Toxicity test of gastropoda extracts of Littorina scabra and Terebralia sulcata

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Research Articles

Toxicity test of gastropoda extracts of *Littorina scabra* and *Terebralia sulcata* from Payung Island, Musi River Estuary, South Sumatera

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ABSTRACT: Gastropoda is one of the potentially most common biotas found in mangrove ecosystems and as bioactive compounds that have not been widely studied. Gastropod species *Littorina scabra* and *Terebralia sulcata* were found in the Payung Island. This research aimed to determine and compare the potential toxicity between two species of gastropod extract of *L. scabra* and *T. sulcata*. This research was conducted in February 2017. The research procedure included sampling and sample preparation, extraction, and toxicity test that Brine Shrimp Lethality Test (BSLT) method. Toxicity test showed LC_{50} 415.58 µg/mL *L. scabra* while LC_{50} value from *T. sulcata* equal to 565.52 µg/mL. From the comparison, the toxicity value of *L. scabra* gastropod was higher than *the T. sulcata* type. @2021 Published by UP2M, Faculty of Mathematics and Natural Sciences, Sriwijaya University

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INTRODUCTION

Indonesia is one of the countries with the highest biodiversity (mega biodiversity) in the world due to the location of Indonesia in the tropics, where more than half of Indonesia is in the form of oceans (Fajarningsih *et al.*, 2006). However, the utilization of the richness and diversity of marine biota still focuses on food products in fish. However, other biotas are not less abundant in addition to fish, such as crustaceans, sponges, algae, mollusks, and other biotas (Martiningsih, 2013; Almaniar et al. 2021). The high biodiversity spurs these biotas to produce secondary metabolite compounds as essential for the survival of the biota (Salamah *et al.*, 2008).

According to Burrens and Clement (1993) in Ali *et al*. (2006), based on data obtained from the National Cancer Institute (Washington) that screened some marine biota, it is known that some biota has biological activity. Antiviral, antibiotic, anti-inflammatory, antileukemic, and anticancer agents are found in the screening process of marine biota.

Harmawan et al. (2012) describe that Gastropods are biota that can produce secondary metabolites used as antibacterial. In addition, according to Pringgenies and Dananjoyo (2012), the class of Gastropod has potential as an antibiotic compound because it can produce secondary metabolite compounds.

One of the earliest methods for cytotoxic testing is the Brine Shrimp Lethality Test (BSLT). BSLT is one of the most widely used methods for searching for new anticancer compounds derived from living things. Fajarningsih *et al*. (2006) mentioned that the toxicity test with Brine Shrimp Lethality Test (BSLT) method could be done quickly, cheaply, and efficiently, so it is widely used as prescreening in a screening of active ingredient extract. The purpose of this toxicity test is to find out whether there are toxic effects or not and assess their safety limits (Ismail *et al.,* 2007 in Putri *et al.,* 2012).

MATERIALS AND METHODS

Site Study

The study was conducted in February 2017. The mollusks (Gastropoda) samples were taken in the eastern region of Payung Island Musi River Estuary, South Sumatera (Figure 1). Sampling treatment and toxicity test will be conducted at Marine Bioecology Laboratory, Marine Science Deparment, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indralaya.

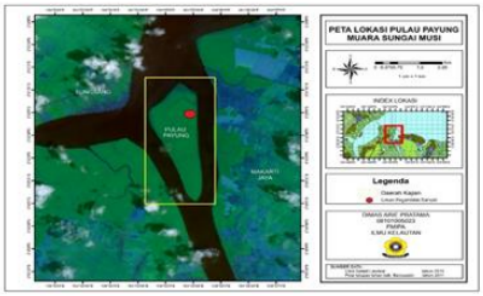

Figure 1. Sampling location

Research Procedure

The samples originated from Payung Island of Musi River Estuary, South Sumatera, and were taken in the East from Payung Island randomly (random sampling). Gastropods were collected so that the sample was obtained 0.5 Kg, then the samples that had obtained samples were placed in a plastic bucket and then inserted into the sample plastic by species and labeled.

Then the sample was taken to the laboratory for identification using the Indonesian Snail and Shell Identification Book (Indonesia Shells) (Dharma B, 1988). The sample was washed thoroughly and separated between the meat and the shell. The sample meat was mashed using a blender for the extraction process.

