Identification of sea urchin gonads chemical compounds using thin-layer chromatography from Bokory island, Southeast Sulawesi

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Abstract. Southeast Sulawesi is one area that has a diversity of potential marine biota as medicinal ingredients, including the sea urchin gonads. Empirically coastal communities have been hereditary, consuming sea urchin gonads as a source of high protein. Considering its potential as a raw material for medicines, it is necessary to conduct a series of tests to guarantee its safety and efficacy. This study aims to determine the chemical compounds of sea urchin gonad extract (Diadema setosum L) by Thin-Layer Chromatography (TLC). The research method uses a Pre- experimental design with a one-shot case study model. Identification of metabolites of sea urchin gonad extracts was carried out in two stages: phytochemical screening and identification by TLC method by UV-Vis. Samples were dried by the Freeze-drying method to get 335.11 g of dry samples and 58.06 g of extracts. The results of the test of the content of water-soluble and ethanol- soluble sea urchin extract were 5.73% and 3.72%. The results showed that in phytochemical screening, ethyl acetate extracts of the positive marine gonads contained alkaloids, saponins, and steroids. In addition, UV-vis 254 nm and 366 nm TLC methods showed alkaloids and saponins. The Rf values are 0.4 and 0.68, respectively.

1. Introduction

Echinoidea, commonly known as sea urchins, are a taxonomic class of marine animals that have a physical appearance with thorns covering their bodies. These marine animals’ spines consist of calcium and vary in length [1]. Biodiversity of marine products is not only a nutritious food ingredient but also has an active biological compound that has medicinal properties [2].

Diadema setosum is a type of sea urchin that is spread on coral reefs, including in sand zones, algae growth zones, seagrass zones to coastal areas [3]. Sea urchins are widely distributed in the world and have an important role in seawater ecosystems. Sea urchin species contain an economic value, nutrition, health are increasingly intensive in the food, pharmaceutical and cosmetics industries. The total world production of sea urchin consumed in 2015 reached 71,229 tons [2,4].

Sea urchins have edible body parts namely eggs and gonads. Gonad has high nutritional value. Several previous studies provide insight into how sea urchin gonads are one of the most nutrients marine commodities to consume [5,6]. Gonads have been consumed by humans since the beginning of history as shown by middens from the Aleutian Islands to the Caribbean Sea and the coast of Chile [7]. Sea urchin gonads are expensive and have a delicacy as traditional Japanese food. One of the most commonly encountered species of sea urchins in Bokori Island, Indonesia is Diadema setosum L. empirically the coastal communities of Bokori island consume sea urchin gonads as a source of protein and reduce cholesterol levels. The references related to studies of sea urchins gonads of different
species have been carried out. Gonads of the green sea urchin *Strongylocentrotus droebachiensis* are rich bioactive compounds, such as polyunsaturated fatty acids (PUFAs) [8], carotenoids [9,10], and phospholipids [11]. Besides, Gonads of sea urchin contains several amino acids such as valine, threonine, glycine, histidine, alanine, glutamic acid, lysine, leucine, I-leucine, methionine, tyrosine, and arginine [12].

Nowadays, health problems are increasing in line with the development of the disease. The problem of health service costs is increasing, so it is necessary to think about increasing product efficiency [13]. In line with this, the concept of life back to nature began to be sought after and supported by the abundance of natural wealth in Indonesia [14]. Sea urchins gonads as a marine product as one of the natural ingredients which have medicinal properties. The use of sea urchin gonads extracts as a burning medicine has been developed in pharmaceutical dosage forms. A good pharmaceutical preparation meets the physical evaluation requirements for topical preparations of sea urchins gonad extract is a gel preparation of 4%, which effectively heals burns by up to 100% for 27 days [15].

Species of sea urchins *Diadema setosum* are widespread on the Bokori Island, Southeast Sulawesi, Indonesia. At present, little information is available about biological and chemical content. Based on the facts above, this research aims to extract sea urchin gonads by the identification of chemical compounds using thin-layer chromatography.

2. Materials and Methods

2.1. Materials

Sea urchin gonad (*Diadema setosum* L) was collected from Bokori island, Southeast Sulawesi in April 2019. After dissection, the fresh gonad tissue was collected. Samples were then dehydrated by indirect sun exposure, and then homogenized, and freeze-dried.

2.2. Extraction

A fresh sample of 2000 g sea urchin gonad was dried by the Freeze-drying method, which obtained dry samples that had passed the dry sorting process 335.11 g. Dry sample extraction was done by the maceration method repeatedly for 3 days then filtered, separated the filtrate and residue, then the residue was macerated again with the same solvent. The maceration process is stopped if there is no change in the color of the solvent. The liquid filtrate was obtained as much as 2030 mL. The liquid extract was evaporated using a rotary evaporator at 50°C. The viscous extract was obtained 58.06 g with a yield value of 17.32%. The high value of yield indicates the number of bioactive components that can be extracted by ethyl acetate [16]. The well-prepared sea urchin gonad extracts were used for phytochemical screening and identification by Thin-Layer Chromatography (TLC).

