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Brine Shrimp Lethality Test (BSLT) of Methanol and Ethyl Acetate Extract of Vinca Flower (*Catharanthus roseus*)

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ABSTRACT

Vinca (*Catharanthus roseus*) is a plant that belongs to the Apocynaceae family. The purpose of this study was to determine the percentage of mortality data and the LC_{50} value of the Vinca flower extract (*Catharanthus roseus*) against the larvae of *Artemia salina* Leach using the Brine Shrimp Lethality Test (BSLT) method. The method used is the Brine Shrimp Lethality Test (BSLT). The methanol extract test consisted of six treatments, namely 0 ppm, 20 ppm, 60 ppm, 100 ppm, 140 ppm, and 180 ppm, and the ethyl acetate extract test consisted of seven concentration treatments, namely, 0 ppm, 200 ppm, 220 ppm, 240 ppm, 260 ppm, 280 ppm, 300 ppm. At each concentration, 10 test animals were used, *Artemia salina* Leach larvae aged 48 hours. The toxic effect of the extract was identified by the percentage of mortality of *Artemia salina* Leach larvae using a probit analysis to obtain a 50% Lethality Concentration (LC_{50}) value. The results of the study in the form of a linear regression equation from the probit analysis is y = 3.0809 x - 12.065 with a value of LC_{50} = 34.599 ppm. The linear regression equation for ethyl acetate extract is y = 15.10178 x- 30.0269 with a value of LC_{50} = 208.636 ppm. The conclusion of this study is that Vinca flowers are toxic because they have an LC50 value <1000 ppm.

Keywords: BSLT, *Catharanthus roseus*, Extract, Toxicity.

INTRODUCTION

Medicinal plants have long been used by the people of Indonesia in an effort to cure and prevent disease, increase endurance and restore fitness. As it is known that Indonesia is a country that has rich biodiversity, around 30 thousand types of plants in Indonesia, more than 1000 types have been used for medicinal purposes [1]. These research centers use the latest scientific methods to analyze biologically active ingredients. Apart from efficacy and quality, a factor that must be considered is the safety of traditional medicines in the use of herbal preparations as treatment [2,3]. One of the medicinal plants is the vinca plant (*Catharanthus roseus*). *Catharanthus roseus* is an important medicinal plant in the *Apocynaceae* family. *Catharanthus roseus* is a species endemic to Madagascar, has two types of flower colors, namely the pink flower of *Catharanthus roseus* and the white flower of *Catharanthus alba* [4]. According to Verrananda (2016), in his research, he stated that the results of phytochemical screening contained alkaloids, flavonoids, phenolics, tannins and terpenoids. The secondary metabolites with IC50 values in *Catharanthus roseus* showed natural antioxidant activity as an antidote to free radicals [5,6]. Various studies have been carried out to examine its contents and benefits, including vinca which has anti-hyperglycemic and hypoglycemic effects, antibacterial, and diabetes effects. According to previous research conducted by Prasetyo (2016), showing that extracts of

vinca flowers with concentrations of 60%, 70%, 80%, 90% and 100% showed that LC50 was achieved at a concentration of 91.2 ppm [7-9].

METHODS

Plant Extraction

5 kg of vinca flowers are taken in the morning, then wet sorting is carried out to separate impurities or other foreign materials and weighed. Furthermore, washing is carried out using a flowing stream to remove soil and other impurities that are attached to the simplicia material. After that, the drying process is in the drying cabinet to obtain simplicia that is not easily damaged, so that it can be stored for a longer time, then blended to obtain simplicia powder [10]. The extraction method used is maceration with a ratio of 1:10 macerated simplicia powder for 5 days, as much as 360 g of simplicia is put into a glass jar then immersed in 2,700 ml of methanol solvent covered with aluminum foil for 3 days (stirring occasionally) then filtered using filter paper and obtained filtrate 1 and residue. The residue was re-soaked using 900 ml of methanol solvent for 2 days (stirring occasionally) then filtered using filter paper and obtained filtrate 2 and residue. Put together the filtrate 1 and 2 then evaporated using a rotary evaporator with a temperature of 400C - 600C until a thick extract was obtained. The same is done using ethyl acetate solvent [11].

