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ACUTE TOXICITY TEST OF ANTHELMINTIC HERBAL ON *Artemia salina* LARVAE

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Abstract: Jamu X is a traditional medicine that is believed to provide anti-inflammatory effects, contains natural ingredients such as *Curcuma xanthorrhiza Robx* and *Curcuma aeruginaosa Robx*, has long been used to treat worm infections in children, but it has not passed preclinical tests. The use of traditional medicine in Indonesia has also increased to 44.3%, 55.3% in liquid form, and the rest in powder form. Therefore, this study aimed to determine the potential acute toxicity of Jamu X to *Artemia salina* larvae presented as its LC_{50} Furthermore, to determine the damaged parts of the larval organs using the Brine Shrimp Lethality Test (BSLT) method. This experimental study used 10000, 1000, 100, 10, 1, and 0.1 ppm, and each concentration contained 10 *Artemia salina* larvae. Then, we obtained the upper limit of 10,000 and the lower limit of 1000. In the following test, the larvae were grouped into six groups consisting of 10 *Artemia salina* larvae, and each group was given Jamu "X" solution with a series of concentrations 10000, 7820, 5640, .168, 1780, and 1000 ppm. Mortality data of *Artemia salina* larvae were analyzed by probit analysis to determine the value of LC_{50}. The results of this study showed the LC_{50} value was 1548.81 µg/mL, and damage was found in the thorax, antennae, antennule, stigma, and abdomen. It shows that Jamu X does not have the potential for acute toxicity or is non-toxic, which is indicated by the value of LC_{50} > 1000 ppm.

Keywords: *Jamu, Toxicity, Artemia salina*

INTRODUCTION

Jamu is part of Indonesian culture and natural wealth included in traditional medicine[1]. The nation's ancestors passed down herbal medicine because it has been used for generations as medicine and health maintenance[2]. Basic Health Research (Riskesdas) from 2010 to 2018 stated that the use of traditional medicines by the community to maintain health has increased to 44.3%. The use of herbal medicine in liquid form reached 55.3%, and others used herbal medicine in powder dosage form [3].

One of the herbs used by the community that is believed to be able to treat anthelmintics in children is a herbal medicine containing *Curcuma xanthorrhiza Robx* and *Curcuma aeruginosa Robx*, which can treat or prevent helminth infections [4]. Jamu is a preparation that has not passed a preclinical test. One of the preclinical tests is the toxicity test, a pharmacological test to assess the toxicity or safety of a natural substance used for treatment [5]. A toxicity test is important to determine the level of damage caused by a compound for biological and non-biological materials. It is useful in developing new drugs in knowing the therapeutic potential produced by a drug [6].

The in vitro Brine Shrimp Lethality Test (BSLT) method is commonly used in toxicity tests using test animals in the form of *Artemia salina* [7]. The Brine Shrimp Lethality Test is used as an initial screening in detecting toxic compounds in a natural compound by looking at the LC_{50} value of the active compound [8]. The advantage of this method is that it is fast, cheap, and accurate enough to be used for the initial safety screening of active extracts using *Artemia salina* shrimp [9].

The community often uses Jamu, believed to be an anthelmintic medicine for children, because it already affects curing helminthiasis. Therefore, to increase the use of herbal medicine, which has only been tested empirically, it is necessary to carry out preclinical tests, one of which is a toxicity test. This study aimed to determine the LC_{50} and the level of damage to the larval organs of *Artemia salina* due to the administration of herbal medicine with various concentrations using the BSLT method.

RESEARCH METHODS

Materials

The materials used in this study were Brand X children's anthelmintic herbs circulating in the market, *Artemia salina* eggs, mineral water, and seawater.

Tools

The tools used in this study were 100 mL cups, 100 mL and 1000 mL micropipettes, 25 mL and 10 mL volumetric flasks, Pasteur pipettes, 50 mL and 2 L measuring cups, 50 mL beaker, incandescent lamp, aquarium thermometer, aquarium, analytical balance, pH stick, binocular light microscope, aerator, and stir bar

Sample Preparation

4 L of seawater was put into a modified aquarium with two sides (bright side and dark side) and separated by a perforated partition. Install the aerator on the aquarium's dark side and check the

water's pH using a pH stick and the temperature of the water with a thermometer. *Artemia salina* eggs were weighed as much as 0.1 g and put into the aquarium on the dark side. The aquarium is lit with a fluorescent lamp on the bright side, and wait up to 3 days for *Artemia salina* eggs to hatch into larvae.

