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TOXICITY TEST OF PANDAN LEAF BEACH ETHANOL EXTRACT (*Pandanus odorifer*) AGAINST SHRIMP LARVAE (*Artemia salina* Leach)

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ABSTRACT

Background: The coastal pandan plant (*Pandanus odorifer*) has biological properties, namely anti-inflammatory, antioxidant, anticancer, antitumor, antiviral, antidiabetic and cholesterol-lowering activities. **Objective:** This study aimed to determine the toxicity level of ethanol extract of coastal pandan leaves (*Pandanus odorifer*) to shrimp larvae (*Artemia salina* Leach) by BSLT method. **Method Research:** The sample used in this study was 200 grams of coastal Pandan simplisia powder (*Pandanus odorifer*) which had been dried at room temperature. 2 maceration processes were carried out using 70% ethanol solvent. The concentration of the test solution is 500 mg/L, 250 mg/L, 100 mg/L, 50 mg/L and 0 mg/L.

Research Results: of the toxicity test of sea pandan ethanol extract on shrimp larvae (*Artemia salina* Leach) obtained an LC50 value of 68.43 mg / L showing that the toxic level in sea pandan leaves has a fairly strong toxic level.

Conclusion: from the results of the research carried out are recommended to conduct further research, namely the identification of pure compounds present in coastal pandan leaves (*Pandanus odorifer*) so that they can be used as herbal medicines or new drug findings.

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PRELIMINARY

Indonesia is a country rich in biological natural resources, including forest holdings and coastlines overgrown with medicinal plants.¹ Herbs are medicinal plants or plants that can be used in traditional medicine against diseases. Treatment of traditional medicine of diseases by using herbs with basic ingredients from plants and something that exists in nature. It is now in great demand by the public because the materials are usually easily available in the surrounding environment.² More scientific developments lead people to an awareness of the superiority of natural ingredients.³ However, traditional medicine still contains chemicals and has side effects if not used carefully. Therefore, in simple terms, the level of safety of traditional medicine can be known whether many traditional ingredients are consumed by the community or not.⁴ Researchers are interested in conducting research on toxicity tests of coastal pandan leaf plant extracts using the BSLT method on shrimp larvae.

The coastal pandanus (*Pandanus odorifer*) is a wild tree and vegetation in a semi-natural seaside environment in tropical and subtropical Pacific areas where it can withstand aridity and storms.⁵ Coastal pandanus lives in coastal areas, especially coastline areas. Several studies on the pandanus genus have been carried out including the energy of *Pandanus odorifer* known as anti-inflammatory, antiviral, antidiabetic, antitumor, antioxidant, anticancer and cholesterol-lowering activity.⁶ Secondary metabolites that plant produced include alkaloids, terpenes, flavonoids, lignin, steroids, curcumin, saponins, phenolics, and glucosides.⁷

Toxicity tests are tests performed on test animals to detect the effects of toxicity on biological systems and to obtain dose response data typical of test preparations.⁸ More or less the chemical content in coastal pandanus plant extracts and potentially toxic

namely terpenoids, tannins, saponins, with flavanoids, at specific concentrations has the potential for toxicity and can cause larval death.⁹ BSLT is one of the screening methods. The purpose of the BSLT method is to be used as a bioassay to examine toxic compounds that exist in plant extracts and to determine the level of toxicity in plants.¹⁰ *Artemia salina* L is used as a test animal in the BSLT method because the classification of this animal is exactly that of mammals, *Artemia salina* L has DNA-dependent RNA polymerase and mammals also have it, RNA polymerase that functions and relies on DNA to form proteins.¹¹

The advantages of toxicity testing using the BSLT method are that the first pharmacological screening procedure is simple, cheap and fast, a reliable method in the search for toxic compounds present in plants that have been extracted coarsely and has a weight of 95% confidence. Toxicity tests should be performed to determine the minimum concentration of poisonous plants. Toxicity is the ability of a chemical substance to cause harm to organisms, both in use and in the environment.¹² Toxicity tests of a compound are divided into two groups, namely general and special toxicity tests.¹³