2.3. Test of the content of water-soluble and ethanol-soluble

A total of 2.5 grams of the extract was filtered for 24 hours with 50 ml of LP chloroform (water-soluble) ethanol 95% (ethanol-soluble), and, water using clogged pumpkin while being shaken repeatedly for the first 6 hours and then left for 18 hours. Strain, steam 10 ml of the filtrate to dry in a shallow flat cup that has been weighed before, heat for 18 hours. Filter quickly by avoiding evaporation of ethanol, then residue at 105 °C. Calculate levels in percent of compounds that dissolve in water, calculated against the initial extract [17].

2.4. Phytochemical screening

The test to determine alkaloid, saponin, triterpenoids, and steroids. Phytochemical screening was carried out to determine the presence of secondary metabolites in sea urchin gonad extracts. About 1 mg/mL of ethyl acetate extract stock solution is prepared [18,19, 20].
2.4.1. Test for saponin
About 1 ml of the stock solution is diluted with 10 mL of distilled water in a test tube. The test tube was closed and shaken vigorously for 15 minutes. The formation foam indicates the presence of saponins in the sample.

2.4.2. Test for flavonoid
About 0.5 mL of aqueous NaOH solution is added to 1 mL of stock solution in a test tube. An intense yellow colour appeared on the test tube. Then, dilute hydrochloric acid it became colorless indicating the presence of flavonoids.

2.4.3. Test for steroids
About 1 ml of the stock solution is dissolved with 2 mL of chloroform in a test tube. Then, slowly added concentrated sulfuric acid. The upper layer turns into a red and sulphuric acid layer showed yellow with green fluorescence indicating the presence of steroids.

2.4.4. Test for terpenoids
About 0.5 mL is dissolved in 0.5 mL chloroform stock solution. Then 1 mL of concentrated sulfuric acid is added to the solution. The formation of reddish-brown color indicates the presence of terpenoids.

2.4.5. Test for alkaloids
About 1 ml of the stock solution was dissolved in 2 mL hydrochloric acid then heated 5 minutes and filtered. The obtained filter added 2-3 drops of dragendorff reagent. Alkaloid compounds are shown in orange

2.5. Identification by Thin-Layer Chromatography
Thin Layer Chromatography (TLC) tests were carried out using gel plates. The extract from the sample is poured at a distance of ± 1 cm from the bottom edge of the plate with a capillary tube, then dried and eluted with each phase of the compound group. The eluent used is Benzen: methanol with a ratio of 9: 1 and Methanol: Ammonia with a ratio of 100: 1.5 After the mobile phase rises to the upper line then the elution is stopped. The testing reagents of each class of compounds are as follows [18,19].

2.5.1. Alkaloids Test
Dragendorf stain reagent is used, if a stain arises on a brownish-black TLC plate indicates the presence of alkaloids.

2.5.2. Saponin Test
H2SO4 reagent stain is used, if the stain on the pink TLC plate indicates the presence of saponins.

3. Results and Discussion

3.1. Sample preparation and extraction
Preparation of the research sample was made in the form of simplisia, and extracted using a recurring maceration method with 96% ethyl acetate as a solvent. The results of the preparation and extraction can be seen in Table 1.

| Fresh sample (g) | Dry simplisia (g) | Liquid extract (g) | extract (g) | Rendemen (%) |
|------------------|-------------------|-------------------|-------------|--------------|
| 2000 g           | 335.11 g          | 2000.30 mL        | 58.06 g     | 17.32        |
3.2. Characterization of Sea urchin gonads
The results of raw material identity were obtained from the determination of sea urchin in the Laboratory of Biology Education Department of Halu oleo, University in Kendari.

| No | Test | Result |
|----|------|--------|
| 1  | Identity of raw materials | **Diadema setosum** L. |
|    | a. Latin name | Black sea urchin, sea urchin |
|    | b. Synonym | Gonad |
|    | c. Sample section | 2b, 3a, 4a, 5b, 6a, 7b, 8a, 9a, 10b, 11b, 12b, 13b, 14b |
|    | d. Macroscopic description | 2b, 3a, 4a, 5b, 6a, 7b, 8a, 9a, 10b, 11b, 12b, 13b, 14b |
| 2  | Organoleptic | The solid yellow-brown solid color smells typical. |
|    | a. Fresh sample | The shape of the powder color is a dark yellow-brown Typical odor. |
|    | b. Dry simplisia | Viscous form of brownish-orange color characteristic odor. |
| 3  | Test of the content of water-soluble | 5.72% |
| 4  | Test of the content of ethanol-soluble | 3.17% |

Based on table 1, this result is reinforced by the previous statement that sea urchin (**Diadema setosum** L.) is a marine biota that lives in a colony, has long bones, is easy to make, the body is a shell, moves freely, is not vertebrate, and its body resembles a ball so that it can move freely [21]. The results of the test levels of water-soluble compounds and ethanol-soluble compounds of sea urchin gonad extract in Table 2, shows that every 2.5 mg of extract the water-soluble content is 5.73%, and the soluble content of ethanol is 3.72%. The purpose of the water-soluble and ethanol content test is to provide an initial overview of the chemicals contained in a water-soluble extract. the amount of chemical content in the water increases with chemical activity. The water-soluble and ethanol extract content are eligible if > 6%. Based on this, the sea urchin gonad extract meets the quality requirements [17].

