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TOXICITY TEST OF ACEHNESE PLANTS USING THE BRINE SHRIMP LETHALITY TEST (BSLT) METHOD

Mastura[1], Mauliza[2], Hasby[3]

[1, 2, 3] *Chemistry Education Study Program, FKIP Samudra University, Jln. Meurandeh Langsa Campus, 24416*

E-mail: *mastura@unsam.ac.id, **mauliza@unsam.ac.id, ***hasby@unsam.ac.id

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Abstract: This study aimed to determine the potential toxicity of the ethanol extract of typical Acehnese plants to shrimp larvae (Artemia salina Leach) using the Brine Shrimp Lethality Test (BSLT) method as indicated by the LC50 value. This study used 1,080 shrimp larvae divided into four groups (three groups of extract concentration series and one negative control group). Each group consists of 15 larvae with three repetitions (triple) of treatment. The four treatment groups were given the suspension of ethanol extract of Cananga flower and leaf extract (Cananga odorata), jeumpa flower and leaf (Michelia alba), and Tanjung flower and leaf (Mimusops elengi) with concentrations of 1000 ppm, 100 ppm, and 10 ppm. The mortality data of shrimp larvae were analyzed by probit analysis to determine the LC50 value. The result of this research is that the ethanol extract of the Cananga flower and leaf (Cananga odorata) has LC50 values of 55.71 ppm and 79.43 ppm, respectively. The ethanol extract of the jeumpa flower and leaf (Michelia alba) had LC50 values of 831.76 ppm and 398.10 ppm, respectively. The ethanol extract of the Tanjung flower and leaf (Mimusops elengi) had LC50 values of 295.12 ppm and 77.67 ppm, respectively.

Keywords: Toxicity, Acehnese plants, BSLT

Abstrak: Penelitian ini bertujuan untuk mengetahui potensi toksisitas ekstrak etanol tumbuhan khas Aceh terhadap larva udang (Artemia salina Leach) dengan menggunakan metode *Brine Shrimp Lethality Test* (BSLT) yang ditunjukkan dengan nilai LC50. Penelitian ini menggunakan 1.080 ekor larva udang yang dibagi menjadi empat kelompok (tiga kelompok seri konsentrasi ekstrak dan satu kelompok kontrol negatif). Setiap kelompok terdiri dari 15 ekor larva dengan tiga kali ulangan (triple) perlakuan. Empat kelompok perlakuan diberi suspensi ekstrak etanol bunga dan daun kenanga (Cananga odorata), bunga dan daun jeumpa (Michelia alba), dan bunga dan daun Tanjung (Mimusops elengi) dengan konsentrasi 1000 ppm, 100 ppm, dan 10 ppm. Data mortalitas larva udang dianalisis dengan analisis probit untuk menentukan nilai LC50. Hasil dari penelitian ini adalah ekstrak etanol bunga dan daun kenanga (Cananga odorata) memiliki nilai LC50 masing-masing sebesar 55,71 ppm dan 79,43 ppm. Ekstrak etanol bunga dan daun jeumpa (Michelia alba) memiliki nilai LC50 masing-masing sebesar 831,76 ppm dan 398,10 ppm. Ekstrak etanol bunga dan daun Tanjung (Mimusops elengi) memiliki nilai LC50 masing-masing sebesar 295,12 ppm dan 77,67 ppm.

Keywords: Toksisitas, Tumbuhan Aceh, BSLT

INTRODUCTION

Indonesia is an archipelagic country with a tropical climate and rich in natural resources, one of which is plants. Indonesian people have long known and used plants as medicines (traditional medicine). Parts of plants that are often used as medicine are roots, tubers, bark, wood, leaves, flowers, or seeds. This traditional medicine is widely used by people in various regions, including the people of Aceh, because it is easy to obtain and the price is more affordable when compared to chemical drugs.

