality) of *Artemia salina* L larvae at each concentration within 24 hours. The percentage of death was obtained from the result of multiplying the ratio by 100%, namely the dead larvae divided by the number of initial larvae multiplied by 100% for each replication. Then compared with the control and data analysis was carried out using SPSS version 25 to obtain the LC₅₀ value. Analysis of the LC₅₀ value is used to determine the relative toxicity of phytochemical compounds and chemical compounds for living organisms (Hadi, 2021).

RESULTS

Based on the results of the toxicity test, a homogeneous data analysis was carried out Oneway ANOVA using SPSS version 25, aiming to determine whether the data set studied has the same characteristics or not. The requirement for a homogeneous test is if the significance value (p) > 0.05 then it is said to be homogeneous, and if the significance value (p) < 0.05 then the data is not homogeneous. The result of the homogeneous data analysis is 0.671. Then a significance test of shrimp larval mortality was carried out, aiming to show the difference in significance between each test concentration.

The requirement for a significance test is if the significance value < 0.05 then it is said to have a significant difference, if the significance value > 0.05 then it is said to have no significant difference.

The level of influence of the concentration of ethanol extract of cardamom leaves (*Amomum cardamomum* L) can be seen in Figure 4.1 on the death of *Artemia salina* L. larvae. The highest percentage of larval mortality was at a concentration of 1000 ppm ($\mu\text{g/ml}$) and 500 ppm ($\mu\text{g/ml}$). While the lowest percentage was at a concentration of 50 ppm ($\mu\text{g/ml}$), 25 ppm ($\mu\text{g/ml}$) and 0 ppm ($\mu\text{g/ml}$) as a negative control. This shows that the higher the concentration of the extract, the higher the total mortality of larvae.

Figure 1. Percentage Graph of the Effect of Cardamom Leaf Extract Concentration (*Amomum cardamomum* L).

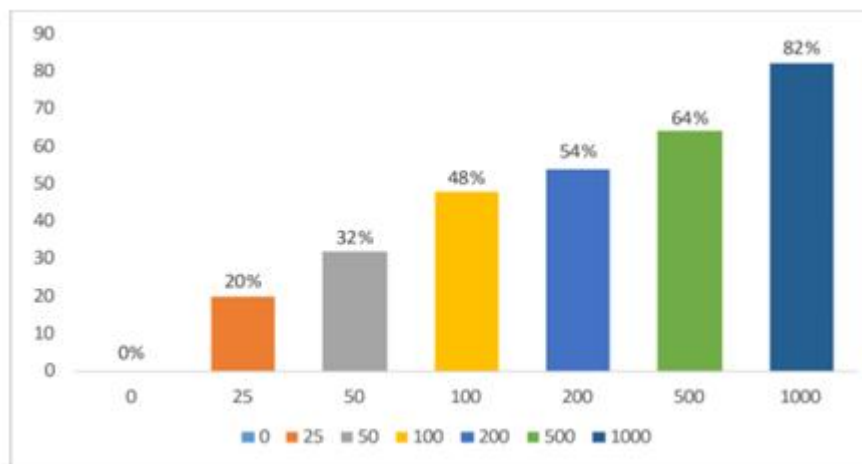
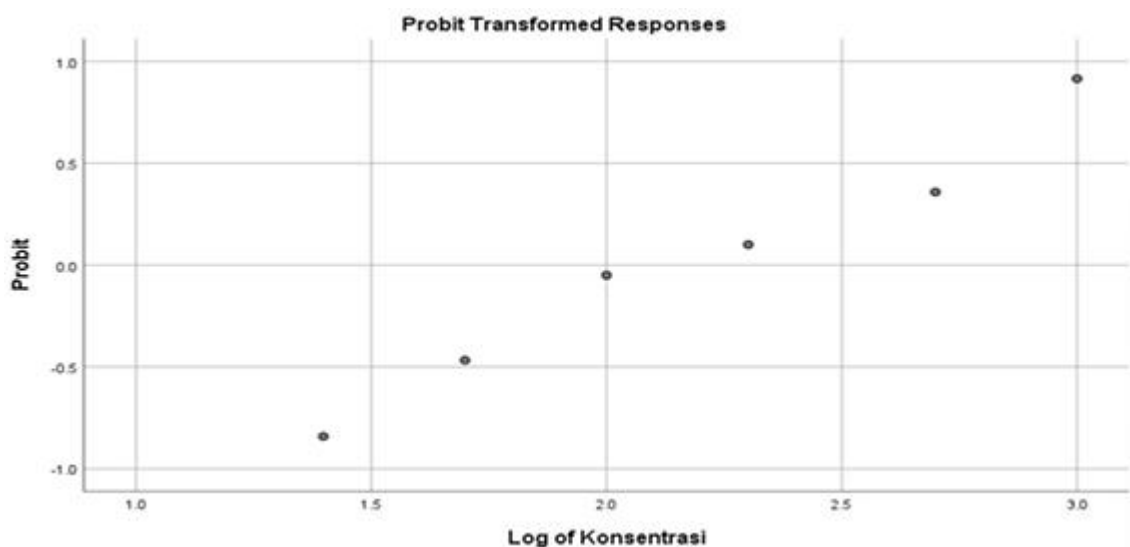


Figure 2. LC₅₀ Probit Analysis Result Curve Using SPSS Version 25



Based on the results of the toxicity test of cardamom leaf extract (*Amomum cardamomum* L) using the BSLT (Brine Shrimp Lethality Test) method in this study is toxic because $LC_{50} < 1000 \mu\text{g/ml}$ so it has the potential as anticancer. According to Mayer (1982) the potential for toxicity activity is based on the LC_{50} value, which is a value that indicates the concentration of toxic substances that can cause death of organisms up to 50% at a concentration $< 1000 \text{ ppm } (\mu\text{g/ml})$ and is said to be non-toxic if the LC_{50} value is $> 1000 \text{ ppm } (\mu\text{g/ml})$. If the LC_{50} value is $< 30 \text{ ppm } (\mu\text{g/ml})$ then the extract is very toxic and has the potential as anticancer. In this study, it was found that the ethanol extract of cardamom leaves (*Amomum cardamomum* L) has high effectiveness so that it is toxic. This is related to the compounds contained in cardamom leaves, namely alkaloids, flavonoids, tannins and saponins, where at certain levels have toxic potential and can cause death of *Artemia salina* L. shrimp larvae.

The mechanism of larval death is thought to be related to the flavonoid and saponin compounds in cardamom leaves inhibiting the larvae's appetite (antifedent). Therefore, the compounds that enter the larvae's body, then the digestive tract will be disrupted, so that these compounds inhibit the taste receptors in the larvae's mouth area.

This causes the larvae to fail to get taste stimuli so that they are unable to recognize food and cause the larvae to die of starvation. (Melisa. 2022).

CONCLUSIONS

From the results of the research that has been conducted, it can be concluded as follows: Ethanol extract of cardamom leaves (*Amomum cardamomum* L) has toxicity activity against *Artemia salina* L using the Brine Shrimp Lethality Test (BSLT) method with an LC_{50} value of 151.671 ppm ($\mu\text{g/ml}$).

SUGGESTIONS

From the results of the research that has been done, there are some suggestions:

- Further research needs to be done with other solvents.
- Further research needs to be done with flowers, fruits, stems and roots of cardamom (*Amomum cardamomum* L).
- Further research needs to be done using zebra fish

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