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Toxicity Test Of Ethanol Extract Of Pletekan Leaves (Ruellia tuberosa L) Using The Brine Shrimp Lethality Test (BSLT) Method

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Abstract

The pletekan plant (Ruellia tuberosa L.) is a family of Acanthaceae Ruellia tuberosa L.) one of the plants used as a medicinal plant, especially the leaves, have been used empirically for therapy as a diuretic, antidiabetic, antihypertensive. Also used in the treatment of syphilis, kidney stones, cancer, heart disease, colds, hypertension and digestive problems. The purpose of this study was to determine the toxicity by determining the LC_{50} in the ethanol extract of pletekan leaves (Ruellia tuberosa L). The analysis carried out was a toxicity test on the ethanol extract of pletekan leaves against Artemia Salina Leach shrimp larvae using the BSLT method with various concentrations, namely 10, 15, 20, 25, 30, 35, and 40 ppm and determining the LC_{50} value with probit analysis. Based on the results of the toxicity test, the LC_{50} value was 96.4595 µg/Ml. These results show that the ethanol extract of pletekan leaves (Ruellia tuberosa L) has a toxic effect on Artemia Salina Leach shrimp larvae.

Introduction

In Indonesia, many medicinal plants are useful as traditional medicine and have been used empirically for a long time to benefit the health of the body and to treat various diseases (1). Traditional medicine is more easily accepted by the public because it is safer, cheaper and easier to obtain (2). However, activity tests and preclinical trials are needed to determine the effectiveness, dosage and safety of this traditional medicine (3).

Pletekan leaves (Ruellia tuberosa L) are one of the plants used as medicinal plants, especially the leaves. Purple Kencana leaves (Ruellia tuberosa L.) have been used empirically for therapy as a diuresis, antidiabetic, and antihypertensive. Apart from that, Purple Kencana leaves (Ruellia tuberosa L.) are also used in the treatment of syphilis, urinary stones, cancer, heart disease, colds, hypertension and digestive problems (4).

A toxicity test is a test to observe the pharmacological activity of a compound that occurs within a short time after exposure or administration in a certain dose (5). The principle of toxicity testing is that bioactive components are always toxic when administered at high doses and become drugs at low doses (6).

Shrimp larvae have thin skin and are sensitive to their environment, so they are widely used in toxicity tests. Foreign substances or compounds in the environment will be absorbed into the body by diffusion and directly affect life (7). These sensitive shrimp larvae will die if the foreign substance or compound is toxic (8). Toxicity tests are used to determine the toxic effects produced by a single dose of a mixture of

chemicals on experimental animals as a pre-screening test for anticancer bioactive compounds (9).

The BSLT method was chosen because this method is often used for pre-screening of active compounds contained in plant extracts because it is simple, fast, cheap, easy, reliable and the results are representative (10). The toxicity test using BSLT can be determined from the number of deaths of Artemia salina Leach due to the influence of extracts or natural compounds. The test results are expressed as LC_{50} (11).

Based on previous research, namely the toxicity test of pletekan leaves (Ruellia tuberosa L) with ethanol and N-hexane solvents using the BSLT method, it showed that the LC_{50} results on ethanol were 147.91 µg/mL and on N-hexane were 123.03 µg/mL. This study aimed to determine the level of toxicity of pletekan (Ruellia tuberosa L) leaves using the BSLT method against Artemia salina leach larvae.

Methods

Materials

The tools used in this research are analytical scales (kren), rotary evaporator (IKA), furnace (pyrex), hot plate (Thermo Scientific Cimarec), evaporating cup (pyrex), desiccator, measuring flask (pyrex), and container. hatching of Artemia salina Leach eggs. The materials used in this research were pletekan leaves (Ruellia tuberosa L), Artemia Salina Leach eggs (Supreme plus), sea salt (Mandanics), 96% ethanol, distilled water, potassium iodide, iodine, mercury (II) chloride, iron (III) chloride 1%, bismuth nitrate, nitric acid, alpha-naphthol, lead (II) acetate, sodium hydroxide 2 N, anhydrous acetic acid, toluene (Emsure), chloroform (Smart lab), concentrated hydrochloric acid, aquadest, sodium hydroxide, concentrated sulfuric acid.