The safety of the use of natural ingredients as traditional medicines in society is guaranteed by the government with the Minister of Health Regulation No.760/Menkes/Per/IX/1992, concerning traditional medicines and phytopharmaca (KepMenKes RI 1995). Before becoming a phytopharmaceutical preparation, every natural ingredient must pass several stages including experimental pharmacology tests, toxicity tests, clinical trials, quality tests and other tests according to requirements for user safety (Jelita et al. 2020).

One of the typical plants from Aceh, cempaka putih (Michelia alba) has many benefits that are not known to ordinary people. In India and Thailand, the leaves and bark of the Cempaka Putih (Michelia alba) plant are used as a remedy for fever and nausea. The essential oil contained in the flowers has a high selling price and is used as the main ingredient in the most expensive perfumes in the world (Zuhrotun, at al 2018). In Indonesia and Vietnam, Kenanga (Cananga odorata) is used as a traditional medicine to prevent malaria and as an anti-depressant. Meanwhile, in the Northern Mariana Islands, the community of healers used the bark and flowers of the ylang plant to treat stomach aches and pneumonia (Tan, at al 2015). In India, a decoction of the bark, flowers and fruits of the cape plant (Mimusops elengi Linn) is traditionally used to clean teeth, while the tender branches are used for toothbrushes. Tanjung seeds are anti-microbial for gram-positive and negative bacteria (Hazra, at al 2007)

Cananga (*Cananga odorata*), Jeumpa (*Michelia alba*), and Tanjung (*Mimusops elengi*) are typical Acehnese plants that are widely used by the people of Aceh as medicine. Cananga flower (*Cananga odorata*) is a flower originating from Southeast Asia, with a stem diameter of 0.1-0.7 meters and a tree height of up to 10 meters. Cananga flowers include compound flowers in umbrella-shaped bouquets, short, and hanging. Consists of 6 pieces of flower petals that are

lanceolate in shape and have a distinctive aroma. Chemical substances contained in cananga flowers are saponins, flavonoids and volatile oil components that contain polyphenolic compounds (Sacchetti in Dusturia et al. 2016). Cananga flower is a plant that can be used as a basic ingredient for making natural medicines and cosmetics.

The jeumpa plant (*Michelia alba*) belongs to the Magnoliaceae family where almost all parts of the plant such as bark, leaves, and flowers can be used as medicine, such as medicine for fever, irregular menstruation, bronchitis, cough, vaginal discharge, inflammation, urinary tract infections, and urination. a little. In addition, the three parts of this plant are also efficacious as expectorants and are diuretics so that they can break down kidney stones, and prevent and cure bad breath. In addition to containing essential oils found in flowers, all jeumpa plants also contain alkaloids, flavonoids, and saponins. The content of these secondary metabolites is spread from roots, leaves, and bark (Bawa 2011).

The cape plant (*Mimusops elengi*) belongs to the Sapotaceae family which has many benefits. Many parts of this plant are used as medicine, namely the bark, leaves, flowers, fruit, and seeds.The bark and fruit of this plant are used as a remedy for diarrhea and dysentery, and a decoction of the bark is used as a mouthwash (Dash et al. 2020).In addition, the tanjung flower can be used as an ingredient for making lotions for wounds and ulcers, the powder from the dried flowers can be used as a braintonic, expectorant, nasal disease, and its smoke is good for asthma (Hadaginhal et al. 2010). Therefore, it is necessary to study the potential of cananga (*Cananga odorata*), jeumpa (*Michelia alba*), and tanjung (*Mimusops elengi*) plant extracts as toxicity to shrimp larvae (*Artemia salina* Leach) using the Brine Shrimp Lethality Test (BSLT) method. The BSLT method was chosen because this method is often used for pre-screening of active compounds contained in plant extracts (Gani, at al. 2021). In addition, this method is also simple, fast, cheap, easy, reliable, and the results are representative. Toxicity test using BSLT can be determined from the number of deaths of shrimp larvae (*Artemia salina* Leach) due to the influence of extracts or compounds of natural ingredients. The test results are expressed as LC50. Based on the above background, this research is proposed with the aim of knowing the potential toxicity of ethanol extract of typical Acehnese plants to shrimp larvae (*Artemia salina* Leach) using the Brine Shrimp

Lethality Test (BSLT) method indicated by the LC_{50} value.

