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Testing the Toxicity, Protein Content, and Anticholesterol of the Ethanol Extract of the Sea Urchin (Diadema Setosum) Gonad as Marine Biodiversity-Based Medicinal Ingredients

Yusuf Sabilu*, Jafriati, Asnia Zainuddin

Public Health Study Program, Public Health Faculty, Halu Oleo University, Kendari, 93231, Indonesia

Abstract: Indonesia has various marine resources that can be used, one of which is a sea urchin (*Diadema setosum*), which can be used as pharmacology and food sources by using their gonad. This study aims to test the toxicity, protein content, and antioxidant activity of the ethanol extract of the sea urchin gonad. The sample tests consisted of several stages, namely: maceration performed the extraction using 96% ethanol solvent; the toxicity test used the Brine Shrimp Lethality Test method with Artemia salina Leach as the bioindicator; the protein content test used the Lowry method; anticholesterol activity test was done with ultraviolet-visible spectrophotometry (UV-Vis) method. The results showed that the ethanol extract of the sea urchin gonad had no toxic activity because the IC50 value was more than 1000 ppm, which was 1318.8639 ppm. The protein content of the ethanol extract of the sea urchin gonad was 0.0257% and had no anticholesterol potential with a value of 0.0002%. This research can be performed by conducting tests to determine aspects of reproductive biology and the anti-inflammatory effects of *Diadema setosum*.

Keywords: *Diadema setosum*, toxicity, protein content, anticholesterol.

測試海膽(王冠瀨戶)性腺的乙醇提取物作為海洋生物多樣性藥物成分的毒性、蛋白質含量和抗膽固醇

摘要：印度尼西亞有多種海洋資源可供利用，其中一種是海膽（毛茸茸的王冠），利用其性腺可作為藥理和食物來源。本研究測試了海膽性腺乙醇提取物的毒性、蛋白質含量和抗氧化活性。樣品測試由幾個階段組成，即：浸漬使用 96%乙醇溶劑進行提取；毒性試驗採用鹵水蝦殺傷力試驗法，以鹵蟲浸出液為生物指標；蛋白質含量測試採用勞瑞法；抗膽固醇活性試驗採用紫外-可見分光光度法法進行。結果表明，海膽性腺乙醇提取物的我知道了 50 值大於 1000 ppm，即 1318.8639 ppm，無毒活性。海膽性腺乙醇提取物的蛋白質含量為 0.0257%，無抗膽固醇作用，值為 0.0002%。這項研究可以通過進行測試來確定生殖生物學的各個方面和毛茸茸的王冠的抗炎作用。

关键词：毛茸茸的王冠，毒性，蛋白質含量，抗膽固醇。

1. Introduction

Indonesia has a very high diversity of marine life that can be used for life. One of the potential marine biota to be used as medicine is a sea urchin. Sea urchins are aquatic biota from the echinoderm phylum scattered in almost all waters. *Diadema setosum* is one spreads in reef waters, including sandy and algae growth zones [1].

According to [2], in Japan, the sea urchin gonad is known as *uni*; on Tomia Island, Wakatobi Regency, known as Kukure. Kukure is a processed product from sea urchins that are only in the form of gonads and packaged in an individual sea urchin shell whose spines

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About the authors: Yusuf Sabilu, Jafriati, Asnia Zainuddin, Public Health Study Program, Public Health Faculty, Halu Oleo University, Kendari, Indonesia

Corresponding author Yusuf Sabilu, yusuf.sabilu@uho.ac.id

have been removed [3]. Meanwhile, the Indonesians consume four sea urchins: *Tripeustas gratilla*, *Diadema setosum*, *Echinothrix calamaris*, and *Toxopneustes pileolus; Diadema setosum* is one of the most popular types of sea urchins for consumption by the Indonesians.

Sea urchins are caught in their habitat, and their gonads are taken for consumption, both raw and cooked. The capture of this sea urchin has even been reported to be overfishing [4]. Sea urchin gonads are one of the delicious foods that have high nutritional content, so they have a high selling value. The very high economic value of sea urchins is a critical reason to get to know this biota more to preserve and develop sea urchin cultivation. Many benefits can be taken from sea urchins. Besides being important for water, sea urchins are also used as food by coastal communities by taking sea urchins for consumption [5, 6].

According to [7], sea urchin organs have economic value. They are used as food ingredient – gonads. Local people know it better than sea urchin eggs. As a reproductive organ, the gonads are seed factories that are certain to have high protein deposits [7]. The gonads of *D. setosum* sea urchins contain 18 unsaturated fatty acids, including omega-3 and omega-6 and 15 types of amino acids [8].