Procedure

Samples of pletekan leaf plants (*Ruellia tuberosa* L.) were taken from the MedanTimur District area, North Sumatra. The part used is the leaves of the pletekan plant (*Ruellia tuberosa* L).

Making Pletekan leaf extract using the maceration method. A total of 500 grams of powder was macerated with 5000 mL of 96% ethanol. Simplicia powder is put into a maceration container then 3750 mL of 96% ethanol is added. Soak for the first 6 hours, stirring occasionally, then leave for 18 hours cover the container leave for 5 days and place in a place protected from sunlight. During the maceration process, stir occasionally. The dissolved simplicia is filtered, and then the filtrate is collected as macerate I. The soaking process is carried out again with 1250 mL of 96% ethanol until macerate II is obtained and left to sit for 2 days. Maserate I and II are combined and then evaporated using a rotary evaporator (12).

Phytochemical Screening

Phytochemical screening was carried out to determine the content of secondary metabolite chemical compounds in simplified kaffir lime leaves, and ethanol extracts of kaffir lime leaves, including alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides (13).

Toxicity Test

Hatching of Artemia salina leach larvae

Egg hatching is done in clear containers using seawater as the medium. The container used is divided into two parts by a perforated partition, the dark part and the light part. The perforated partition becomes a way for the hatched larvae to move naturally towards the light container filled with one litre of artificial seawater. Then one spoonful of eggs is placed in the dark part. The dark container was covered with aluminium foil or black duct tape. The bright part of the container is illuminated with lamplight so that the hatching temperature of 25-30°C is maintained. Shrimp eggs are left submerged for 48 hours until the eggs hatch. Eggs will hatch within 24-36 hours and will move naturally towards bright areas so that the shrimp larvae are separated from the eggs or eggshells, larvae that have been actively moving are ready to be used as test animals in research (14).

Preparation of Stock Solution

The stock solution was made with a concentration of 1000 ppm, and weighed as much as 0.1 gram and 50 mL of seawater, from the stock solution was made with concentrations of 10, 15, 20, 25, 30, 35 and 40 ppm by dilution. Control (0 ppm) is done without the addition of extracts (15).

Toxicity Testing

10 artemia larvae that have been 48 hours old are taken and put in a vial containing extracts with certain concentrations. Then seawater as much as 10 mL. Each test was accompanied by a negative control and 3 replications were made. The vials were kept illuminated. After 24 hours, the number of larvae that died was counted to determine the probit value and analyzed to determine the LC_{50} value (16).

Data Analysis

The effect of pletekan leaf extract against Artemia salina Leach is done with the calculation of probit analysis. The calculation is done by comparing the dead larvae to the total number so that the percent mortality is seen in the probit table value. From the data be known probit value is entered into the regression equation, to get the LC_{50} value.

Result and Discussion

Extract Results

Weighed as much as 500 grams of pletekan leaf simplisia powder with maceration method using 96% ethanol solvent as much as 5 liters then evaporated with a rotary evaporator and concentrated so that a thick extract of 167.375 grams was obtained.

Phytochemical Screening

Phytochemical screening results show that ethanol extracts of pletekan leaves contain flavonoids, alkaloids, steroids and triterpenoids. The results of screening extract can be seen in Table 1.

Table 1. Phytochemical Screening

No	Chemical compound group	Simplisia
1	Alkaloids	(+)
2	Flavonoids	(+)
3	Saponins	(-)
4	Tannins	(-)
5	Steroids/triterpenoids	(+)
6	Glycosides	(-)

Information:

+ : contains the substance being examined

- : does not contain the substance being examined

Based on the results obtained in Table 1, it show that the extracts leaves positively contain alkaloid compounds, flavonoids, and glycosides.

