

Extraction and Phytochemical Test of Toxic Extract of *Xestospongia testudinaria* Sponge

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Abstract This research aims to extraction and phytochemical test of the toxic extract of the *Xestospongia testudinaria* sponge collected from Sanur beach, Bali, Indonesia. Extraction of the sponges was carried out by methanol at room temperature. Toxicity screening test was done based on Brine Shrimp Lethality Test (BSLT). The compounds of the toxic extract were performed by phytochemical test. Based on the results, it was found that the methanol extract of *X. testudinaria* sponges has toxic activity with LC₅₀ of 31.62 ppm. The toxic extract contained alkaloid, steroid, polyphenol, and saponin compounds.

Keyword: phytochemical test, toxic extract, *Xestospongia testudinaria* sponge

I. INTRODUCTION

The requirement for new anticancer medicines is increasingly urgent, because the medicines used are expensive and low selectivity. The search for new sources to produce anticancer compounds continues to be done, one of them from marine organisms. The bio-prospecting potential of marine biota for basic materials of pharmaceutical, cosmetics, bio-energy, and other industries in Indonesia is huge, estimated to reach economic value of 40 billion US dollars per year [1]. In 1995, trade results for bio-prospecting medicines reached US \$ 14 billion [1]. In fact, Indonesia still has not been able to produce basic chemicals for the production of medicines with nearly 90% of the basic chemical ingredients is still imported.

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 [2].

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 [2]. Sponges are potential marine organisms for exploration of new anticancer compounds because sponges produce the antiviral and cytotoxic compounds [3]. One of the sponges found on the coast of Sanur, Bali is *Xestospongia testudinaria* (*X. testudinaria*). Various studies have been conducted to isolate the compounds of the type sponge *X. testudinaria*. Quinn and Tucker [4] isolated brominated bis-acetylenic acetic acid. Further more Quinn and Tucker [5] also isolated two new compounds of brominated acetylenic acids. Pham et al. [6] isolated brominated acetylenic fatty acid and two esterified sterol. Jiang et al. [7] isolated four brominated aliphatic hydrocarbon and sterol. Lee et al. [8] isolated *Marinobacter xestospongiae* sp. Nov. Sun et al. [9] isolated the new compound bisabolane sesquiterpenoid. Nguyen et al. [10]. reported isolating anti-fouling compound 26,27-cyclosterol. Toxicity of *X. testudinaria* has been reported by Zhou et al. [11]. According to their report, the five compounds contained in *X. testudinaria*

(Sapinofuranone; Xestospongiic acid; 24-hydroperoxy-24-vinyl-cholesterol; Saringosterol; and 29-hydroperoxystigmasta-5,24-dien-3 β -ol) were toxic towards larvae of *A. Salina* with LC₅₀ values varies between 0.56 and 6.99 μ M.

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 (LC₅₀) is determined using bioindicator of *A. salina* larvae. If a substance has a toxicity (LC₅₀) lower than 1,000 ppm, then the material has the potential to have anticancer activity, then it can be tested for cancer cells [12].

II. METHODS AND PROCEDURES

A. Materials

Sponges *X. testudinaria* was collected from Sanur beach, Bali, Indonesia, on April 25th, 2017. The sample was cleaned of impurities with tap water until clean, then it dried at room temperature for several days. After drying, subsequently it ground to the degree of fineness of 100 mesh.

B. Metabolites Extraction

A total of 200 grams of powder samples was extracted with methanol until submerged and left to stand for 24 hours, than filtered. The filtrate was collected and the waste was added again the same solvent until submerged. This work was repeated 3-4 times until all the estimated extractable compounds. The filtrated collected was evaporated with a rotary vacuum evaporator until all the solvent evaporates, thus obtained crude extract.