METHOD

Sample Preparation

Samples in the form of tanjung leaves, tanjung flowers, jeumpa leaves, jeumpa flowers, cananga leaves, and cananga flowers were taken from the Langsa area. The sample was cut into small pieces and dried at room temperature and then ground to a fine powder using a blender to a powder.

Sample Extraction

The extraction method was carried out by maceration. The sample was aerated until brown and mashed using a blender to form a dry powder and then soaked in ethanol for 48 hours. Then evaporated to obtain a thick extract.

Phytochemical Screening

Examination of the content of secondary metabolites (phytochemical screening) was carried out to identify the presence of flavonoid compounds, alkaloids, terpenoids, saponins, and tannins. In the flavonoid test, a 2 mL sample of plant ethanol extract was put into a test tube, then added a few mg of Mg powder and 3 drops of HCl, so that an orange to red color was formed, this result indicates a positive test for flavonoids (Nastiti et al. 2017). In the alkaloid test, the ethanolic plant extract sample was added with 2N HCl and the solution was divided into 2 tubes. Tube 1 was dripped with Mayer's reagent, tube 2 was dripped with Wagner's reagent, so that the extract was orange or brown and a white precipitate was formed indicating a positive test result for alkaloids (Faskalia & Wibowo 2014). In the terpenoid test, a sample of 2 mL of plant ethanol extract was evaporated, then the residue obtained is redissolved in 0.5 ml of chloroform, then 0.5 acetic acid is added. If a brownish or violet ring is formed on the boundary of the two solvents, it indicates the presence of terpenoids (Rafiqah et al. 2019). In the saponin test, 1 mL of the extract was put into a test tube, 2 mL of distilled water was added and it was shaken for 1 minute, then 2 drops of 1N HCl were added. Positive for saponins if the foam formed remains stable for 7 minutes (Setiabudi & Tukiran 2017). In the tannin test, it is carried out by taking 2 mL of each sample that has been extracted with ethanol solvent, then heated for approximately 5 minutes. After being heated, each was added a few drops of 1% $FeCl_3$.

Toxicity Test with Brine Shrimp Lethality Test (BSLT) Method

Shrimp Larva Hatching (*Artemia salina* Leach)

Shrimp larvae (*Artemia salina* Leach)

were weighed as much as 1 gram of shrimp eggs were put into an erlenmeyer containing 500 mL of filtered seawater and then installed an aerator. Leave it for 48 hours with lighting until the shrimp eggs hatch into shrimp larvae (nauplii) and are ready to be used for testing (Baud et al. 2014).

Sample Preparation

Making extract solution 2000 ppm. A total of 40 mg of the extract was weighed carefully and then dissolved in 20 mL of sea water, for extracts that are difficult to sea, 1% DMSO (5 drops) can be added to increase the solubility. Furthermore, variations in the concentration of the sample extract were made. A concentration of 200 ppm was made by pipetting 2 mL of a 2000 ppm extract solution and adding seawater to 20 mL. A concentration of 20 ppm was made by pipetting 2 mL of a solution with a concentration of 200 ppm and adding seawater to 20 mL. A sample solution with a concentration of 1000 ppm was made by pipetting 5 mL of a 2000 ppm extract solution and adding 5 mL of seawater. The sample solution with a concentration of 100 ppm was made by pipetting 5 mL of a 200 ppm extract solution and adding 5 mL of seawater.