Preliminary Test

A preliminary test is the first before a real test on toxicity. This test aims to estimate the actual test's upper and lower limit concentrations [10]. The preliminary test was carried out by preparing a test solution for children's anthelmintic herbal medicine brand X and providing 6 cups of 100 mL containing six test solutions and one control.

Herbal medicine brand X was weighed as much as 7 g using an analytical balance. The herbal medicine that has been weighed is dissolved using 50 mL of warm mineral water in a beaker glass and stirred with a stirring rod until dissolved. Six concentration variants of 10 mL were made using a volumetric flask from this solution. The concentrations were 10000, 1000, 100, 10, 1, and 0.1 ppm with multilevel dilutions. The solution with each concentration was transferred into a 100 ml cup, and 10 *artemia salina* larvae were added at each concentration using a Pasteur pipette. Observation was carried out for 24 hours on the death of shrimp larvae. After the death data is obtained, the concentration for the real test is searched using the formula [11].

$$F = \sqrt[r]{l}$$

Information :
F = Scatter factor
l = Lower limit / upper limit
r = number of concentration variations – 1

The lower limit is the test solution's lowest concentration, which causes larvae death. In contrast, the upper limit is the test solution's highest concentration, which causes larvae death. Six concentration ranges were made from the lower and upper limits used for the actual test.

Actual Test

Jamu anthelmintic "X" was weighed as much as 7 g using an analytical balance. Dissolve the herbal medicine weighed using 50 mL of warm mineral water in a beaker glass and stir using a stir bar until dissolved. Six concentration variants of 10 mL were made using a volumetric flask from this solution. The concentrations with multilevel dilution consisted of 10.000, 7820, 5640, 3168, 1780, and 1000 ppm. The solution with each concentration was transferred into a 100 ml cup, and 10 *Artemia salina* larvae were added at each concentration using a Pasteur pipette. After being left for a 24-hour, the number of surviving and dead larvae was counted in each test cup [12].

The control was compared to the above procedure without adding brand X anthelmintic herbs. Observations were made for 24 hours on the death of shrimp larvae. The data obtained for each concentration is entered into table [13]. Calculation of Lc 50 is done using probit analysis of Microsoft Excel software [14].

Morphological Observations of *Artemia salina Larvae*

Artemia salina larvae were taken in the test solution and left for 24 hours using a Pasteur pipette. Each larva was placed on a glass slide and observed under a light microscope with a magnification of 100x. The morphology of Artemia salina was observed to determine whether there was damage.

RESULTS AND DISCUSSION

Preliminary tests on anthelmintic herbs following the procedures in the method produced the data listed in Table 1.

Table 1. Preliminary results of the 24-hour acute toxicity test solution for children's anthelmintic herbal preparations "X"

Concentration (ppm)	Larval Mortality
10000 (a)	10
1000 (b)	1
100	0
10	0
1	0
0.1	0

Description : (a) = Upper limit ; (b) = lower bound

The upper limit for the concentration or the highest concentration that causes death is found at a concentration of 10000 ppm, while the lower limit for the concentration or the smallest concentration that can still cause death is at a concentration of 1000 ppm. The data is then entered into equation (i) and obtained results worth 1.78 as a multiplier factor to determine the concentration of the test solution in the actual test with an upper limit of 10000 ppm and a lower limit of 1000 ppm in order to obtain the concentration of the test solution for the actual test of 10000, 7820, 5640, 3168, 1780 and 1000 ppm respectively. Then an actual test was carried out with the results in Table 2.

Table 2. Results of the 24-hour acute toxicity test solution for children's anthelmintic herbal medicine preparations "X

Concentration (ppm)	Logarithm of Concentration	Number of Larval Mortality			% Death	Probit
		P	R1	R2		
10000	4	10	10	10	100	6.97
7820	3.9	8	9	9	87	6.13
5640	3.8	8	8	9	83	5.95
3168	3.5	6	7	7	67	5.44
1780	3.3	6	6	6	60	5.25
1000	3	4	4	3	37	4.67