[9] stated that until now, *Diadema setosum* is more on the inside of its body, which is used for food, while the shell and other outer parts have not been maximally used. Information about published research results on sea urchins revolves around the cytotoxic activity. Chloroform extracts of sea urchin shells and spines have cytotoxic potential with a lethal toxicity value (LC50) against *Nauplius Artemia sp.* of 133.58 ppm [9, 17].

Sea urchin gonads have been used as a traditional food for the community by using the gonads consumption, both raw (fresh) and processed products. However, the use of sea urchins as medicinal products and health food has not been widely used. Based on this description, it is necessary to research assess the toxicity, protein content, and anticholesterol activity of the ethanol extract of the gonads of the sea urchins as a medicinal ingredient based on marine biodiversity.

2. Research Method

A sampling of sea urchins was carried out in Wakatobi waters. Sample preparation and extraction were carried out at the Pharmacy Research Laboratory, Faculty of Pharmacy, Halu Oleo University. The tests of toxicity, protein content, and anticholesterol activity were carried out at the Biochemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University of Makassar.

2.1. Materials and Tools

The tools used are Toolbox, autoclave, analytical balance, set of glassware, UV-Vis spectrophotometer, vortex, measuring cup, beaker glass, test tube, blender, water heater, rotary vacuum evaporator, Petri dish, micropipette and tip, dropper, sieve, Eppendorf tube, vial, insulated container and dark lid, volumetric flask, mortar and stamper, hotplate, cuvette, pipette, glass funnel, volumetric flask, and water bath.The materials used in this study were sea urchin (*D. setosum*), 96% ethanol, distilled water, DMSO, labels and markers, *Artemia salina L. larvae*, zinc powder, 2 N hydrochloric acid, concentrated hydrochloric acid, anhydrous acetic acid, 10% NaOH, $FeSO_4$, 92% cholesterol standard, concentrated sulfuric acid (H_2SO_4), aqua dest, acetone, boric acid powder, oxalic acid powder, and ether.

2.2. Preparation and Extraction of Raw Materials

The sea urchin (*Diadema setosum*) was sampled at low tide in Wakatobi waters. Then the gonads were taken and stored in a toolbox containing ice cubes to maintain the freshness of the sea urchin gonads during the trip to the laboratory. The gonad preparation of the sea urchin began by weighing the sample. The next stage is the extraction of the active ingredients. The extraction method is the maceration. The solvent used in this research was 96% ethanol solvent. 96% ethanol was used as a solvent in the maceration process with the consideration that ethanol is more selective, antibacterial, non-toxic, and miscible with water in various ratios [10].

The sample weighed as much as 150 g and was macerated with 1100-mL ethanol solvent for 48 h. The result of maceration, in the form of a solution, was then filtered with filter paper to obtain the filtrate and residue. The filtrate was evaporated until the solvent separated from the extract using a Rotary Vacuum Evaporator at a temperature of less than 50^{o}C. This extract was developed by adding diluents or aqua dest as a solvent. The extraction results were evaporated to obtain a thick extract of sea urchin gonads. Furthermore, the toxicity, protein content, and anticholesterol activity of the ethanol extract of the gonads of the sea urchins were tested (Fig. 1).

Fig. 1 Sea urchin (*Diadema setosum)* gonad sample

2.3. Toxicity Test

Toxicity tests of ethanol extract of sea urchin gonads used the Brine Shrimp Lethality Test (BSLT) method, which referred to the method of [11]. A total of

10 shrimp larvae aged 48 h and 1 mL of seawater were put into four different vials. Then to each vial, add 1000, 100, and 10 sample solutions, and add to 2-mL seawater so that each concentration was obtained at 1000 ppm, 100 ppm, and 10 ppm, and for each concentration was repeated three times, while the control was made by adding 10 shrimp larvae and 2-mL seawater without adding the test solution. Observations were made every 24 h by counting the number of live and dead shrimp larvae.

2.4. Determination of Protein Content

The protein content was determined by the Lowry method [12]. The Sea urchin extract reacted with Lowry's reagent, allowed to stand for ten minutes at room temperature. Then, Folin-Ciocalteu reagent was added and incubated for 30 min. After that, the absorbance was measured at the maximum wavelength obtained. Measurements were repeated thrice. The results of the average absorbance were entered into the linear regression equation of the protein standard curve, y = ax + b. The protein standard used was Bovine Serum Albumin (BSA). The protein content in sea urchins was obtained using the following formula:

