Toruntju 2020 IOP Conf

by Pak Laban 7

Submission date: 11-Oct-2022 12:35AM (UTC+0700)
Submission ID: 1921771836
File name: untju_2020_IOP_Conf._Ser.__Earth_Environ._Sci._465_012038_1.pdf (416.58K)
Word count: 3592
Character count: 20213

Identification of secondary metabolite contents on marine rabbit extract (dolabella auricularia)

Identification of secondary metabolite contents on marine rabbit extract (dolabella auricularia)

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Abstract. The aim was to identify all forms of secondary metabolites contained in one of the marine biota namely sea rabbit (Dolabella Aurikularia). ***Method,*** Sampling and sample preparation were carried out at Toronipa Beach, Soropia District, Konawe Regency, Southeast Sulawesi. Then the sea rabbit sample was tested at the Phytochemical Laboratory of the Faculty of Pharmacy, University of Halu Oleo. The tools used are several tools in the phytochemical laboratory, pharmacy at Haluoleo University. Ingredients namely sea rabbits and some chemical reagents used. The extraction process is then carried out and identifies secondary metabolic components (phytochemical analysis), including examinations: Alkaloids, Flavonoids, Tannins, Saponins, and Terpenoids. The results, The extraction process is then carried out and identifies secondary metabolic components (phytochemical analysis), including examinations: Alkaloids, Flavonoids, Tannins, Saponins, and Terpenoids. Conclusion, there is Sea rabbit (*Dolabella Aurikularia*), positively contains several secondary metabolic compounds, qualitatively, specifically: Flavonoids, Alkaloids, Saponins, Tannins, and Terpenoids. These compounds are very useful for health, especially in the pharmaceutical world.
Keywords: Sea rabbit, Alkaloid, Flavonoid, Saponin, Terpenoid dan Tanin.

1. Introduction

The use of natural food in addition to meeting the needs of side dishes, also as a treatment effort is increasingly showing progress today. Various research results and scientific studies show that recovery of disease using natural ingredients is increasingly being used by experts today. Back to foods naturally, this motto is right to use today. Especially in using natural food which is very much available in various regions, especially the sea or waters that provide various types of marine animals [1]. How many marine animals are not yet fully known and consumed by the community, especially fishing communities in coastal areas [2]. The use of sea rabbits (*Dollabela aurekularia*), is one of the marine animals that live on most of Indonesia's east coast, specifically the Toronipa beach, Soropia District Konawe Regency and Wabula beach, Wabula District Buton Regency, Southeast Sulawesi. Especially in Wabula beach, the rabbit mollusk habitat is quite a lot when compared to other mollusk animals, such as sea cucumbers, sea worms, etc., [3]. Marine invertebrates have good potential to be developed in the pharmaceutical and food fields. One of the biota included in marine invertebrates that is widely used as a bioactive producer is gastropods [4].

Gastropods are organisms that will Characterize Amino Acids, Fatty Acids, and Minerals. Indonesian fishery product processing communities are widely spread in various regions both in waters and land, this group is widely consumed by the community and is believed to have enormous benefits for health.

Sea rabbit mollusks (*Dolabella auricularia*) are a group of animals that are often found in sea areas, with the condition of sea sand and coral or on seaweed which is the body directly from the seabed. Sea rabbits (*Dolabella auricularia*) can be found from tropical ocean waters to polar waters. Sea rabbit mollusk is one of the seafood products as a source of nutrients, especially animal protein is not widely known by the public. Recent research has found that sea rabbits are very rich in amino acids such as aspartic acid, glutamate, and glycine, moreover, the amino acid arginine which is an essential amino acid for growing children [1]. In addition to primer metabolic nutrients, sea rabbits (*Dolabella auricularia*) also contain several secondary metabolic nutrients, such as saponins, flavonoids, and tannins. Flavonoid compounds have a variety of important functions for health, including reducing the risk of cardiovascular disease, blood pressure, atherosclerosis, and anti-oxidants and increasing levels of erythrocytes[5].

Phytochemical examination results prove that sea rabbit extracts contain several secondary metabolic active compounds, such as Saponin Flavonoids and Tannins. Above was stated that the increase in hemoglobin levels was probably caused by the presence of secondary metabolic substances contained in sea rabbits, specifically: Flavonoids, Saponins, Tannins. It is known that phytochemical compounds especially tannins and flavonoids have been able to increase hemoglobin levels. Research by Ufelle *et al*.[6] reports that the active phytochemical compounds namely flavonoids and tannins play a major role in hematological processes. This hematological process will take place more quickly if the availability of active phytochemicals is supported by sufficient primer metabolic substances. In the last few decades, research on active substance search from marine biota that has increasingly intensive pharmacological activities has been carried out including sea cucumbers [7].