Description: P = treatment 1 ; R1 = replication 1 ; R2 = replication

The tests that have been carried out show a clear relationship between the concentration level and mortality of *Artemia salina* larvae, as indicated by the increase in the mortality rate of *Artemia salina* larvae. The table above shows that the higher the concentration of the test solution, the higher the death rate. The toxicity of a test compound can be identified based on the value of LC_{50}. LC_{50} (Lethal Concentration 50) is the concentration of a substance or compound needed to kill 50% of the organisms tested under specified conditions [15]. The smaller the LC50 value, the higher the ability of the substance or compound to kill shrimp larvae, indicating a high toxicity [16]. The results of the actual toxicity test of Jamu Anak "X" by the Probit method showed a value of LC_{50} 1548.81 ppm. Extracts with LC_{50} < 1000 ppm are considered toxic, while extracts with LC_{50} > 1000 ppm are considered non-toxic [17]. Jamu Anak "X" is included in the non-toxic category based on the specified toxicity category. The validity of these results is supported by 0% mortality in the negative control, which indicates that the death of the larvae is only caused by the "X" Children's Herbal Medicine test solution. In addition, the relationship between larval mortality and the concentration of the test solution is depicted in Figure 1.

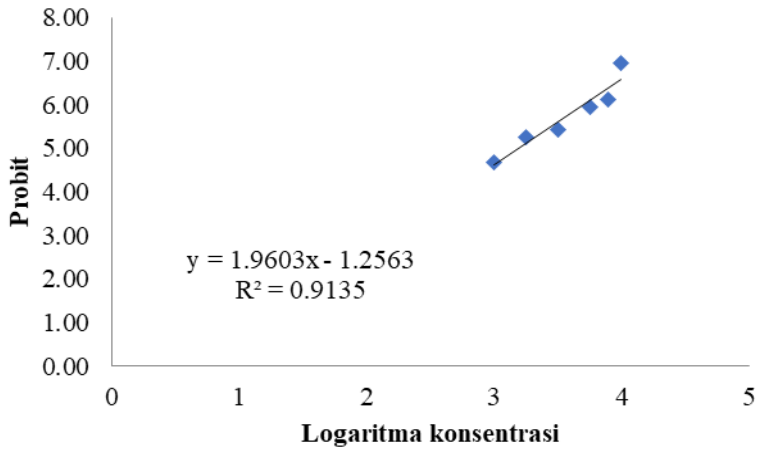

Figure 1. The curve of the relationship between the probit value and the logarithm of the concentration

The curve in Figure 1 shows that the probit value representing the percentage of mortality of *Artemia salina* larvae shows a very strong relationship with a coefficient of determination of 0.9135. If the value of the coefficient of determination (R^2) is closer to 1, which means that the influence of the independent variable on the dependent variable is getting bigger; this indicates that the model used can explain the result of these variables well [18].

Based on the results of microscope observations with a total magnification of 100x, the results of the morphology of *Artemia salina* larvae were as follows:

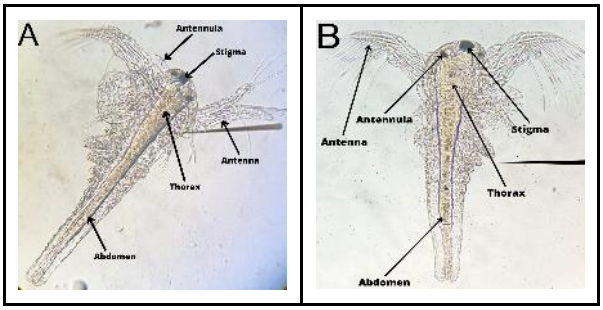

Figure 2. Morphology of *Artemia salina* larvae (A) negative seawater control and (B) 1000 ppm test solution. Negative control (A) and 1000 ppm test solution (B) showed no morphological damage.

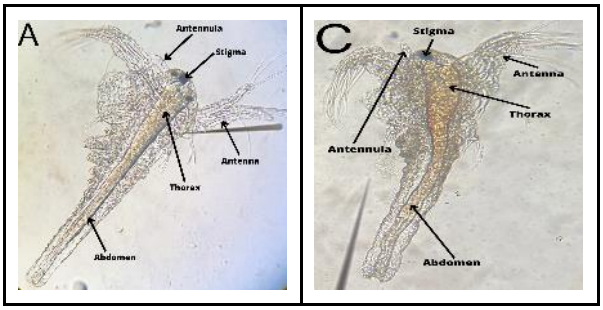

Figure 3. Morphology of *Artemia salina* larvae (A) negative seawater control and (C) 1780 ppm test solution. Negative control (A) and 1780 ppm test solution (C) There was no evidence of morphological damage.