Protein content = $C.fp.V_{\text{sample weight extract}}\,(g)\;x$ 100%

2.5. Anti-Cholesterol Activity Test

The thick extract of the sea urchin made a series of concentrations of 200 ppm, 400 ppm, and 600 ppm. 5.0 mL of each concentration was added to a test tube, each was added to 5.0 mL of standard cholesterol solution, and then 2.0 mL of anhydrous acetic acid and 0.1 mL of concentrated H_2SO_4 were added. The solution was left in a dark place for 5 min until the color changed to green. The color results obtained were measured using a UV-Vis spectrophotometer at a wavelength of 668 nm [13]. The absorbance obtained from the measurement of the ethanol extract of the sea urchin (*Diadema setosum*) was compared with the standard cholesterol solution to determine the percentage of cholesterol reduction. Calculation of the percentage of cholesterol-lowering levels used the formula:

$$A = \frac{C - B}{C} x\ 100\%$$

where A = % of cholesterol reduction; B = Average amount of cholesterol after treatment; C = Average amount of initial cholesterol.

3. Results and Discussion

3.1. Toxicity Test

The toxicity test results on the gonadal extract of the sea urchin (*Diadema setosum*) were obtained with three repetitions, and the total larvae of *Artemia salina Leach.* The results can be seen in Table 1 and Fig. 2.

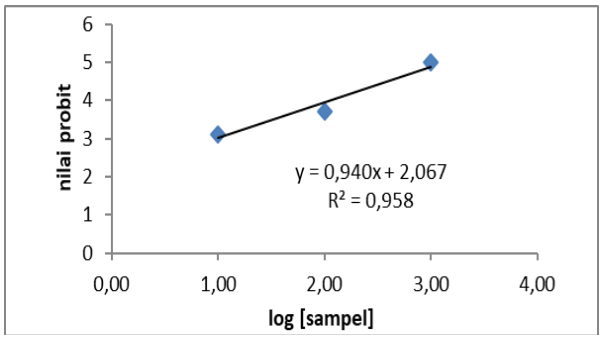

Fig. 2 Probit analysis graph

Table 1 Percentage of larval mortality

Sample code	Absorbance	Measurable Protein (mg/mL)	Sample mass (mg)	Sample Solution Volume (mL)	Measurable Protein (%)	Average Measurable Protein (mg/mL)	Average Measurable Protein (%)
Simplo sea urchin	0.092	0.0009	2	1000	0.0002	0.0009	0.0002
Duplo sea urchin	0.092	0.0009	2	1000	0.0002		

Based on Table 1, it can be seen that the higher the concentration, the greater the larval mortality rate.

For the LC 50 (x), the probit value is 5 (y), entered into the regression equation of

y = 0.940x + 2.067
y – 2.067/0.940 = x
5 – 2.067/0.940 = 3.1202
Thus, log x = 3.1202
x = antilog 3.1202
x = 1318.8639 ppm
LC 50 sample = 1318.8639 ppm

The toxicity test in this study used the BSLT method, which uses aquatic animals in the form of shrimp larvae (*Artemia salina L.*). This method can be carried out quickly, cheaply, and easily as an initial stage in assessing a material's toxicity level. This method is recommended for screening the activity of various extracts with pharmacological effects [10, 14]. One of the initial methods for cytotoxic testing was the Brine Shrimp Lethality Test (BSLT) [11]. The sea urchin gonad extract was obtained using 96% ethanol solvent from the maceration process. Maceration is the simplest extraction method by immersing the sample (simplicia) with a solvent that can penetrate the cell wall and remove the active substances in the sample cells [10].

Table 1 shows that the mortality of shrimp larvae increased along with the increase in extract concentration, which means that the higher the extract concentration, the higher the toxicity produced. However, the test material's toxicity level was inversely proportional to the Lethal Concentration of 50% LC50. Based on the results of the probit analysis of the relationship between the concentration of sea urchin gonad extract and the percentage mortality of shrimp larvae, the LC50 value was 1318.8639 ppm, which means that it was not toxic. It refers to the statement [11] that an extract is considered very toxic if it has an

LC50 value below 30 ppm; it is considered toxic if it has an LC50 value of 30–1000 ppm, and it is considered non-toxic if the LC50 value is above 1000 ppm. The high and non-toxic LC50 value was due to the low mortality of *A. salina* larvae at a concentration of < 1000 ppm, i.e., at this concentration, mortality did not reach 50% of the number of larvae tested [11]. Additionally, it is also suspected that the sample extract did not contain secondary metabolite compounds, or the compounds in the extract were not detected at the desired concentration, so they were non-toxic. A compound can be toxic if, in a short time, it can kill 50% of *A. salina* larvae.