Bioactivity Test

The bioactivity test was carried out by inserting 15 prawn larvae (*Artemia salina* Leach) aged 48 hours into a bottle containing a solution of extract and seawater, for each concentration 3 repetitions were carried out (triplo). As a control, sea water was not given the sample extract. Experimental bottles were stored under TL lamp lighting. Observations were made after 24 hours. The number of dead shrimp larvae was recorded and then the mortality percentage (death) was calculated. The data obtained was processed using probit analysis (Rafiqah et al. 2019)

Data analysis

Data processing for toxicity test uses probit analysis which is carried out using manual probit analysis method, so the probit value can be known by converting the percent value of larval mortality at each concentration to the probit value.

$$\text{Percentage of deaths} = \frac{\textit{Jumlah larva mati}}{\textit{Jumlah larva total awal}} \; x\ 100\%$$

After getting the percent mortality, the probit value of each group of test animals was searched in the probit table. Then determine the concentration log and make a graph with a straight-line equation of the relationship between the probit

value and the concentration log with the formula y = mX + b (Juniarti et al. 2009).

Note:

y = probit number
X= log concentration

Slop value (m) Calculated by the formula:

$$\frac{\sum(X)\sum(XY) - \sum(X^2)\sum(Y)}{(\sum(X))^2 - n\sum(X^2)}$$

The intercept value (b) is calculated by the formula:

$$\frac{\sum(X)\sum(Y) - n\sum(XY)}{(\sum(X))^2 - n\sum(X^2)}$$

The analysis method can also use Microsoft Office Excel by making a graph of a straight line equation of the relationship between the probit value and the concentration log. The LC_{50} value can be calculated from the straight-line equation by entering the value 5 (probit of 50% of test animal deaths) as y so that x is produced as the log concentration value. The antilog of the x value is the LC_{50} value (Caroline, et al., 2019). LC_{50} (Lethal Concentration 50) is the concentration of substances that cause death in 50% of experimental animals, namely shrimp larvae (*Artemia salina* Leach) (Kurniawan & Ropiqa 2021).

According to Rampe & Tombuku (2015), the level of toxicity of a sample can be categorized into four (4) levels based on the LC_{50} value obtained, which can be seen in Table 1.

Table 1. Toxicity Level Based on LC_{50}.Value

No	LC50 value (µg/ml)	Toxicity Level
1	0-250	Very Toxic
2	250-500	Currently
3	500-1000	Weak
4	>1000	Non Toxic

RESULTS AND DISCUSSION

Sample Extraction

The extraction method is carried out by maceration, the maceration method was chosen in this study because it is easy and does not need heating so it is unlikely that natural materials will be damaged or decomposed. Maceration is carried out by immersing the simplicia into a suitable solvent in a vessel and placing it at room temperature and waiting for some time for 2-3 days. This aims to maximize the process of taking chemical compounds contained in the sample.

After obtaining the results of maceration, then concentrated by evaporation at a temperature of 40oC. This was done to evaporate the remaining solvent so that in this study larval death occurred due to the influence of the extract without being influenced by the ethanol solvent. After being evaporated, the extract yields were calculated from each sample of cananga flower and leaf (*Cananga odorata*), jeumpa flower and

leaf (*Michelia alba*), and cape leaf and flower (*Mimusops elengi*). The results of the yield value (%) obtained in each sample can be seen in Table 2.

Table 2. Sample Extraction Results

Sample	Simplicia Weight (g)	Extract Weight (g)	Yield Percentage (%)
BK	27,620	8.007	28,989
BJ	44,480	13,219	29,718
BT	70	33,752	48,217
DK	200	28,199	14,099
DJ	200	14,043	7,021
DT	200	45,782	22,891

Information:
BK = Cananga Flower; BJ= Jeumpa Flower; BT = Tanjung Flower; DK = Cananga Leaf; DJ = Jeumpa Leaf; and DT = Tanjung Leaf.

Based on Table 2. shows that the extraction results obtained by each sample are different. The percentage yield obtained from the extraction indicates the ability of the solvent and the method used to extract the compound in the simplicia. The results obtained in the ethanol extract of all samples after being evaporated the consistency became thick, semi-solid, and black in color. The ethanol extract of cananga flower and leaf (*Cananga odorata*) obtained the yield percentage of 28.989% and 14.099%, respectively. The ethanol extract of jeumpa flower and leaf (*Michelia alba*) obtained the yield percentage of 29.718% and 7.021 %, respectively. The ethanol extract of tanjung flower and leaf (*Mimusops elengi*) obtained the yield percentage of 48.217% and 22.891%, respectively.