Extraction

The composite sample was weighed by biomass for further maceration process (n-hexane, ethyl acetate, and methanol) at a ratio of 1: 5 (g / v). Then extracts were separated between filtrate and residue using filter paper to be evaporated using a rotary evaporator with a temperature of 60 °C to form a paste. The result of this evaporation was the extract that will be used in the toxicity test (Asshidiq et al. 2020).

Preparation of Larva *Artemia salina*

Preparation of larvae was conducted by taking 1 g of *A. salina* Leach eggs. Hatching was conducted using glass jars by soaking the eggs in the seawater as 100 mL and given lighting with incandescent lamps and aerated for 48 hours (Purwaningsih and Deskawati, 2014; Puspitasari et al. 2018).

Preparation of Test Solutions

To make a test solution that was the extract of Gastropoda obtained in Payung Island made by making mother liquor as much as 10 mL with concentration 10000 µg/mL. Prepare vial tubes that had been filled with 10 mL of seawater, then enter 0.1 g of crude extract. Prepared test solution of 5 test solutions with each done three times repetition with concentrations of 2000, 1000, 100, 50, and 25 µg/mL, and also made a control solution that is seawater media.

Toxicity Test

Each test solution and a control solution were included 10 *A. salina* larvae incubated under a 15 watt TL lamp for 24 hours and observed every 6 hours. The number of dead *A. salina* larvae was calculated to determine the percentage of death.

Data Analysis

Toxicity tests were performed by calculating and observing the mortality of *A. salina* larvae as test animals at each concentration (Martiningsih, 2013). The effects of toxicity were analyzed from observations with the percent of deaths of Nurhayati *et al*. (2006) determined by the formula:

$$\%\ Larvae = \frac{number\ of\ dead\ larvae}{number\ of\ test\ larvae} \times 100\%$$

If there were larvae that die on the control, then used the formula abbot (Meyer *et al.,* 1982) to determine the % death:

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

Where :

T = Number of dead testing larvae (Ind)
K = Number of controlling dead larvae (Ind)
10 = Number of test larvae (Ind)

After knowing the % death of *A. salina* larvae, then search probit value through probit table and linearly regression. Percentage transformation values of probit were presented in Table 1.

Table 1. The value of percentage transformation to probit by Finney (1952) in (Okomoda *et al*. 2013)

Percentage	Probit									
	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.5	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.8	4.82	4.85	4.87	4.9	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.2	5,23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.5
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
99	7.33	7.37	7.41	7.41	7.46	7.58	7.65	7.75	7.88	8.09

Sudjana (2005) explained that regression analysis could be used with data consisting of two or more variables. The relationship obtained was generally expressed in mathematical equations, which express the functional relationship between variables. In this toxicity test, the variables were probit (Y) and concentration logarithm (X). After obtained values of a and b, then searched LC_{50} value by using linear regression equation previously X and Y had been calculated and known value (Sudjana, 2005).

$$y = a + bx$$

Where

Y = Probit value
a = Regression concentration
b = *Slope*/slope regression
X = Logarithm of test concentration

The toxicity category of the extract was determined by the concentration value of LC_{50}, as presented in Table 2.

Table 2. Value and category of toxicity (Meyer *et al.* 1982)

No	Value (µg/ml)	Category of toxicity
1	< 1000	Toxic
2	> 1000	Non-Toxic

RESULT AND DISCUSSION

Gastropoda in Payung Island

Littorina scabra

The species *L. scabra* was one of the most abundant species in the mangrove ecosystem. This species had an average size of 2.5 cm and an average width of 1 cm with a small drill-shaped shape with a white base color with stripes and dots brown. This species had a valve that opens and closes. In addition, this species had a mucus that allows this species to stick in the shadows. This species is commonly found in twigs and stems of mangroves because this species consumes mangrove leaves as food.

Figure 2. *Littorina scabra*

Terebralia sulcata

The *T. sulcata* species had an elongated shell like a greenish-brown trumpet with an average length of 4 cm and an average width of 1 cm to 2.5 cm, at the foot of this species did not have a valve that covers the legs and had a sticky mucus. This species is found in mud and mangrove root where there are many mangrove leaf litter.

Figure 3. *Terebralia sulcata*

Mortality of *Artemia salina*

Littorina scabra

The results of mortality tests on *Artemia salina* larvae to *Littorina scabra* meat extract can be seen in Table 3. Table 3 shows that the mortality of *A. salina* larvae in *L. scabra* Gastropoda extracts was started at 25 µg / mL concentration of 0%, 50 µg / mL (10%) concentration, 100 µg / mL concentration (16.67%). At the concentration of 10000 µg / mL, the average mortality of *Artemia salina* larvae obtained a 100% mortality percentage. At 2000 and 1000 µg / mL concentration obtained mortality of over 50% i.e, 73.33% and 56.67%. In comparison, the control of the test solution without extracts obtained no mortality in larvae. That result was in line with the statement of Harborne (1994) in Nurhayati *et al.* (2006), which said that the greater concentration of extracts tested will further increase the percentage mortality of the larvae.