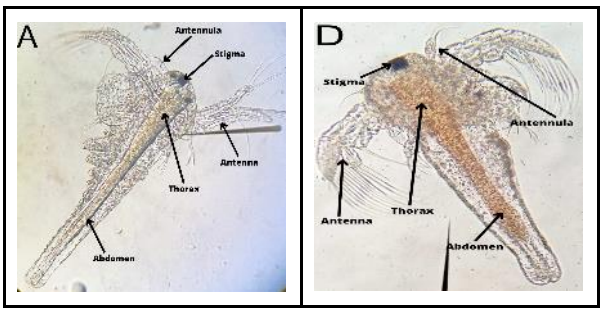

Figure 4. Morphology of *Artemia salina* larvae (A) negative seawater control and (D) 3168 ppm test solution. Negative control (A) and 3168 ppm test solution (D) Showed no evidence of morphological damage.

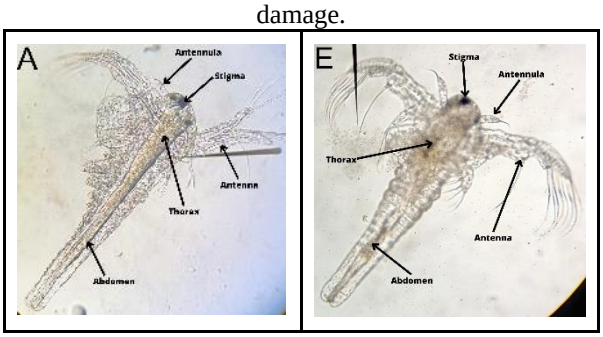

Figure 5. Morphology of *Artemia salina* larvae (A) negative seawater control and (E) 5168 ppm test solution. The 5168 ppm (E) test solution caused damage to the abdominal and thoracic organs with a darker color change compared to the negative control seawater (A).

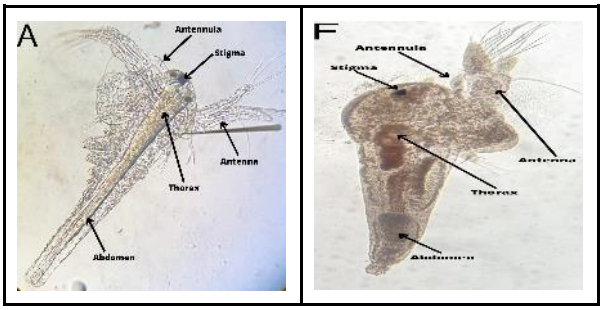

Figure 6. Morphology of *Artemia salina* larvae (A) negative seawater control and (F) 7820 ppm test solution. The 7820 ppm (F) test solution causes damage to the Abdomen, Antenna, Antennula, Stigma, and Thorax organs with a darker color change compared to the 5168 ppm (E) test solution.

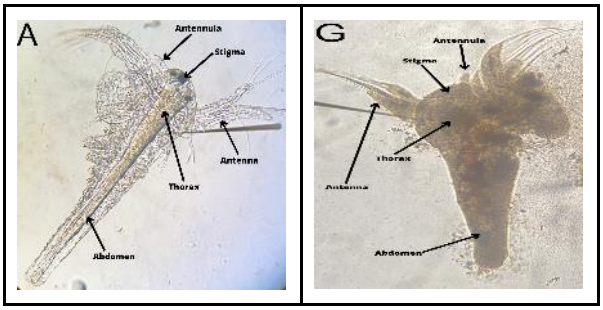

Figure 7. Morphology of *Artemia salina* larvae (A) negative seawater control and (G) 10000 ppm test solution. The 10000 ppm (G) test solution for damage to the organs is the same as in the 7820 ppm (F) test solution, namely Abdomen, Antenna, Antennula, Stigma, and Thorax. However, the color change is darker when compared to the 5168 ppm (G) test solution.

The death of Artemia saliana larvae is caused by toxic compounds that enter through the larvae's mouth and are absorbed into the digestive tract so that there is a process of damage to metabolic reactions in larvae [19,20].

CONCLUSION

Based on the results, the anthelmintic herb "X" LC value was 1548.81 µg/mL, included in the non-toxic category. Description of damage to *Artemia salina* larvae due to exposure to anthelmintic herbs occurs in the thorax, antennae, and abdomen organs.

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