Toxicity Test Results of Pletekan Leaf Ethanol Extract

The results of the toxicity test of the Ethanol Extract of Pletekan Leaves using the Brine Shrimp Lethality Test (BSLT) method can be seen in Table 2.

Table 2. Percentage of mortality in ethanol of pletekan leaves

No	Concentration (µg/mL)	% Mortality	Log Concentration	Probit value
1	Blanko	0	0	0
2	10	13,3	1	3,8877
3	15	26,8	1.1760	4,3811
4	20	33,3	1.3010	4,5684
5	25	50	1.3979	5,0000
6	30	63,3	1,4771	5,3398
7	35	66,7	1,5440	5,4316
8	40	73,3	1,6020	5,6219

Based on the research results obtained from different larval mortality. From the table above, it can be seen that the percentage of mortality from the lowest concentration of 10µg/mL and the highest concentration of 40µg/mL has a percentage of 73.3% while the blank has no mortality value against larvae. This is in accordance with the theory that the higher the concentration of the extract the more the number of larvae that die.

In addition, from the percentage of larval mortality, it can be concluded that the higher the

concentration of the extract, the higher the number of larval death, the number of larval deaths is also higher.

The data obtained were then analyzed using a probit analysis table to obtain the LC_{50} value. The LC_{50} value can be calculated with the straight line regression equation by entering the value (probit 50% mortality of test animals) as y so that x is produced as the log concentration value. The antilog x value is the LC_{50} value. The parameter that is shown to determine the biological activity of the compounds against test animals is by counting the number of larvae that die due to the effect of giving compounds with a predetermined concentration. After probit analysis, the graph of the straight line equation Y = 2.9406x + 0.8999 can be seen as figure 1.

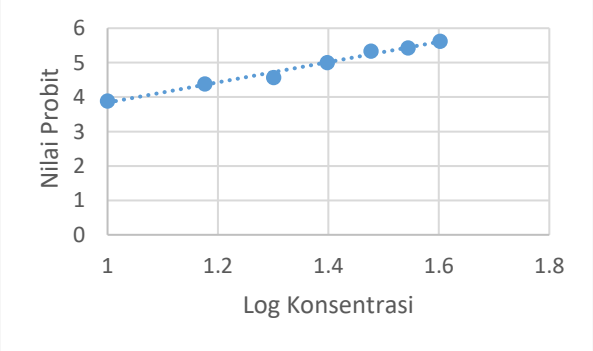

Figure 1. Observation data graph of shrimp larval mortality using probit analysis

The x-value antilog becomes the parameter of LC_{50} which indicates the presence of biological activity in a compound against testanimals, namely by calculating the number of larvae that die due to the effect of giving the compound that has been determined. The probit analysis of the feather clam shell chitosan obtained a graph of the regression equation y=2.9406x +0.8999. The probit value of 50% of the test animals obtained an x value of 1.3979, and the antilog LC_{50} value of 1.9843 is 96.4595µg/mL.

A compound is said to be toxic if LC_{50} < 1000 and is said to be non-toxic if LC_{50} > 1000. From the results obtained, the compound is potentially toxic. This low and toxic LC_{50} value is due to the high mortality of Artemia salina leach larvae at each concentration, namely at this concentration mortality reaches 50% of the number of larvae tested.

Conclusion

Secondary metabolite compounds contained in the ethanol extract of pletekan leaves (Ruellia tuberosa L) by phytochemical screening method are flavonoids, alkaloids, and steroids / tripernoids. Based on the results of toxicity tests using the BSLT method with concentrations of 10, 15, 20, 25, 30, 35 and 40 ppm, the LC_{50} value = 96.4595µg/mL was obtained, which indicates that the ethanol extract of pletekan leaves has toxic properties.

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