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Toxicity of Sponge Extract *Xestospongia testudinaria*

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Abstract: *Sponge is a highly prospective bioactive material from the ocean. This study aims to determine the toxicity of Xestospongia testudinaria sponge extracts collected from Sanur beach, Bali, Indonesia. Sponges are one biota that contains bioactive compounds from the sea that are very prospective. Nearly 5,000 compounds have been successfully isolated from these animals with various activities such as antimicrobials, antifungal, antiviral, and anticancer. The preliminary test for potentially anticancer material was to determine the toxicity of the material. The method used to determine the toxicity was the Bhrine Shrimp Lethality Test (BSLT) method. Based on the results of this study showed that X. testudinaria sponge methanol extract was toxic to Artemia salina larva with LC_{50} of 31.62 ppm, while that of n-hexane extract showed less toxic because of its LC_{50} value of 177.83 ppm.Based on the results of this study, it can be concluded that the methanol extract of X. testudinaria sponge collected from Sanur beach, Bali, Indonesia was toxic against A. salina larvae with LC_{50} of 31.62 ppm.*

Keywords*: Sponge Xestospongia testudinaria; Toxicity; Artemia salina*

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I. Introduction

Cancer is a malignant disease that is feared by society. Several types of modern medical therapies such as surgery, chemotherapy or radiation have been widely used. However, the remaining constraints are the varying degrees of success depending on the stage and type of cancer, the onset of side effects, and the high cost of modern therapy. Therefore, exploration of the active compounds with anticancer activity has began in many countries, especially developed countries. The study has also expanded on marine natural ingredients and provided a variety of new cancer-fighting alternatives [1].

Sponges are one of the many biota in the sea. In Indonesian waters it is estimated that there are more than 1,000 species (types) of sponges. Reportedly sponge is a highly prospective bioactive material from the ocean. Nearly 5,000 compounds have been successfully isolated from these animals with activities such as antimicrobials, antifungals, antivirals, and anticancer drugs [1]. Sponges are potential marine organisms for exploration of new anticancer compounds because sponges produce the antiviral and cytotoxic compounds [2]. One of the sponges found on the coast of Sanur, Bali is *Xestospongia testudinaria*.

Various studies have been conducted to isolate the compounds of the type spoge *Xestospongia testudinaria* (*X. testudinaria*). Quinn and Tucker [3] isolated brominated bis- acetylenic acetic acid. Further more Quinn and Tucker [4] also isolated two new compounds of brominated acetylenic acids. Pham *et al* [5] isolated brominated acetylenic fatty acid and two esterified sterol. Jiang *et al* [6] isolated four brominated aliphatic hydrocarbon and sterol. Lee *et al* [7] isolated *Marinobacter xestospongiae* sp. Nov. Sun *et al* [8] isolated the new compound bisabolane sesquiterpenoid. Nguyen *et al* [9] reported isolating anti-fouling compound 26,27-cyclosterol. Toxicity of *X. testudinaria* has been reported by Zhou *et al*.[10]. According to their report, the five compounds contained in *X. testudinaria* (Sapinofuranone; Xestospongic acid; 24-hydroperoxy-24-vinyl-cholesterol; Saringosterol; and 29-hydroperoxystigmasta-5,24-dien-3β-ol) were toxic towards larvae of *A. Saline* with LC_{50} values varies between 0.56 and 6.99 μM.

Prescreening test of a substance potentially having anticancer activity is by toxicity test using Bhrine Shrimp Lethality Test (BSLT) method. Concentration sample with the 50% of mortality (LC_{50}) is determined using bioindicator of *Artemia salina* larvae. If a substance has a toxicity (LC_{50}) lower than 1,000 ppm, then the material has the potential to have anticancer activity, then it can be tested for cancer cells [11].

II. Material And Methods

2. 1 Materials:

X. testudinaria sponges were collected at Sanur beach, Bali, Indonsia, on April 25th to Mei 5th, 2017. Methanol, n-hexane, and ethyl acetate were purchased in Merck, Germany. Brine shrimp *Artemia salina* Eggs

was purchased in American Technology. Sel HeLa Line was purchased in Primate Study Center, Bogor Agriculture Institute.

2. 2 Sample preparation:

Wet sponges *X. testudinaria* were cleaned with fresh water, then sliced into small size. Furthermore, they dried in a place that is not subject to direct sunlight for approximately 10 days. After drying, the sample was smoothed using a blander and sieved to a smoothness of 100 mesh.

2. 3 Extraction:

Dry powder samples of 250 grams each were extracted using methanol and n-hexane by maceration method. Every 24 hours the extract was isolated and the residue was added with a new solvent. Substitution of this solvent was done up to 3 times. Each of the extracts obtained was then vaporized using a rotary vacuum evaporator until a crude methanol and n-hexane extract was obtained.