Toxicity Test with Brine Shrimp Lethality Test (BSLT) Method

Toxicity testing begins with hatching shrimp (*Artemia salina* Leach) larvae, sample preparation, and bioactivity test. The results of the bioactivity test of the sample extract on larval mortality can be seen in Table 3.

Table 3. Effect of Various Concentrations of Sample Ethanol Extract on Shrimp Larva (*Artemia salina* Leach)

Sample	Larvae Mortality Rate of 15 Larvae			
	Extract Concentration (ppm)			
	1000	100	10	Control
BK	12;15;15	7;8;5	3;5;5	0
BJ	7;7;8	5;6;5	2;2;1	0
BT	13;11;8	6;4;4	1;1;2	0
DK	11;15;8	7;6;7	5;5;6	0
DJ	8;7;10	5;6;8	2;5;6	0
DT	13;10;12	8;6;9	2;2;1	0

Information:
BK = Cananga Flower; BJ= Jeumpa Flower; BT = Tanjung Flower; DK = Cananga Leaf; DJ = Jeumpa Leaf; and DT = Tanjung Leaf

Based on Table 3. shows that the total mortality of shrimp (*Artemia salina* Leach) larvae increased with each increase in concentration. In addition, the presence of secondary metabolites in the sample can affect the mortality of shrimp larvae during toxicity testing.

Ethanol extracts of typical Acehnese plants, in this case cananga flowers and leaves (*Cananga odorata*), jeumpa flowers and leaves (*Michelia alba*), and cape flowers and leaves (*Mimusops elengi*) have potential as toxicity. This is related to the presence of secondary metabolites that were chemically identified in each sample extract, such as secondary metabolites of flavonoids, alkaloids, terpenoids, saponins, and tannins. These compounds play a role in causing the death of shrimp larvae (*Artemia salina* Leach).

The mechanism of action of the death of shrimp larvae (*Artemia salina* Leach) is estimated that the presence of secondary metabolites contained in the sample extract can inhibit the larval feeding power (antifeedant) by acting as stomach poisoning. Therefore, when these compounds enter the body of shrimp larvae, their digestive organs will be disturbed (Jelita et al. 2020). Stomach poison attacks the main digestive organs of insects (larvae), namely the ventriculus. Alkaloid compounds have toxin, repellent and antifeedant characteristics on insects (larvae), thus disrupting the growth and development of larvae. In small amounts, alkaloids are only antifeedant and kill larvae slowly due to decreased appetite and will only cause death in some time due to starvation. But in large quantities, alkaloids work as contact poisons and digestive poisons that will directly kill the larvae and cause death because they attack vital organs such as the nervous system and affect heart activity. Flavonoid compounds have a way of working as respiratory toxins and metabolic toxins that can directly cause death in a short time (Gokok, 2017). Flavonoid compounds can also inhibit the digestive tract of insects and are also toxic. In addition, this compound inhibits taste receptors in the mouth area of the larvae. This causes the larvae to fail to get a taste stimulus so they are unable to recognize their food so that the larvae starve to death (Cahyadi 2009). Alkaloids work as contact poisons and digestive poisons that will directly kill larvae and cause death because they attack vital organs such as the nervous system and affect heart activity. Flavonoid compounds have a way of working as respiratory toxins and metabolic toxins that can directly cause death in a short time (Gokok, 2017).

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Furthermore, a comparison of the percentage of larval mortality from each sample extract was carried out, and processing was carried out based on probit analysis, in order to obtain the Lethal Concentration (LC_{50}) value of each sample extract, which can be seen in Table 4.