RESEARCH METHODS

This study using the BSLT Brine Shrimp Lethality Test (BSLT) method is one of the methods used to determine the toxic ability to cells (cytotoxic) of a compound produced by plant extracts using *Artemia salina* Leach shrimp larvae as bioindicators. The independent variable in this study was the concentration of the test solution. The test solution used is a concentration of 500 mg / L, 250 mg / L, 100 mg / L, 50 mg / L and 0 mg / L d. The variable tied to the study is the LC₅₀ value of the toxicity test. The population in this study was larvae of *Artemia salina* leach.¹⁴ The sample used in this study was 200

g of coastal pandan simplisia powder (*Pandanus odorifer*) that had been mashed.¹⁵ Data analysis is the process of organizing and sorting data into forms, categories, and units of basic description so that themes can be found and working hypotheses can be inferred as suggested by the data.¹⁶ The number of LC₅₀ can be calculated by a straight-line equation by entering 5 (50% probability of death of test animals) into Y, creating X as the sum of logarithmic concentrations.

The antilogue of the number X is the number LC₅₀.¹⁷

$$\% = \frac{\text{Death rate}}{\text{number of larvae}} \times 100$$

RESEARCH METHODS

A total of 200 (g) simplisia of coastal pandanus plants (*Pandanus odorifer*) was macerated with 2000 mL of 70% ethanol solvent. The extraction process for 5 days is carried out at room temperature that is not directly exposed to sunlight, with occasional stirring every 24 hours. Maserat I is filtered and squeezed after 5 days, producing filtrate 1 and residue. Then the residue of maserat I was macerated with 1000 mL of 70% ethanol for

3 days, after 3 days maserat II was filtered to produce filtrate 2 and residue. Filtrates 1 and 2 are combined and then the solvent is evaporated with the help of a fan until it becomes a viscous extract and the yield is calculated. The yield yield of coastal pandan leaf ethanol extract can be seen in Table 1.

Table 1. Beach Pandan Leaf Ethanol Extract Marinade

Sample	Weight of simplisia powder (g)	Extract weight (g)	Extract Marinade (%)
Pandan Leaf Beach	200 (g)	15 (g)	7,5

To determine the toxicity level of coastal pandan leaves (*Pandanus odorifer*) using the LC₅₀ method, it is necessary to conduct experiments on *Artemia salina* L larvae whose test solution concentrations are 500 mg / L, 250 mg / L, 100 mg / L, 50 mg / L and 0 mg / L and carried out two replications within 1 day. After 1 day the death of the larvae can be assessed on each vial containing a concentration solution.

Table 2. Toxicity Test Results

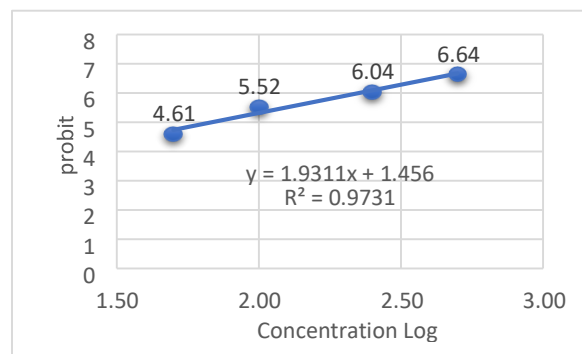
Test Solution Concentration (mg/L)	Concentration Log	Number of Deaths		Average Death	% Death	Probit
		Replication I	Replication II			
500	2.70	10	9	9.5	95	6.6
250	2.40	9	8	8.5	85	6.0
100	2.00	7	7	7	70	5.5
50	1.70	4	3	3.5	35	4.6
0	-	0	0	0	0	-

From the data above, a linear regression equation is created with the help of Microsoft excel where the x axis is the concentration log while the y axis is the probit, obtained the linear regression equation $y = 1.9311x + 1.456$. Next, the value of x is calculated by substituting the number 5 (probit 50% mortality of test animals) into a straight-line equation. In the equation of the number of Y, the amount of LC_{50} when converted to the X antilog, the LC_{50} value is 68.43 mg / L..