The mechanism of death of *Artemia salina L*. larvae is related to the function of alkaloid and flavonoid compounds that inhibit the larval feeding power (antifeedant). The way these compounds act as stomach poisoning or stomach poison. Therefore, the digestive system will be disturbed when these compounds enter the larva's body. This compound will block the taste receptors in the mouth area of the larvae. The larvae failed to get a taste stimulus, so they could not recognize their food.

Consequently, the larvae starved to death [15]. In this study, sea urchin gonad extract was not toxic to shrimp larvae because sea urchin extract did not contain flavonoids [15]. The ethanol extract of sea urchin (*Diadema setosum*) gonads contained alkaloids, steroids, triterpenoids, and tannins, while flavonoids were not detected [16].

It is different from the other body parts of sea urchins, namely, sea urchin shells, which have toxic activity. As revealed by Aprillia *et al.* in 2012, the chloroform extract of sea urchin shell had a toxic activity against Artemia with an LC50 value of 133.58 ppm. The body of a sea urchin is 95% made up of very fragile and poisonous spines [17]. The poison in sea urchins comes from serotonin, glycosides, steroids, cholinergic, and bradykinin-like substances [18]. Sea urchin toxins can be used in medicine; they have the potential as an antibiotic. Sea urchins are also known as biota that produces high natural calcium carbonate [19].

3.2. Protein Content Test

The Lowry method measured the protein content in the ethanol extract of sea urchin (*Diadema setosum*) gonads. The results of the average protein content can be seen in Table 2.

Table 2 Average protein content results

Sample code	Absorbance	Measurable Protein (mg/mL)	Sample mass (mg)	Sample Solution Volume (mL)	Measurable Protein (%)	Average Measurable Protein (mg/mL)	Average Measurable Protein (%)
Simplo sea urchin	0.093	0.0242	1000	10	0.02	0.0257	0.0257
Duplo sea urchin	0.100	0.0272	1000	10	0.03		

Table 2 shows that the protein content of the ethanol extract of the sea urchin gonad is 0.0257%. The reason for the low protein content lies in many factors, including the influence of stability and changes in water content. Additionally, differences in protein content in the fresh ingredients also affect the final protein amount. Protein levels of sea urchin gonads will certainly be different before and after being extracted. Sea urchins have three important biochemical components: protein, fat, and carbohydrates. These three components are energy providers for sea urchins and structural elements in the formation and development of eggs [20].

Habitat and type of sea urchin can also influence the protein content. The nutritional content of protein in sea urchins depends on the size of the gonads, and yellow gonads have more protein content [21].

3.3. Anticholesterol Activity Test

The anticholesterol activity test of the ethanol extract of the sea urchin gonad used the UV-Vis spectrophotometric method (Table 3).

Table 3 Anticholesterol activity average results

Sample code	Absorbance	Measurable Protein (mg/mL)	Sample mass (mg)	Sample Solution Volume (mL)	Measurable Protein (%)	Average Measurable Protein (mg/mL)	Average Measurable Protein (%)
Simplo sea urchin	0.092	0.0009	2	1000	0.0002	0.0009	0.0002
Duplo sea urchin	0.092	0.0009	2	1000	0.0002		

The results in Table 3 show that the ethanol extract of the sea urchin gonad does not have low anticholesterol potential, with a value of 0.0002%.

Plants containing flavonoids are effective in lowering cholesterol. In contrast, the ethanol extract of sea urchins does not contain flavonoids, so it does not have anticholesterol potential. The ethanol extract of the sea urchin (*Diadema setosum*) gonad contained alkaloids, steroids, triterpenoids, and tannins, while flavonoids were not detected [16]. The compounds thought to play a role in lowering cholesterol levels are phenolics, flavonoids, and vitamin C. The hydroxyl group in cholesterol reacts with the ketone group in flavonoids to form hemiacetals [22].

The findings of this study were to determine the toxicity, protein content and anticholesterol activity of the ethanolic extract of the gonads of the sea urchin *Diaedema setosum*. This information will be helpful in the management and cultivation of *Diaedema setosum* sea urchins. This research can be conducted by conducting tests to determine aspects of reproductive biology and the anti-inflammatory effects of *Diadema setosum*.

4. Conclusion

The toxicity test using the BSLT method showed that the gonad extract of the sea urchin (*Diadema setosum*) was classified as non-toxic, with an LC50 value of 1318.8639 ppm.

The protein content test using the Lowry method showed that the ethanol extract of the sea urchin gonad was 0.0257%.

The anticholesterol activity test using UV-Vis spectrophotometry showed that the ethanol extract of the sea urchin gonad did not have anticholesterol potential with a value of 0.0002%.

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