A study conducted by Manulang *et al*.[4] reported that sea rabbits from the Southeast Sulawesi region contained many active components in the form of Tanin and Saponin. Antioxidant activity for methanol extract of sea rabbit is very low, with IC 50 value of 5123.25 μg / ml and inhibition of the α-glucosidase enzyme is also very low with IC 50 value of 23453.45 μg / ml, so it is very useful in the hematological process.

The purpose of this study is to identify all forms of secondary metabolic compounds contained in one of the marine biota namely sea rabbit (*Dolabella aurikularia*). The results of this study are expected to know the existence of secondary metabolic compounds in sea rabbits, to provide information to the community on an alternative food source of animal protein.

2. Methods

The study was conducted for 6 months from July to December 2018. Sampling and sample preparation were carried out at Toronipa Beach, Soropia District, Konawe Regency, Southeast Sulawesi. Then the sea rabbit sample was tested at the Phytochemical Laboratory of the Faculty of Pharmacy, University of Halu Oleo.

2.1 Tools and Materials

Tools. There are several laboratory equipment used, among others: autoclaves, vacuum evaporators, analytical scales, water baths, glassware sets, UV-Vis spectrophotometers (Genesys 6) and vortices.

Materials. There are several laboratory equipment used, among others: autoclaves, vacuum evaporators, analytical scales, water baths, glassware sets, UV-Vis spectrophotometers (Genesys 6) and vortices.

2.2 Catching and Preparing Sea Rabbit Samples

Catching. Catching rabbits is done at low tide and night. This is because during the day the rabbit does not come out of the sea sand. It must be at night because the sea rabbit sample must avoid direct sunlight because it can damage many secondary metabolic compounds.
The sample to be extracted is a sea rabbit (*Dolabella auricularia*).

Preparation. Seabirds are taken as many as 90 heads (30 kg), cleaned, separated between intestinal shells and other organs from sea rabbit meat. Then clean the sea bare flesh and rinse with fresh water 5 times to reduce the salt content. Furthermore, sea rabbits are dried at temperatures <50 oC, while being kept away from direct sunlight, this is to avoid damage to many secondary metabolic compounds. After drying, 2.8 kg of sea rabbits were obtained.

2.3 Procedure for Making Sea Rabbit Extract
Dried rabbit, 2.8 kg dry. Then smoothed, then macerated using 10 liters of Ethanol solvent. This maceration is carried out 3 times, with a repeat of soaking every 24 hours. After that, further filtering is done to get the rough extract.Then this coarse extract is browned using a rotary evaporator until a coarse extract is obtained in the form of a paste.

2.4 Identification of Secondary Metabolite Components (Phytochemicals)
Phytochemical analysis is the science that describes the chemical aspects of a material. Harborne, 2006[8] statedthat phytochemical analysis is carried out to determine the characteristics of the bioactive component of a crude extract that has toxic or other pharmacological effects that are beneficial when tested with a biological or bioassay system.

The procedure for identifying secondary metabolites according to [8], includes identification of alkaloids, steroids/triterpenoids, saponins, flavonoids, and polyphenols.

Table 1: Test Results of several types of phytochemicals in sea rabbits

Phytochemical Name (Secondary metabolites)	**Type of Test**	**Name of Reagent**	**Positive Reaction (+)**
- Flavonoid	Sinode test	A few drops of Dragon	Brown
- Alkaloid	Culvenor test	NaOH 10 %	Red Sediment
- Saponin	Foam Test	10 mL Hot H_2O	Stable foam at ± 10 seconds
- Tanin	Phenol Test	$FeCl_3$ 1 %	Purple Blue black
- Terpenoid/Steroid	Lieberman test	Anhidrida asetat	Orange-red or purple

2.5 Data Analysis
The analysis included extraction analysis and phytochemical screening analysis. Data from observations were carried out in descriptive data analysis.

3. Results

3.1.Morphology Sea rabbit
The results of catching sea rabbits as described above.

The following are photos of an adult sea rabbit and a large category.