Sea Water Artificial Preparation and Hatching Shrimp Larvae

Artificial Seawater is prepared by dissolving 150 grams of NaCl in 10 liters of distilled water. Then the sea water is first measured for pH using a pH meter, obtained pH 8-9 [12,13]. The hatchery of shrimp larvae is carried out in the aquarium. Previously, the aquarium was divided into light and dark sections, then given a barrier in the form of a styrofoam whose bottom edge had been perforated so that the hatched eggs could come out of the hole. The aquarium is then filled with sea water so that the two holes in the styrofoam are submerged [12,13]. In a dark room filled with 1 egg spoon, then covered with black duct tape and aluminum foil. In a bright room, it is illuminated using fluorescent lights to stimulate hatching. Then in the bright room an aerator is installed to provide oxygen to the eggs that hatch into larvae and move to the bright room. After the eggs hatch into larvae that are 24 hours old, then they are transferred to another container until they are 48 hours old. Larvae that are 48 hours old can be used as test animals in the BSLT method experiment [12-13].

Sample Preparation

The solution is made from 2 grams of extract that has been weighed and then dissolved with 2 ml of DMSO and added with distilled water until the volume reaches 1000 ml so that the main solution concentration is 2000 ppm (15). After obtaining 2000 ppm of mains solution, dilution is carried out to obtain a test solution with a concentration of 20 ppm, 60 ppm, 100 ppm, 140 ppm, 180 ppm in methanol extract. Meanwhile, the ethyl acetate extract was diluted to obtain a test solution with a concentration of 200 ppm, 220 ppm, 240 ppm, 260 ppm, 280 ppm, 300 ppm.

Brine Shrimp Lethality Test (BSLT)

In the methanol extract, 10 larvae of *Artemia salina* Leach shrimp that were 48 hours old were put into a test tube and piped the test solution with a dilution concentration of 20 ppm, 60 ppm, 100 ppm, 140 ppm, 180 ppm. then stir with sea water up to 10 ml. Each concentration was repeated 3 times and compared with negative control. Counted the number of larvae that died after 24 hours in each test tube. In the ethyl acetate extract, 10 larvae of *Artemia salina* Leach shrimp that were 48 hours old were put into a test tube and piped the test solution with a dilution concentration of 200 ppm, 220 ppm, 240 ppm, 260 ppm, 280 ppm, 300 ppm. then stir with sea water up to 10 ml. Each concentration was repeated 3 times and compared with negative control. The number of larvae that died after 24 hours in each test tube was counted. The count was carried out using a magnifying glass loop or under a lamp. The dead larvae were identified from the absence of movement during observation [13].

DATA ANALYSIS

To determine the percentage of larval mortality for each concentration using Microsoft Office Excel, graph the straight-line equation of the relationship between the probit value and the log of concentration. The LC_{50} value can be calculated from the straight-line equation by plugging in the value 5 as y. The value of 5 is obtained based on the probit value of 50% of the deaths of the tested animals. So that the x value is generated as the log of concentration. The LC_{50} value is the antilog to the x value (15). In the manual probit analysis method, the probit value is known by converting the percent mortality value of larvae for each concentration to the probit value in the table by determining the log of concentration and making a straight line equation $y = mx + b$, where y is the probit value and x is the concentration log [13,14].

RESULTS AND DISCUSSION

Plant Extraction Result

Catharanthus roseus flowers were extracted using methanol and ethyl acetate solvents then filtered and evaporated using a rotary evaporator to evaporate the solvent so that a thick extract of vinca flower was obtained as a solvent of methanol as much as 64.16 grams with a yield of 17.82% and extract of vinca flowers. As much as 7.41 grams of ethyl acetate solvent with a yield yield of 2.47%.

BSLT Result

The observation results of *Artemia salina* Leach larvae after giving methanol and ethyl acetate extracts of *Catharanthus roseus* flowers with various concentrations after 24 hours can be seen in table 1. The regression equation graph can be seen in Figures 1 and 2.