2. 4 Toxicity test:

The medium for larvae hatching was made by filtering the seawater sufficiently. Sea water was put in aquarium that was divided into two parts, one dark and the other light. A total of 50 mg *A. salina* egg was placed or immersed in the dark part and left for 48 hours until it hatched into a mature larvae and ready for use for testing. Twenty milligrams of each methanol and n-hexane extract were dissolved with 2 mL of solvent. This solution was taken as 500 µL, 50 µL and 5 µL, then each was inserted into the test tube and the solvent was evaporated. Fifty µL of dimethyl sulphoxide, 1 mL of seawater, and 10 larvae were fed into a test tube containing the sample (the solvent was evaporated), then seawater was added to the volume of 5 mL, to obtain the extract with concentration of 1000, 100, and 10 ppm. The concentration of 0 ppm was prepared as a control without the addition of the extract. After 24 hours, the death of *A. saline* larvae was observed. The standard for assessing larvae mortality wass when the larvae did not show movement for several seconds of observation [12]. The number of live and dead larvae were recorded, then data analysis was done to find the lethal concentration (LC_{50}).

III. Result And Discusion

3. 1 Sample preparation and extraction:

The wet sponge samples of 7,634 grams were cleaned, sliced, and dried. It produced dry sponge sample of 893 grams. All of the dried samples were mashed and sieved with a fineness level of 100 mesh producing 784 grams of fine sample. Each 250 gram sample was extracted with methanol and n-hexane resulting in 757 mg of methanol extract and 327 mg of n-hexane extract.

3. 2 Toxicity:

The n-hexane and methanol extract was assayed their toxicity by *A. salina* larvae. Accumulation and Percentage of Deaths of larvae in the extracts were shown that Table 1.

Table 1. Accumulation and Percentage of Deaths of larvae in n-hexane and methanol extract

Extract	Concentration (ppm)	The number of live larvae	The number of dead larvae	Accumulation		% Mortality
				Live	Dead	
n-hexane	1000	1	9	1.0	14.0	93
	100	7	3	8.0	5.0	38
	10	8	2	16.0	2.0	11
Methanol	1000	0	10	0.0	20.0	100
	100	2	8	2.0	10.0	83
	10	8	2	10.0	2.0	17

From the data in Table 1 there can be made a correlation between the log of the sample concentration and the percentage of mortality of each extract as Table 2.

Table 2. The correlation between log of sample concentration and % mortality of n-hexane and methanol extracts

n hexane extract		Methanol extract	
Log of sample concentration	% Mortality	Log of sample concentration	% Mortality
0	0	0	0
1	11	1	19
2	38	2	83
3	93	3	100

Based on Table 2, can be made the graph correlation between the log of sample concentration and the percentage of mortality of each sample such as Figure 1.

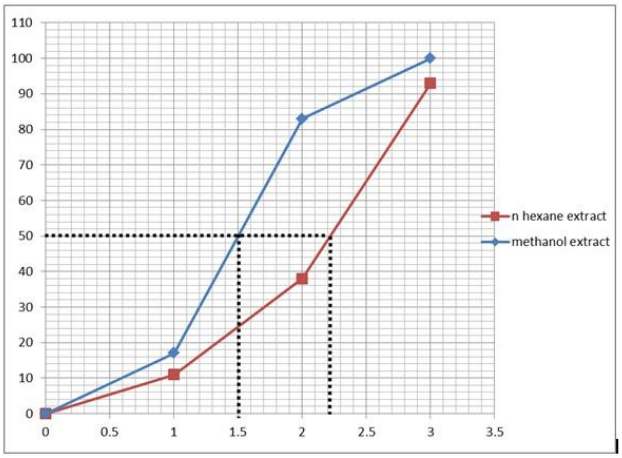

Figure 1: The graph correlation between log of sample concentration and % mortality in n-hexane and methanol extracts

Based on Figure 1 above, 50% mortality of n-hexane extract was obtained at log concentrations of 2.25. So the LC_{50} value for n-hexane extract is 177,83 ppm. 50% mortality of methanol extract also was obtained at log concentrations of 1.50. So the LC_{50} value for methanol extract is 31.62 ppm. From all the above data, it can be summarized as in Table 3 below.

Table 3. Result of Toxicity Test of *X. testudinaria* Sponge n-hexane and methanol extracts

Extracts	Concentration	The number of dead larvae				%	LC_{50}
	(ppm)	1	2	3	Average	Mortality	
n-hexane	1000	9	9	9	9	93	177.83
	100	2	3	4	3	38	
	10	2	2	1	2	11	
	0	0	0	0	0	0	
Methanol	1000	10	9	10	10	100	31.62
	100	8	7	8	8	83	
	10	2	2	1	2	17	
	0	0	0	0	0	0	

Based on the results in Table 3 above, it showed that *X. testudinaria* sponge methanol extract was toxic to *A. salina* larvae with LC_{50} of 31.62 ppm. The results of this study were in line with research conducted by Zhou *et al*.[10]. He obtained that five compounds in the *X. testudinary* sponge, namely sapinofuranone; xestospongic acid; 24-hydroperoxy-24-vinylcholesterol; Saringosterol; and 29-hydroperoxystigmasta-5,24-dien-3β-ol are toxic to *A. saline* larvae with LC_{50} values varying between 0.56 to 6.99 μM.

IV. Conclusion

Based on the results of this study, it can be concluded that the methanol extract of *X. testudinaria* sponge collected from Sanur beach, Bali, Indonesia was toxic against *A. salina* larvae with LC_{50} of 31.62 ppm.

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