Table 3 Result mortality test of *Littorina scabra* extract

Sample Code	Concentration (µg/mL)	Log 10 Concentration (µg/mL)	Test Larvae	Average Mortality *A.salina*	% Average Mortality *A.salina*	Probit Value
LS	10000	4.00	10	10.00±0.0	100.00	8.09
	2000	3.30	10	7.3±0.58	73.33	5.61
	1000	3.00	10	5.67±0.58	56.67	5.15
	100	2.00	10	1.67±0.58	16.67	4.01
	50	1.70	10	1.0±0.0	10.00	3.72
	25	1.40	10	-	-	-
Control	0	-	10	-	-	-

Terebralia sulcata

The mortality tests on *Artemia salina* larvae of *Terebralia sulcata* meat extract can be seen in Table 4. Based on Table 4. The results of *A. salina* larvae test on the extract of Gastropoda type *Terebralia sulcata* began at a concentration of 25 µg/mL obtained percentage mortality 0%, concentration 50 µg/mL (3%), concentration 100 µg/mL (17%). At the concentration of 10000 µg/mL, the average mortality of *Artemia salina* larvae obtained 100% mortality percentage. At 2000 and 1000 µg/mL concentrations obtained 50% mortality i.e, 57%, and 50%. At a concentration of 50 µg/mL test solution, mortality was found to be one larval. The control i.e., test solution without extracts showed that there was no larval mortality. That shows that the extract of Gastropoda type, *Terebralia sulcata* has the toxic ability only until the concentration of 1000 µg/mL upward.

Table 4. Result mortality test of *Terebralia sulcata* extract

Sample Code	Concentration (µg/mL)	Log 10 concentration (µg/mL)	Test Larvae	Average mortality *A.salina*	% Average mortality *A.salina*	Probit value
TS	10000	4.00	10	10.00±0.0	100.00	8.09
	2000	3.30	10	5.67±0.58	57.00	5.18
	1000	3.00	10	5.00±0.0	50.00	5.00
	100	2.00	10	1.67±0.58	17.00	4.05
	50	1.70	10	0.3±0.58	3.00	3.12
	25	1.40	10	-	-	-
Control	0	-	10	-	-	-

LC_{50} of Extract

A toxicity test that has been done on the extract of ethyl acetate *L. scabra* and *T. sulcata* to *A. salina* larvae is presented in Table 5. Toxicity testing using Brine Shrimp Lethality Test (BSLT) method on A.salina larvae was done by looking at the mortality rate of larvae caused by crude extract of the sample. Furthermore, the mortality rate of larvae was analyzed to determine LC_{50} (lethality concentration 50%). The LC_{50} value is the amount of concentration of test extract that can cause the death of *A. salina* larvae by 50% after being observed for 24 hours (Meyer et al. 1982).

Table 5. LC_{50} of *L. scabra* dan *T. sulcata* extract

Sample code	Linear Regression			LC_{50} Value (µg/mL)	Category
	a	b	R^2		
LS	0.4362	1.7428	0.9023	415.58	Toxic
TS	-0.0912	1.8497	0.8771	565.52	Toxic

Based on Table 5. The result of the toxicity test of extract from the extract of two types of Gastropoda *L. scabra* and *T. sulcata* showed that *L. scabra* extract had 41558 µg/mL, and $LC_{50}$the value from *T. sulcata* extract was 565.52 µg/mL. That shows that the extract concentration *L. scabra* of 415.58 µg/mL will cause the death of *A. salina* larvae by 50%, and the extension of *T. sulcata* of 565.52 µg/mL will cause the death of *A. salina* larvae as much as 50%. So it can be seen that the extract from *L. scabra* has LC_{50} higher than the extract from *T. sulcata*.

CONCLUSION

Brine Shrimp Lethality Test (BSLT) test showed that *L. scabra* and *T. sulcata* gastropod extracts had cytotoxic potential. From the testLC_{50} results, *L. scabra* 415.58 µg/mL and *T. sulcata* 565.52 µg/mL, where the *L. scabra* extract showed a bit more toxicity potential than the *T. sulcata*.

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