Table 1. Effect of extract concentration on mortality

Sample	Concentration (ppm)	Mortality	Percentage of Mortality (%)	LC50 Value
Methanol Extract	0	0	0	34.599
	20	10	33	
	60	20	66	
	100	26	86	
	140	27	90	
	180	30	100	
Ethyl Acetate Extract	0	0	0	208.593
	200	16	53	
	220	19	63	
	240	22	73	
	260	26	86	
	280	28	93	
	300	30	100	

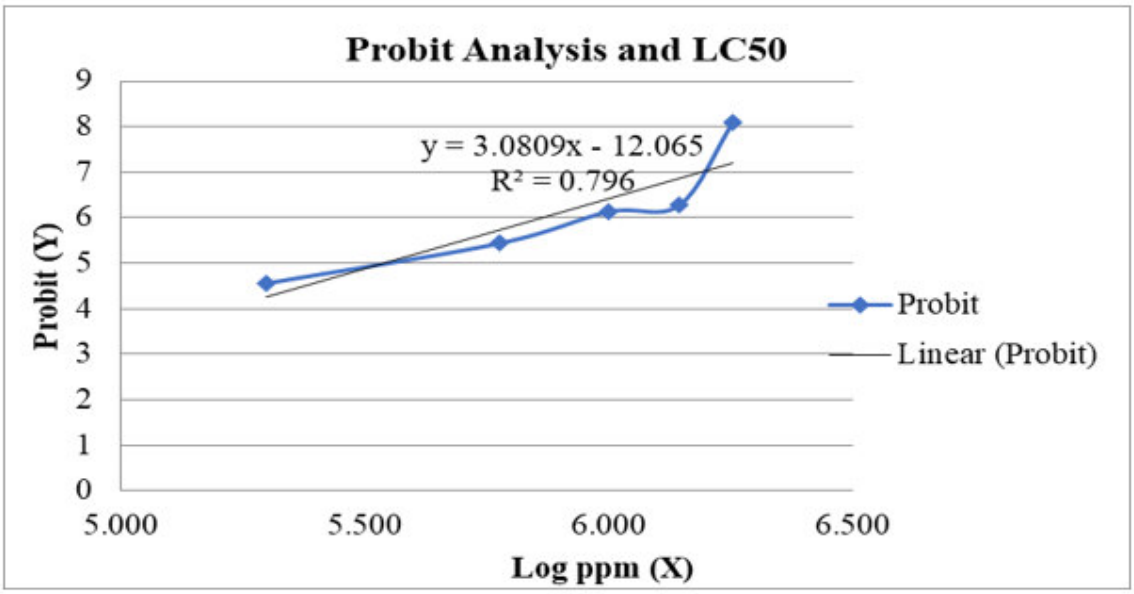

Figure 1. Regression equation of methanol extract

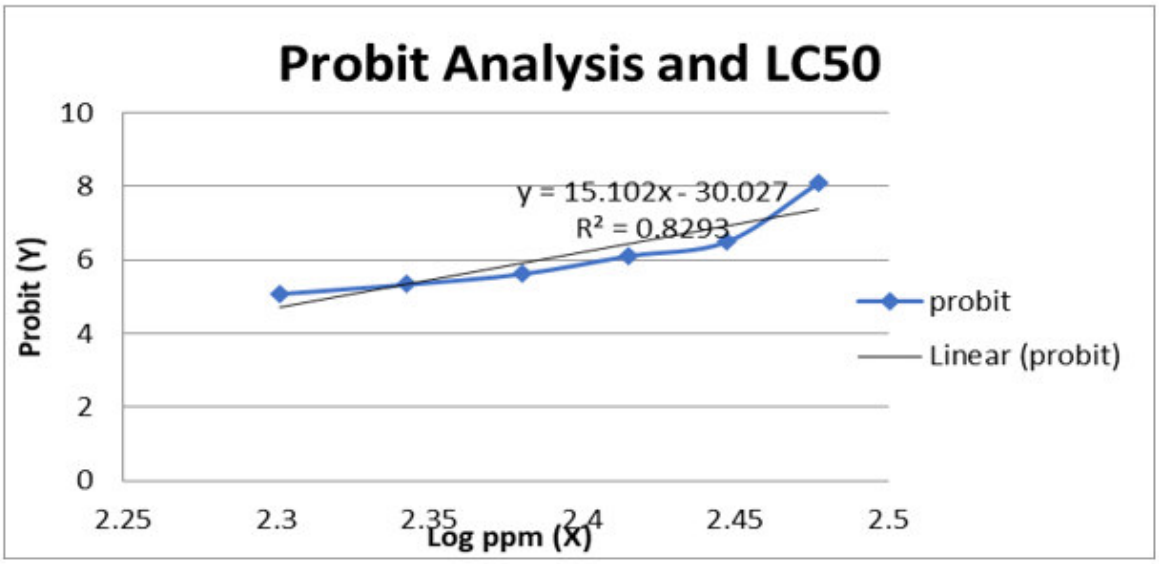

Figure 2. Regression equation of ethyl acetate extract

In the table above shows the percentage of mortality of *Artemia salina* Leach larvae with 3 repetitions. In the methanol extract of *Catharanthus roseus* flowers with concentrations of 0, 20 ppm, 60 ppm, 100 ppm, 140 ppm, and 180 ppm, the total mortality of larvae was 0.10, 20, 26, 27, 30, and an average mortality of 0, 0.33, 0.66, 0.86, 0.90, 1 with the percentage of deaths of 0%, 33.33%, 66.66%, 86.66%, 90%, 100%. Whereas in the ethyl acetate extract concentrations of 0 ppm, 200 ppm, 220 ppm, 240 ppm, 260 ppm, 280 ppm and 300 ppm had total larvae mortality of 0, 16, 19, 22, 26, 28, 30, an average mortality of 0 , 0.53, 0.63, 0.73, 0.86, 0.93, 1, with the percentage of deaths of 0%, 53%, 63%, 73%, 86%, 93%, 100%. This shows that the higher the level of extract concentration, the higher the total mortality of larvae. Figure 1 shows the relationship between the percentage of mortality of *Artemia salina* Leach larvae and the log of vinca flower methanol extract concentration. The linear regression equation from the graph above is y = 3.0809 x - 12.065. Whereas in Figure 2 log the concentration of ethyl acetate extract with the linear regression equation from the graph above is y = 15.10177 x + 30.02690. In this study, it was found