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ACUTE TOXICITY TEST OF BARK AND STEM ETHANOL EXTRACT OF SOPANG (*Caesalpinia sappan Linn*) BY BRINE SHRIMP LETHALITY TEST

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ABSTRAK

Sopang (Caesalpinia sappan Linn) empiris dikenal sebagai tanaman obat. Uji toksisitas akut dengan Uji Brine Shrimp Lethality berusaha untuk mengevaluasi Konsentrasi Lethal Median (LC50) dari kulit kayu dan batang ekstrak etanol Sopang (Caesalpinia sappan Linn). Bark dan ekstrak etanol batang Sopang (Caesalpinia sappan Linn) yang beracun. LC50 untuk ekstrak etanol kulit Sopang (Caesalpinia sappan Linn) adalah 204.644 ppm dan LC50 untuk ekstrak etanol batang Sopang (Caesalpinia sappan Linn) adalah 239.883 ppm.

ABSTRACT

Test sought to evaluate Median Lethal Concentration (LC50) of bark and stem ethanol extract of Sopang (Caesalpinia sappan Linn). Bark and stem ethanol extract of Sopang (Caesalpinia sappan Linn) are toxic. LC50 for bark ethanol extract of Sopang (Sopang (Caesalpinia sappan Linn) empirical known as medicine plant. Acute toxicity test with Brine Shrimp Lethality Caesalpinia sappan Linn) is 204,644 ppm and LC50 for stem ethanol extract of Sopang (Caesalpinia sappan Linn) is 239,883 ppm.

Keywords: *Bark and stem ethanol extract of Sopang (Caesalpinia sappan Linn), Brine Shrimp Lethality Test (BST), Artemia salina Leach, Median Lethal Concentration (LC50).*

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INTRODUCTION

Utilization of biological diversity (biodiversity) for welfare has been done in a traditional, historical or through application of modern technology. However, there are still many who have not explored the potential of forests to be developed as a source phytopharmaca or modern medicine (Ohlstein et al. 2000). Research and development with a distinct and systematic stages needed in the utility of traditional medicine. The stages include the selection of materials based on the use of public information and literature about the chemical content of plants, biological screening test (screening biologically) that include acute toxicity test, subacute toxicity test, chronic and specific toxicity test, formulation development and clinical trials in humans (Bawa 2009).

Sopang (*Caesalpinia sappan Linn*) is a native plant in East Kalimantan which has empirically proven as a medicinal plant. One of method to acute toxicity test of bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) is Brine Shrimp Lethality Test (BST) on shrimp larvae (Artemia salina Leach). This test is important as information about mortality response relationship with the concentration of the experimental animal that is response 50% mortality of larvae shrimp (Artemia salina Leach) of bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*), which is applied to determine the value of the Median Lethal Concentration (LC50) after 24 hours of treatment (Sutisna 2000, Widyastuti 2008).

MATERIALS AND METHODS

Type of research is experimental of laboratory with research design with post test only control group design. Form of experimental research and data collection conducted at the Laboratory of Pharmacology Faculty of Medicine Mulawarman University and Chemical Wood Laboratory Forestry Faculty Mulawarman University Samarinda in East Kalimantan from December 2009 through March 2010. The samples were Sopang (*Caesalpinia sappan Linn*) bark and stem taken from Sekolah Darat Subdistrict, Kutai Barat. Most herbarium specimens are made and photographed. Determination is assisted by botanists Dendrology and Ecology Laboratory of Forestry Faculty Mulawarman University to get certified the validity of the material specimen.

The samples were grinded extracted using maceration method, which uses absolute ethanol solvent to soak the sample for 5 days. After obtaining the filtrate, then filtered with a glass funnel equipment and filter paper and vacuum suction to separate the extract from the plants. The extract was concentrated by rotary evaporator to be at a temperature of 40º C to obtain extract material similar to thick syrup. And then dried by entering into a desiccator. Shrimp eggs included in the breeding box of shrimp larvae were divided in half with a perforated septum. Eggs are placed on the left (the dark box). After 48 hours the egg will hatch and move into the light close to the 40 watt ball incandescent.