DISCUSSION

Beach pandan leaves (*Pandanus odorifer*) taken in Luari Village, North Tobelo District, in July 2022 as samples for making simplisia. The sample is done wet sorting first, this is done to separate foreign materials or impurities attached to the simplisia. After wet sorting, washing of the material is carried out. Washing is done with clean and running water, this is to remove soil or other impurities that are still attached to simplisia. After the material has been washed, cutting / displaying the purpose of the display process is to accelerate simplisia to dry when carrying out the drying process. After the display process is carried out the following steps, namely the drying process, drying is done by aeration, this aims to reduce the water content, by reducing the water content can prevent damage to simplisia. After the dried simplisia is then sorted dry, this is done to prevent if there are still foreign objects attached to the simplisia or separate damaged simplisia. After the simplisia is sorted dry, the material grinding is carried out with a refining tool in the laboratory, the purpose of grinding the material is to make it easier for the solvent and the material to mix evenly.

The extraction process used in this study is by maceration method, this is done because when the simplisia soaking process is about to separate the cell wall and cell



Picture 1. Probit Value Graph

membrane in simplisia until it causes the compounds in the simplisia to break and dissolve in the organic solvent carried out. 41 with 70% ethanol solvent and as much as 200 grams of simplisia powder of beach pandan leaves that have been mashed twice macerated, namely mafiber I, simplisia powder added ethanol solvent as much as 2000 mL and maserat II added solvent as much as 1000 mL. Beach pandan leaf simplisia powder (*Pandanus odorifer*) is macerated for 5 days and every 24 hours stirring is carried out, stirring is carried out with the aim of speeding up the extraction process.¹⁸ The storage must be protected from direct sunlight so as not to affect the extraction process. After the extraction process is carried out for 5 days, then further filtering is carried out so that the filtrate from maserat I and the residue from maserat I is squeezed and macerated again with 1000 mL ethanol for 3 days and stirred every 24 hours. After 3 days the filtrates of maserat I and II are combined to produce a filtrate of 2500 mL, then the combined filtrates I and II are evaporated by aerating with the help of a fan, this is done so that the evaporation process evaporates faster and becomes a viscous extract.

The toxicity test in this study was carried out using the BSLT method which aims to determine the dose of the load of compounds that have the ability as toxic

compounds in cell growth. This research was also carried out 2 times replication, with the aim of confirming that the research to be carried out gets more accurate results. After the evaporation process is complete and the extract has become viscous and the viscous extract is weighed and dissolved using seawater of 1000 mL to produce a solution supply of 1000 mL, toxicity tests are carried out by inserting 10 larvae of Artemia salina Leach shrimp aged 48 hours along with seawater into vials that have contained each Leach shrimp larvae using the BSLT method with the results of the amount of LC50 as much as 68.43 mg / L, it can be concluded that the amount of LC50 found in coastal pandan leaves (*Pandanus odorifer*) with a concentration of 500 mg / L, 250 mg / L, 100 mg / L, 50 mg / L and 0 mg / L have a level of poison that can be said to be strong if not with a certain dose, the death of larvae is solely due to the reaction of each concentration in the vial tube. After 24 hours, the number of larval deaths was checked before calculating LC50 with the help of Microsoft excel.

CONCLUSION

From the results of the toxicity test of coastal pandan leaf extract (*Pandanus odorifer*) in animal tests of Artemia salina Leach shrimp larvae using the BSLT method with the results of the amount of LC50 as much as 68.43 mg / L, it can be concluded that the amount of LC50 found in coastal pandan leaves (*Pandanus odorifer*) with a concentration of 500 mg / L, 250 mg / L, 100 mg / L, 50 mg / L and 0 mg / L have a level of poison that can be said to be strong if not with a certain dose..

SUGGESTION

From the results of the research carried out, it is recommended to conduct further research, namely the identification of pure compounds present in coastal pandan

concentration of extract solution, Then the test tube is placed in a safe place and not exposed to direct sunlight, this is done so that

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leaves. (*Pandanus odorifer*) in order to be used as herbal medicine or new drug findings.

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