that the methanol and ethyl acetate extracts of vinca flowers had high effectiveness so that they were toxic. This is related to the compounds contained in vinca flowers, namely alkaloids, phenolics, flavonoids, terpenoids, and tannins, which at certain levels have toxic potential and can cause the death of Artemia salina Leach shrimp larvae. The phase used in this study is the nauplius phase because in this phase Artemia salina Leach is in a very active phase to divide by mitosis which is identical to cancer cells which also divide by mitosis. This is why the BSLT test is often performed as a preliminary test of anticancer activity [14,15]. Based on the toxicity test of methanol and ethyl acetate extracts of vinca flowers with the BSLT (Brine Shrimp Lethality Test) method, this study is toxic because LC_{50} < 1000 ppm so it has the potential as an anticancer. According to Meyer (1982) and Anderson (1991), reported that an extract showed toxicity activity in BSLT if the extract could cause the death of 50% of the test animals at a concentration less than 1000 ppm. Based on the above statement, the methanol extract of the vinca flower is toxic. This is shown by the acquisition of methanol extract data reaching LC50 at a concentration of 34.599 ppm while the data obtained from ethyl acetate extract reached LC50 at a concentration of 208.5931 ppm [15].

CONCLUSIONS

Methanol and ethyl acetate extracts of vinca flowers (*Catharanthus roseus*) have potential toxicity against *Artemia salina* Leach using the Brine Shrimp Lethality Test (BSLT) method. The results of the LC50 value of the methanol extract determined by probit analysis were 34.599 ppm while the ethyl acetate extract was 208.5931 ppm.

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