C. Toxicity Test

Toxicity test using bio-indicator of *A. salina* larvae followed the method of Meyer [12]. Media for *A. salina* larvae hatch was made by filtering sea water. The sea water was put into the aquarium divided into two parts, one part was made of dark covered with black paper and the other part was left open. *A. salina* eggs laid moderation on the part of dark and left to stand for 2 x 24 hours so that the eggs hatch into larvae that was ready for testing. Weighing 20 mg of substance to be tested was dissolved in 2 mL of n-hexane. From this solution was taken 500; 50; and 5 μ L, then each inserted into a test tube and the solvent evaporated. Into each test tube added 1 mL of seawater, 50 μ L of dimethyl sulfoxide, 10 larvae. Then the sea water was add to get volume of 5 mL, in order to obtain the concentration of the extract on each tube: 1000; 100; and 10 ppm. Extract with concentration of 0 ppm (without the addition of extract) was made as a control. Each test tube was covered with aluminum foil and hollowed out a bit and then left at room temperature. After 24 hours, the observation on larval mortality was carried out. The standard for assessing larvae mortality was when the larvae did not show movement for several seconds of observation [13]. The number of dead larvae were recorded, then count of LC₅₀.

D. Phytochemical Test

The toxic extract were identified the group chemical compounds including: testing of alkaloid, flavonoids, triterpenoids/steroids, polyphenols, and saponins [14].

III. RESULTS

A. Metabolites Extraction

Extraction of 200 grams of *X. testudinaria* sponge powder samples using methanol was produced 11.57 grams of dark green methanol extract. According to Sadek [15], methanol has polarity index of 5.4 and has a 100% solubility in water will dissolve the compounds that a polar to non-polar.

B. Toxicity Test

The 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

Concentration (ppm)	The number of live larvae	The number of dead larvae	Accumulation	Percentage of Deaths (%)	
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 the extract as Table 2.

TABLE 2. THE CORRELATION BETWEEN LOG OF SAMPLE CONCENTRATION AND % MORTALITY

Methanol extract	
Log of sample concentration	% Mortality
0	0
1	19
2	83
3	100

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

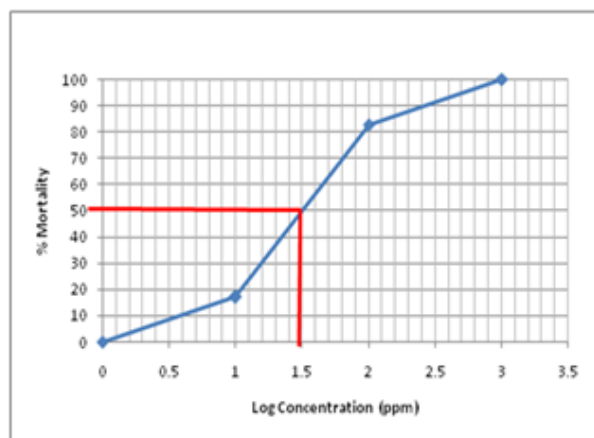


Fig. 1. The graph correlation between log of sample concentration and % mortality

From on Figure 1 above, the 50% mortality was obtained at the concentration log of 1.50. So the LC_{50} value for methanol extract is 31.62 ppm.

Based on the results, 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. [11]. He obtained that five compounds in the *X. testudinaria* sponge, namely sapinofuranone; xestospongic acid; 24-hydroperoxy-24-vinylcholesterol; Saringosterol; and 29-hydroperoxystigmasta-5,24-dien-3 β -ol are toxic to *A. salina* larvae with LC_{50} values varying between 0.56 to 6.99 μ M.

D. Phytochemicals Test

The toxic extract were tested phytochemically to determine the compound. Phytochemical test results was presented in Table 3.

TABLE 3. RESULTS OF PHYTOCHEMICAL TEST OF TOXIC EXTRACT

Group of compounds	Reagents	Changes of colour	Conclusion
Alkaloids	Dragendrof Mayer Wagner	Red precipitate White precipitate Brown precipitate	Positive alkaloids
Flavonoids	Na OH 10% Test Wilstatter Test Bate-Smith and Metacalf	No changes No changes No changes	Negative flavonoids
Triterpenoids/ Steroids	Lieberman-Burchard H_2SO_4 10%	Green Green	Positive Steroids
Polyphenols	$FeCl_3$ 10%	Black	Positive Polyphenol
Saponins	Uji Busa/Froth +HCl 2%	Foam formed	Positive Saponins

IV. CONCLUSION

Based on the above results it can be concluded:

1. 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.
2. The phytochemical test results showed that the toxic extract was positive containing alkaloids, steroids, polyphenols, and saponins.

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