Based on Table 4. shows that from the results of the toxicity test, the LC_{50} value of each sample extract can be obtained. The ethanol extract of cananga flower and leaf (*Cananga odorata*) had LC_{50} values of 55.71 ppm and 79.43 ppm, respectively. The ethanol extract of jeumpa flowers and leaves (*Michelia alba*) had LC_{50} values of 831.76 ppm and 398.10 ppm, respectively. The ethanol extract of tanjung flowers and leaves (*Mimusops elengi*) had LC_{50} values

of 295.12 ppm and 77.67 ppm, respectively. This shows that each sample extract has the potential for toxicity, namely flower and leaf extract of cananga cananga (*Cananga odorata*), and tanjung leaf (*Mimusops elengi*) has a very strong toxic level. The extracts of tanjung flower (*Mimusops elengi*) and jeumpa leaf (*Michelia alba*) have a fairly strong (moderate) toxic level.

Table 4. Results of Determination of LC_{50} in Each Sample Extract

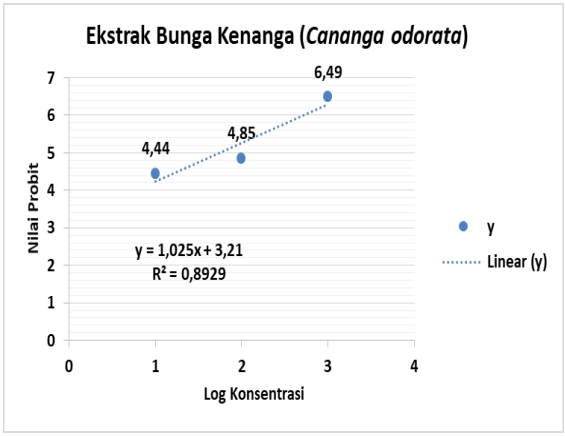
Sample	Concentration (ppm)	% Death	Probit (Y)	LC_{50}
	1000	93.33	6.49	
BK	100	44.44	4.85	55.71
	10	28,88	4.44	
	1000	48.88	4.96	
BJ	100	35.55	4.62	831.76
	10	11.11	3.77	
	1000	71.11	5.55	
BT	100	31.11	4.50	295.12
	10	8.88	3.64	
	1000	75.55	5.69	
DK	100	44.44	4.85	79.43
	10	35.55	4.62	
	1000	55.55	5.13	
DJ	100	42.22	4.80	398,10
	10	28,88	4.44	
	1000	77.77	5.76	
DT	100	5.02	5.02	77.67
	10	28,88	4.44	

Information:

BK = Cananga Flower; BJ= Jeumpa Flower; BT = Tanjung Flower; DK = Cananga Leaf; DJ = Jeumpa Leaf; and DT = Tanjung Leaf

Then, the probit analysis was performed from the concentration log value (X) and the probit value (Y) using Microsoft Office Excel, in order to obtain a linear regression comparison chart for each sample extract. This is done to prove the similarity of the calculation results between manual probit analysis using Microsoft Office Excel, as shown in Figure 1.

Based on the linear regression graph in Figure 1 shows that the results of calculations using the manual probit method and Microsoft Office Excel show the same LC_{50} value as shown in Table 4., so there is a similarity in the LC_{50} value after being calculated by the two calculation methods.



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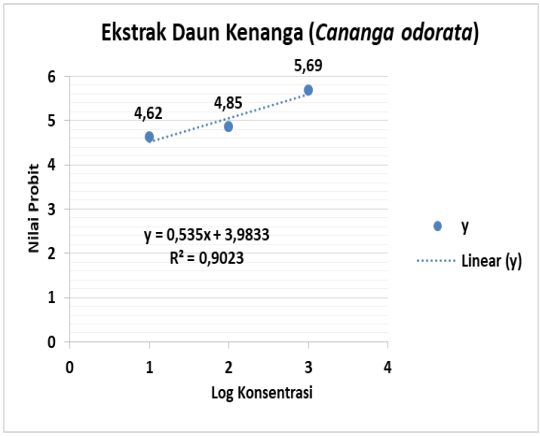