Figure1. Sea Rabbit (*Dolabella Auricularia*)

The result of direct observation on sea rabbits is a sea animal that 97% of its body surface is slimy. This slime will come out after the sea rabbit is touched by something. In addition to sea rabbit mucus also has a dark purple liquid which is released as a weapon to fool its prey. Morphologically, sea rabbits have an average length of 15 cm to 20 cm, with a diameter of 8 cm to 13 cm and weigh up to 300 grams in wet conditions. Organisms belonging to the Dolabella class can be used as an alternative nutritious alternative food source, because in addition to containing secondary metabolic compounds also contain several essential nutrients or primer metabolic compounds. As reported by Manulang *et al*[4] that sea rabbits contain the highest amount of essential amino acids, arginine, the highest non-essential amino acid is glycine. The highest fatty acid for Saturated Fatty Acid (SAFA) is palmitate, for Mono Unsaturated Fatty Acid (MUFA) the highest is oleic and the highest linoleic fatty acid for Poly Unsaturated Fatty Acid (PUFA). The highest mineral content of sea rabbits is calcium. The high calcium content is useful as a prevention of osteoporosis.

3.2. Phytochemical Test Analysis

A qualitative analysis of several secondary metabolites has been carried out, through phytochemical analysis, the following hashes have been obtained :

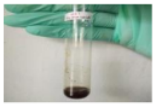
The following is a sample photo of the test results :

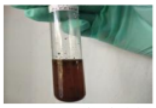
a. Alkaloid Test

In a few mL of sea rabbit extract, it was dissolved in 10 ml of aqua dest and 2 N (9: 1) hydrochloric acid, then heated on a bath for 2 minutes. Then cooled and filtered. The filtrate obtained was used as a solution of the experiments carried out as follows :

a.1. Take 1 ml of trial solution, then add 2 drops of Mayer, showing positive results in the presence of alkaloids, namely the formation of a white precipitate.

a.2. Take 1 ml trial solution, then add 2 drops dragendorff, showed a positive result in the presence of alkaloids, namely the formation of brownish-orange deposits.


Alkaloid Test Before


Alkaloid Test After

b. Tannin Test

In a few mL of sea rabbit extract, 10 drops of 10% FeCl3 are added. Positive extracts contain tannins if they produce a blackish-green or a blackish-blue color.


Tannin Test Before


Tannin Test After

c. Flavonoid Test

In a few mL of sea rabbit extract, added with 100 mL of hot water, boil for 5 minutes, then filtered. Take 5 mL of filtrate, then add 0.05 g of Mg powder and 1 mL of concentrated HCl, then shake it vigorously. Positive Test Results are indicated by the formation of red, yellow or orange.

Flavonoid Test Before Flavonoid Test After

d. Saponin Test

In a few mL of sea rabbit extract, add 10 mL of water while shaking for 1 minute. The results appear that if the foam formed remains stable for approximately 7 minutes, this shows that the extract is positive to contain saponins.

Saponin Test Before Saponin Test After

e. Steroid Test

In a few mL of sea rabbit extract, 10 drops of glacial CH3COOH were added and 2 drops of concentrated H2SO4 were added The solution is shaken slowly and left for several minutes. Steroid positive test if it produces blue or green, while triterpenoids produce red or purple [8].

Steroid Test Before Steroid Test After

The above description shows that the results of the examination of secondary metabolic compounds in sea rabbits (*Dolabella auricularia*). Positively said that the sea rabbit contains several secondary metabolic compounds namely: Flavonoids, Alkaloids, Saponins, Tannins, and Terpenoids.

4. Discussion

4.1. Alkaloid

Alkaloids are the largest organic compounds of natural material, both in terms of quantity and distribution. Alkaloids according to Winterstein and Trier are defined as basic compounds, containing nitrogen atoms derived from collisions and animals. [9] revealed that none of the satisfactory definitions of alkaloids, but generally alkaloids are secondary metabolic compounds which are alkaline, contain one or more nitrogen atoms, usually in heterocyclic rings, and are biologically active.

The structure of alkaloids varies from simple to complex, from biological effects that refresh the body to toxic. One simple example is nicotine. Nicotine can cause heart disease, lung cancer, oral cancer, high blood pressure, and disorders of pregnancy and the fetus.

4.2. Flavonoid Compound

Flavonoids are a group of phenol compounds that are mostly found in nature. These compounds are responsible for red, purple, blue, and some yellow dyes in plants. In line with the results of this study that when a rabbit is touched, it will emit a deep purple liquid, as a weapon to protect itself from enemy attacks [3]. All flavonoids, according to their structure, are derived from the parent compound "flavon", which is the name of a type of flavonoid which is the largest and also commonly found, which is in the form of white flour in Primula plants.