Acute Toxicity Test Method Brine Shrimp Lethality Test (BST)

Sea water is used as a medium for the observation of shrimp larvae. Media is placed into the aquarium, then the aquarium is divided into two parts. Furthermore, created a section to a dark aquarium with a black background paper and put shrimp eggs sufficiently in that section. Eggs left for 48 hours until they hatch with the help of radiation to be larval shrimp that will be used as materials in the test observations. Prepared the clean tubes and made the bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) concentrations of 10, 50, 100, 250, 500, and 1000 ppm with sea water. Created control solution for each concentration using sea water without the addition of extract. Added 10 shrimp larvae in each sample and were observed for 24 hours. Calculated and recorded the number of mortal shrimp larvae are carried by at least two people. Three times replication was done. Percent mortality of shrimp larvae is calculated using the following formula:

$$\% \text{ of larvae mortality} = \frac{A}{B} \times 100\%$$

Where:
A = sigma of mortality of larvae Artemia salina Leach
B= sigma of larvae of Artemia salina Leach

From the data of mortality of larvae Artemia salina Leach, then construct the linear regression:

$$Y = Bx + A$$

Where:
Y= log concentration
X= % of larva mortality

LC50 values calculated to measure how far the effect of each concentration bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) that have been made to the mortality of shrimp larvae to enter a value of 50% mortality. If the control larvae died there, then the percent (%) mortality of larvae is calculated with the formula of Abbott (Meyer et al. 1982), as follows:

$$\% \text{ of larvae mortality} = \frac{T - K}{10} \times 100\ \%$$

Where :
T = sigma of mortality of larvae Artemia salina Leach test, K= sigma of mortality of larvae of Artemia salina Leach control

Descriptively, the data presented in tables and graphs. While analytically using Probit test to determine LC50 values from bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) against the larvae of Artemia salina Leach.

RESULTS

Observations of Artemia salina Leach larvae mortality after 24 hours on bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) are shown in Table 1.

Table 1. The Percentage Mortalities of Larvae of Artemia salina Leach on Bark Ethanol Extract of Sopang (*Caesalpinia sappan Linn*)

No.	Concentration (ppm)	Number of Mortality of Larvae Artemia salina Leach (larvaes)				Mean	Mortality (%)
		Control	Replication I	Replication II	Replication III		
1.	10	0	1	0	1	0.067	6.667
2.	50	0	1	1	0	0.067	6.667
3.	100	0	2	1	3	2.000	20.000
4.	250	0	6	5	7	6.000	60.000
5.	500	1	6	8	9	7.667	73.333
6.	1000	3	9	9	8	8.667	76.667

From Table 1, several things can be put forward are as follows: bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) shows percent mortality of larvae of Artemia salina Leach 6.667 to 76.667%. At a concentration of 10 ppm percent mortality of larvae of Artemia salina Leach at 6.667%, at a concentration of 50 ppm percent mortality of 6.667%; at a concentration of 100 ppm percent mortality rate of 20%, at a concentration of 250 ppm percent mortality of 60%, at a concentration of 500 ppm percent mortality amounted to 73.333%; at a concentration of 1000 ppm percent mortality of 76.667%. In control solution, percent mortality of larvae of Artemia salina Leach at a concentration of 10 ppm, 50 ppm, 100 ppm and 250 ppm at 0%; at a concentration of 500 ppm for 10%, and at a concentration of 1000 ppm at 30%. From Table 1 above, the concentration of bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) are transformed in logarithmic values. This is so that the range between small concentrations, so that it can reduce the heterogenity. This value is shown in Table 2. The relationship between mortality percentage of Artemia salina Leach with the log concentration of bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) is shown in Figure 1.

Based on linear regression graph in Figure 1, an increase in the percentage mortality of larvae of Artemia salina Leach, along with the increasing concentration of bark ethanol extract of Sopang (*Caesalpinia sappan Linn*). Linear regression formula which describes the relationship between these two variables is y = 0.020 x + 1.311. Coefficient of determination (R2) of 0.852 is quadrate of the correlation coefficient (0.9232 = 0.852) or equal to 85.2%. That is, 85.2% mortality of larvae of Artemia salina Leach was influenced by the concentration of bark ethanol extract of Sopang (*Caesalpinia sappan Linn*), while the remaining 14.8% could be influenced by other factors that can not be controlled by the researchers (eg durability larvae or trauma experienced by larvae when taken with a pipette).

The linear regression equation y = 0.020 x + 1.311 can be used to search for the LC50 values by entering the 50% rate as of x. Y value shows the log concentration of bark ethanol extract of Sopang (*Caesalpinia sappan Linn*). To get the numbers in units of ppm concentration, then the y values obtained from the regression equation must be made antilogarithms form. Thus, the concentration of bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) is the antilog value of 2.311 is 204.644. This means that the mortality of experimental animals reached 50% when the concentration of extract compounds reached 204.644 ppm. Observations Artemia salina Leach larvae mortality after 24 hours on stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) shown in Table 3.