3.3. Phytochemical screening
Testing of alkaloid compounds by adding HCl and Dragendorff to the sample showed a change in color to orange accompanied by the formation of orange deposits. The Bi₃⁺ ion from bismuth nitrate in dragendorff reagents react with KI to form a black BiI₃ precipitate which then dissolves in KI to form K[BiI₄]. Nitrogen in dragendorff is used to form covalent coordinate bonds with K⁺ which is a metal ion. This formation causes the color change to orange [22]. Saponin test was carried out by
the Forth method, in which 2 ml of extract was dissolved in 10 ml of aqua dest and reacted with 10 drops of HCl formed foam as high as 3 cm. The emergence of foam indicates the hydrolysis of glycosides into glucose [22]. Based on previous research, saponins in exclusive animals are found in echinoderms. The biological function of saponins in Echinoderms is related to the defense system against marine fungi, predators, and parasites [16].

Table 3. Phytochemical screening results of sea urchin gonads ethyl acetate extract by color reaction method

| Color reaction test | Observation results | Evidence |
|---------------------|---------------------|----------|
| Alkaloids           | Formation of red-orange deposits, A change in color occurs orange | ++       |
| Saponin             | Formation of a stable foam ± 10 minutes and a deep yellow discoloration occurs | ++       |
| Triterpenoids       | There is no change in color to violet | -        |
| Steroid             | Steroids Green color changes occur bluishly | +        |

Steroids and triterpenoids are saponin glycoside compounds that have steroid core groups and terpene groups [15]. The test used was the Liberman Buchard (LB) reaction, which is a mixture of chloroform, anhydrous acetic acid, and concentrated H2SO4. With the addition of concentrated H2SO4 in acetic acid anhydride, the color changes to bluish-green. The color formed is due to OH groups on steroids reacting with LB reagents. Based on previous research, steroids are fat-soluble, so they are found in animal tissues, especially Echinoderms [16].

Figure 1. Dragendroff test reaction of alkaloid compounds

Figure 2. The hydrolysis reaction of saponins in water
3.4. Identification of sea urchin gonads extract by Thin-Layer Chromatography (TLC)

Based on table 4, the identification of compounds using a comparison standard, to be used as a standard for determining the results by looking at the similarity value or similarity of Rf and visible blemishes (fig 4). In the identification of alkaloid compounds, the samples have similarities to spots with standard standards with almost the same Rf values. So it was concluded positive Alkaloids. Based on previous research, alkaloids are found in many marine biotas especially Echinoderms phylum [23]. In saponin identification, the sample has the same blemishes on the standard with the same Rf value (fig 5). Given the similarity of Rf values, it is suspected that the identified saponins are b-sitosterol types. B-sitosterol is a steroid compound of the phytosterol group found in plants, but b-sitosterol is also identified in marine animals namely sponge [23]. This is supported by other studies that phytosterol, b-sitosterol, and positive campesterol phytosterols are found in marine biota, namely microalgae [24]. In marine biota animals, saponins are exclusively detected in the echinoderms phylum [16].

| Identification | treatment | Eluent | Spotting stains after spraying dragoff (Alkaloids), LB reagents (saponins) | Rf value (cm) |
|----------------|-----------|--------|---------------------------------------------------------------------------|---------------|
| Alkaloids      | Sampels   | Methanol: ammonia (100:1.5) | Brick red Brown Black-brown | 0.4           |
|                | standar   |        | Brick red Brown Black-brown | 0.8           |
| Saponin        | Samples   | Benzene : methanol (9:1) | Pink Brown Blue | 0.68          |
|                | standar   |        | Pink Brown Blue | 0.68          |

Figure 4. Identification of Alkaloids using caffeine standard with methanol: ammonia (100: 1.5) eluent, (a) Visual; (b) UV 254 nm; (c) UV 366 nm
Figure 5. Identification of Saponin compounds using b-sitosterol standards with Benzen with Benzene : methanol (9:1) eluent, (a) Visual; (b) UV 254 nm; (c) UV 366 nm

Research conducted by Septiadi et al by using samples from phylum echinoderms (sea cucumbers) obtained results in accordance with this study namely sea cucumber extract containing triterpenoid bioactive components, steroids, and saponins in ethyl acetate [25].

4. Conclusion
Identification of sea urchin gonads extract by phytochemical screening contains alkaloids, saponins, and steroids and Thin-Layer Chromatography (TLC) contains alkaloids and saponins. The Rf values are 0.4 and 0.68, respectively.

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