Most of the flavonoids found in plants are bound to sugar molecules as glycosides, and in mixed form, they are rarely found as a single compound. Besides that, a mixture of different classes of flavonoids is often found. For example anthocyanins in a red petal are almost always accompanied by flavones or colorless flavonoids. At present, it is estimated that around 3,000 flavonoid compounds have been isolated.

Flavonoids in plants have four functions: 1) As a color pigment, 2) Physiological and pathological functions, 3) Pharmacological Activities, and 4) Flavonoids in food. Pharmacological activities are ascribed to routine (flavonol glycosides) which are used to strengthen the capillary structure, reduce permeability and fragility of blood vessels. Gabor stated that flavonoids can be used as medicine because they have various kinds of bio-activity such as anti-inflammatory, anti-cancer, anti-fertility, antiviral, anti-diabetes, anti-depressant, diuretic [8].

4. 3. Terpenoid Compound

At first, it was a class of compounds consisting only of atoms C and H, in a ratio of 5: 8 to the empirical formula C5 H8 (isoprene unit), which joined head to tail (head-tail). Therefore terpenes are commonly called isoprenoids. Terpenoids are the same as terpenes but contain other functional groups such as hydroxyl groups, aldehydes, and ketones. Today both terpenes and terpenoids are classified as terpenoids (isoprenoid) Example: Limona in citrus fruits, Geraniol in roses.

Based on the number of isoprene units it contains, terpenoid compounds are divided into 1) Monoterpenes (two isoprene units), 2) Seskiterpenes (three isoprene units), 3) Diterpena (four isoprene units), 4) Triterpene (six isoprene units), 5) Tetraterpena (eight isoprene units), and 6) Politerpena (many isoprene units). Monoterpenes and sesquiterpenes are the main components of essential oils (essential oils) that can be obtained by refining. Vitamin A is a diterpenoid, squalene classified as triterpenoid found in fish liver oil, red and yellow pigment carotenoids are classified as tetraterpenes, latex (natural rubber) is polyterpenes [10].

4. 4. Steroid Compound

Steroid is a group of compounds that has the basic framework of cyclopentane peridrofenantrena, has four integrated rings. These compounds have certain physiological effects. Some important steroids are cholesterol, which is the most widespread animal steroid found in almost all animal tissues. Bladder stones and egg yolks are a rich source of this compound. The sex hormone produced mainly in the testes and ovaries is a steroid. Male hormones are called androgens and female hormones estrogen, and pregnancy hormone progestin.

4. 5. Saponin Compound

Saponin is a complex glycoside compound that is a compound from the condensation of a sugar with an organic hydroxyl compound which when hydrolyzed will produce sugars (glycans) and non-sugars (aglycones). These saponins are composed of two groups: triterpenoid saponins and steroid saponins. Saponins are widely used in human life, one of which is found in silver which can be used for washing cloth (batik) and as a shampoo.

Saponins can be obtained from plants through the extraction method. Some secondary metabolite compounds produced by marine invertebrates and symbiotic microorganisms have the prospect of being active substances in drugs from various diseases such as infections, neurology (Parkinsons, Alzheimer's), heart disease, immunology, anti-inflammatory, anti-virus, and anti-cancer.

4.6. Tannin Compound

Formation of blackish-green or dark blue on the extract after adding FeCI3, because of the tannin will form complex compounds with FeCi3 [11]. The results of another study as stated by Laily [12] that the results of the identification of tannin compounds in ethanol extracts of the Carica Pubescens Lenne and K.Koch fruit plants, in the Bromo Cangar and Dieng Plateau regions, were also positive for tannins.

4.7 Sea Rabbit Extract (Dolabella auricularia) Compound

The results of tests on sea rabbit extract, apparently contains several secondary metabolic compounds, including flavonoids, alkaloids, saponins, and steroids. This is ska i further proves that generally marine mollusks contain several metabolic compounds. As is the case that in the testing of sea cucumbers other Echinoderms members such as sea cucumbers (*Holothuria atra*) are known to contain secondary metabolites such as alkaloids, steroids, Triterpenoids, and saponins [13].

Conclusion

Sea rabbit (*Dolabella aurikularia*), positively contains several secondary metabolic compounds, qualitatively, specifically: Flavonoids, Alkaloids, Saponins, Tannins, and Terpenoids. These compounds are very useful for health, especially in the pharmaceutical world.

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