Table 2. Percent Mortality Data larvae of Bark Ethanol Extract of Sopang (*Caesalpinia sappan Linn*) on Artemia salina Leach with Log Concentration

Observation (i)	% of Mortality (Xi)	Concentration (in ppm)	Long Concentration (Yi)
1	6.67	10	1.0000
2	6.67	50	1.6990
3	20.00	100	2.0000
4	60.00	250	2.3979
5	73.33	500	2.6990
6	76.67	1000	3.0000

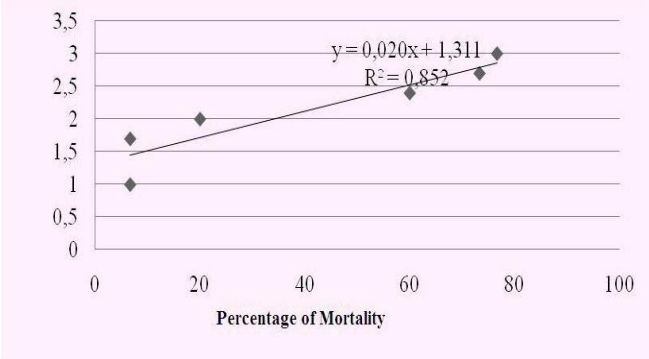

Figure 1. The relationship between percentage mortality of larvae Artemia salina Leach and the log concentration of bark ethanol extract of Sopang (*Caesalpinia sappan Linn*).

Table 3. The percentage mortalities of larvae of Artemia salina Leach on Stem Ethanol Extract of Sopang (*Caesalpinia sappan Linn*)

No.	Concentration (ppm)	Number of Mortality of Larvae Artemia salina Leach (larvaes)				Mean	Mortality (%)
		Control	Replication I	Replication II	Replication III		
1.	10	0	0	1	1	0.067	6.667
2.	50	0	0	1	2	1.000	10.000
3.	100	0	1	2	0	1.000	10.000
4.	250	0	4	7	3	4.667	46.667
5.	500	1	7	6	9	7.333	70.000
6.	1000	3	10	8	8	8.667	76.667

From Table 3, several things can be mentioned among others: the percentage of mortality of larvae of Artemia salina Leach on stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) amounted to 6.667 to 76.667%. At a concentration of 10 ppm percent mortality of larvae of Artemia salina Leach at 6.667%, at a concentration of 50 ppm percent mortality of 10%, at a concentration of 100 ppm percent mortality of 10%, at a concentration of 250 ppm percent mortality of 46.667%; at a concentration of 500 ppm percent mortality amounting to 70%, at a concentration of 1000 ppm percent mortality of 76.667%. In control solution, percent mortality of larvae of Artemia salina Leach at a concentration of 10 ppm, 50 ppm, 100 ppm and 250 ppm at 0%; at a concentration of 500 ppm for 10%, and at a concentration of 1000 ppm at 30%. From Table 3 above, the concentration of stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) is transformed into logarithmic values. This is so that the range between small concentrations, so that it can reduce the heterogenity. This value is shown in Table 4 as follows.

Table 4. Percent Mortality Data larvae of Stem Ethanol Extract of Sopang (*Caesalpinia sappan Linn*) on Artemia salina Leach with Log Concentration

Observation (i)	% of Mortality (Xi)	Concentration (in ppm)	Long Concentration (Yi)
1	6.67	10	1.0000
2	10.00	50	1.6990
3	10.00	100	2.0000
4	46.67	250	2.3979
5	70.00	500	2.6990
6	76.67	1000	3.0000

The relationship between mortality percentage of Artemia salina Leach with the log concentration of stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) is shown in Figure 2.

Based on linear regression graph in Figure 2, an increase in the percentage mortality of larvae of Artemia salina Leach, along with the increasing concentration of stem ethanol extract of Sopang (*Caesalpinia sappan Linn*). Linear regression formula which describes the relationship between these two variables is y = 0.020 x + 1.380. Coefficient of determination (R2) of 0.820 is quadrate of the correlation coefficient (0.9052 = 0.820) or equal to 82%. That is, 82% mortality of larvae of Artemia salina Leach was influenced by the concentration stem ethanol extract of Sopang (*Caesalpinia sappan Linn*), while the remaining 18% can be influenced by other factors that can not be controlled by the researchers (eg durability trauma experienced by larvae or larvae at when taken with a pipette).