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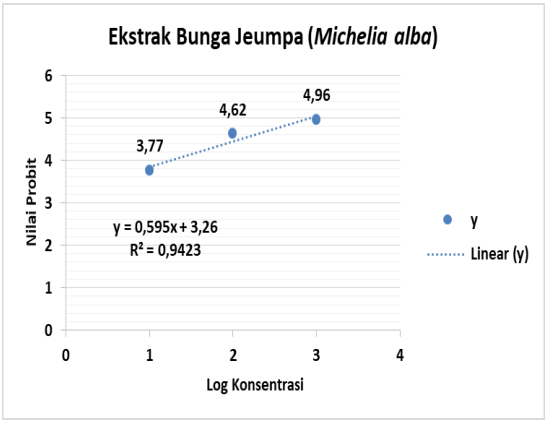

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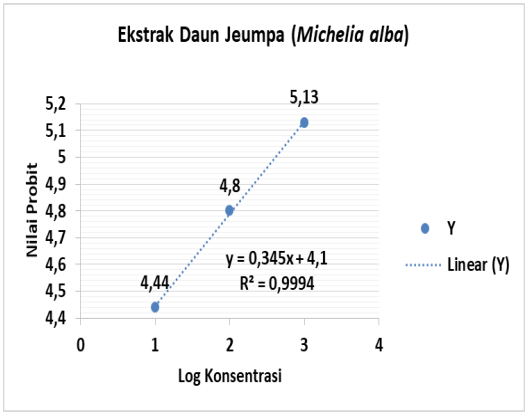

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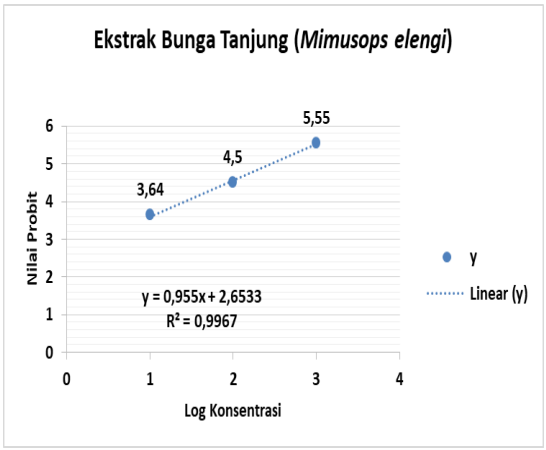

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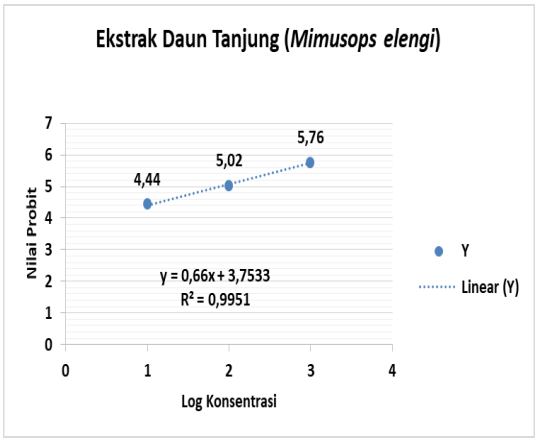

(f)

Figure 1. Linear Regression Graph of Sample Extract Concentration to Probit . Value

CONCLUSION

The ethanol extract of Acehnese plants has the potential for toxicity to shrimp larvae (*Artemia salina* Leach) using the Brine Shrimp Lethality Test (BSLT) method which is indicated by an LC_{50} value of less than 1,000 ppm. Cananga flower and leaf extract (*Cananga*

odorata), and tanjung leaf (*Mimusops elengi*) have very strong toxic levels, namely LC_{50} values of 55.71 ppm, 79.43 ppm, and 77.67 ppm. The tanjung flower extract (*Mimusops elengi*) and jeumpa leaf (*Michelia alba*) have a fairly strong (moderate) toxic level, namely the LC_{50} values of 295.12 ppm and 398.10 ppm, while the jeumpa flower extract (*Michelia alba*) has a weak toxic level. namely the LC_{50} value of 831.76 ppm.

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