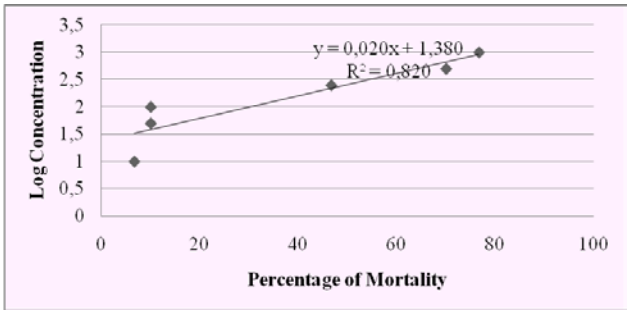

Figure 2. Graph showing the relationship between percentage mortality of larvae Artemia salina Leach and the log concentration of stem ethanol extract of Sopang (*Caesalpinia sappan Linn*).

The linear regression equation y = 0.020 x + 1.380 can be used to search for the LC50 values by entering the 50% rate as of x. Y value shows the log concentration of stem ethanol extract of Sopang (*Caesalpinia sappan Linn*). To get the numbers in units of ppm concentration, then the y values obtained from the regression equation must be made antilogarithms form. Thus, the concentration of stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) is the antilog of 2.38 is 239.883. This means that the mortality of experimental animals reached 50% when the concentration of extract compounds reached 239.883 ppm. From Figure 1 and 2 above, it appears that the greater the value of the concentration of bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) mortality in the larvae of Artemia salina Leach also increased significantly.

DISCUSSION

Based on the results of acute toxicity test of bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) with the BST method indicates that extracts of bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) is toxic, due to its LC50 value below 1000 ppm. Results of calculations performed by probit analysis for bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) is 204.644 ppm. This value indicates that at 204.644 ppm concentration of bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) was able to kill the larvae of Artemia salina Leach up to 50% of the population. While the results of calculations performed by probit analysis on stem ethanol extract of Sopang

(*Caesalpinia sappan Linn*) is 239.883 ppm. This value could indicate that at 239.883 ppm concentration stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) was able to kill the larvae of Artemia salina Leach up to 50% of the population. The lower the LC50 values of a sample, the higher bioactivity (Kadarisman 2000).

From this research, we found that bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) is more toxic when compared with the stem ethanol extract of Sopang (*Caesalpinia sappan Linn*). The presence of active chemical components toxic in the bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) can be developed with further testing to determine the toxic chemical components and potential as anticancer (Bawa 2009).

The active chemical components of the alleged role in the mortality of the larvae of Artemia salina Leach is a flavonoid. The presence of flavonoids in the environment causes cell OH- group on the flavonoid binding to the cell membrane integral proteins. This causes active transport of Na + - K + resistible. Active transport is stopped causing inclusion of Na + ions uncontrolled into the cell, causing rupture of cell membrane. Rupture of the cell membrane that causes cell death (Nurhayati et al. 2006). Flavonoids also work as a respiratory inhibitors that interfere energy metabolism in mitochondria by inhibiting electron transport system. The existence of barriers on electron transport system would block the production of ATP and cause a decrease in oxygen consumption by mitochondria (Rahayu 2009).

In this study, larvae of Artemia salina Leach, who died in the control. Artemia who died in the control due to natural mortality, which has decreased its durability against external factors that can not be controlled by the researcher. This can be known from the behavior of Artemia salina Leach just before death. Artemia who died in the control decreased activity. The longer, weaker Artemia in control and continues to be at the bottom of the tube. While Artemia who died in a test tube because of the treatment, disoriented motion (movement disorder). Artemia in the tube is still active, but still around in circles at one point (Nurhayati et al. 2006).

The results of this study showed that bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) has a toxic chemical component is active. Extracts of which have the LC50 value below 10 ppm is very potent as an anticancer (Meyer et al. 1982). If the LC50 values below 1000 ppm is active, and if the LC50 value of more than 1000 ppm are considered inactive. LC50 values in bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) is 204.644 ppm and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) is 239.883 ppm. This indicates that bark and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) is toxic, but can be expanded with more specific methods as anticancer (Kintoko & Azimahtol 2005, Mukhtar et al. 2007). Toxicity to larvae of Artemia salina Leach may provide the initial screening data important to assist in the selection of plant extracts with potential as anticancer agents (Bawa 2009, Kintoko & Azimahtol 2005). The mortality rate of Artemia larvae will give meaning to the potential as an anticancer activity (Mukhtar et al. 2007). The toxicity test with this method has a significant correlation with the cytotoxic of anticancer compounds, although the toxicity test with the BST method is not specific for anti-cancer (Mukhtar et al. 2007, Nurhayati et al. 2006).

CONCLUSION

Bark ethanol extract of Sopang (*Caesalpinia sappan Linn*) is toxic with LC50 value of 204.644 ppm and stem ethanol extract of Sopang (*Caesalpinia sappan Linn*) is toxic with LC50 values at 239.883 ppm.

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