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EVALUATION OF ETHANOL EXTRACT OF RED ALGAE (*Kappaphycus alvarezii* Doty): TOTAL PHENOLIC AND FLAVONOID CONTENT

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Abstract. This study aims to determine the characterization of red algae simplicia and the amount of yield obtained from the manufacture of red algae ethanol extract using the soxhletation method. Red algae were taken from Banggai Islands Regency, South Sulawesi and processed into dry simplicia and characterized by macroscopic examination, determination of water content, content of water soluble extract and ethanol, total ash content and acid insoluble ash content. Simplicia was extracted using 96% ethanol solvent by soxhletation method and calculated the amount of yield obtained. Macroscopic observation of red algae simplicia obtained a brown coarse powder accompanied by a distinctive taste and odor. Determination of the water content of simplicia obtained 7.21%, water soluble extract content 31.54%, ethanol soluble extract content 17.27%, total ash content 33, 96% and 11.42% acid insoluble ash content. The ethanolic extract of red algae using the soxhletation method was obtained as much as 68.92 grams with a total extract yield of 13.78%. Based on the results of the study, it can be concluded that the simplicia characterization of red algae meets the requirements and has a large extract yield.

Keyword: Red Algae, characterization, soxhletation.

Abstrak. *Penelitian ini bertujuan untuk mengetahui karakterisasi simplisia ganggang merah dan jumlah rendemen yang diperoleh dari pembuatan ekstrak etanol ganggang merah menggunakan metode sokletasi. Ganggang merah diambil dari Kabupaten Banggai Kepulauan, Sulawesi Selatan dan diolah menjadi simplisia kering serta dikarakterisasi meliputi pemeriksaan makroskopik, penetapan kadar air, kadar sari larut dalam air dan etanol, kadar abu total dan kadar abu tidak larut asam. Simplisia diekstraksi menggunakan pelarut etanol 96% dengan metode sokletasi dan dihitung jumlah rendemen yang diperoleh. Pengamatan makroskopik simplisia ganggang merah diperoleh serbuk kasar berwarna coklat disertai rasa dan bau yang khas. Penetapan kadar air simplisia diperoleh 7,21%, kadar sari larut air 31,54%, kadar sari larut etanol 17,27%, kadar abu total*

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33,96% dan kadar abu tidak larut dalam asam 11,42%. Ekstrak etanol ganggang merah dengan menggunakan metode sokletasi diperoleh sebanyak 68,92 gram dengan jumlah rendemen ekstrak sebesar 13,78%. Berdasarkan hasil penelitian dapat disimpulkan bahwa karakterisasi simplisia ganggang merah memenuhi persyaratan dan memiliki rendemen ekstrak yang besar.

Keyword: *Ganggang Merah, karakterisasi, sokletasi.*

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1. Introduction

Approximately 2/3 of the total area of Indonesia is waters consisting of oceans which contain a lot of natural resources and have potential as medicines and food ingredients that can be used to prevent degenerative diseases and one of them is seaweed [1]. Seaweed is a low-level plant that does not have a different arrangement of roots, stems and leaves so that it does not have true roots, stems or leaves, but only resembles a stem called a thallus, grows in nature by attaching itself to coral, mud, sand, rocks and other hard objects. Taxonomically, it is grouped into the division Thallophyta. Red seaweed is one of four groups of seaweeds that have quite a lot of habitat found in Indonesian waters [2];[3]. Red seaweed (Eucheuma cottonii) is a type of red seaweed and changed its name to Kappaphycus alvarezii because the carrageenan produced includes the kappa-carrageenan fraction. This species is taxonomically called Kappaphycus alvarezii [4].

Seaweed belonging to the Rhodophyceae class (red algae) contains pigments, including chlorophyll a which is responsible for the photosynthesis process and seaweed survival or to compete with other organisms, supporting pigments such as chlorophyll d, and carotene, lutein, zeaxanthin which functions to protect chlorophyll a from photo-oxidation, phycocyanin which plays a role in photosynthesis as a light-receiving pigment and phycoerythrin which is the most dominant pigment that plays a role in the absorption of blue/green light and plays a role in revealing red color. Phycoerythrin is the dominant pigment that causes the red color of red algae [5], [6].

Previous Previous research mentionsRed algae showed the presence of alkaloids, saponins, phenols, steriods, proteins, phytosterols, amino acids, sugars, reducing sugars, flavonoids, tannins and the absence of terpenoids and anthraquinones. Red algae have significant anti-inflammatory activity due to the inhibition of the hyaluronidase enzyme [7].

The results of testing the antioxidant activity of red algae using the DPPH free radical scavenging method showed that the ethanolic extract of red algae had an IC50 of 3.03 ppm, which is a very strong antioxidant activity [8]. Red algae are rich in protein, crude fiber, essential fatty acids, minerals, carotenoids, vitamin A and vitamin C [9]. The content of other active compounds in

algae is part of carotenoids (astaxanthin), which can help reduce rough skin and aging, protect sebum from oxidation [10]. This type of red algae was investigated also contains carrageenan (polysaccharide derivative) and abundant minerals which also have hydrating, therapeutic and moisturizing effects [11].

Soxhletation is an extraction method using a solvent that is always new which is generally carried out with a Soxhlet apparatus whose sample is wrapped in filter paper so that continuous extraction occurs with a relatively constant amount of solvent in the presence of reverse cooling [12]. Soxhletation method is included in the hot extraction method which can produce more extract by having advantages in solvent and time efficiency, namely the use of less solvent and faster time and the sample used in the extraction process can be extracted perfectly because it is carried out repeatedly. and biological activity is not lost when heated so that it can be used in the search for parent drugs [13].

2. Materials and Methods

2.1 Location of the study

The research was conducted at the Biology Laboratory, Faculty of Pharmacy, University of North Sumatra.

2.2 Sample collection

Red algae were collected purposively from the Banggai Islands Regency, South Sulawesi.

2.3 Making red algae simplicia

Red algae were collected, wet sorting, washed under running water, drained, and weighed. The red algae are then dried in a drying cabinet to dry, sorted dry, then weighed, and stored in tightly closed plastic containers [14].

2.4 Simplified characterization

2.4.1 Macroscopic examination

The simplicia macroscopic examination was carried out observing the shape, texture and size as well as organoleptic examination by observing the color, taste and smell of fresh plants, simplicia and red algae simplicia powder [15].

2.4.2 Determination of water content

200 mL of toluene and 2 mL of distilled water were put into a round bottom flask, distilled for 2 hours. The toluene was cooled for 30 minutes and the volume of water in the receiving tube was read. Simplicia powder as much as 5 g that has been weighed carefully put into the flask, then heated carefully for 15 minutes. After the toluene begins to boil, the drip rate is adjusted to approximately 2 drops per second, until most of the water is distilled, then the distillation rate is

increased to 4 drops per second. After all the water has been distilled, the inside of the cooler is rinsed with saturated toluene. Distillation was continued for 5 minutes, then the receiving tube was allowed to cool to room temperature. After the water and toluene are completely separated, the volume of water is readread in accordance with the water content contained in the material being examined. Moisture content is calculated in percent [16].

Formula :

$$\% \text{ water content} = \frac{V2 - V1}{\text{Sample Weight}} \times 100\%$$

2.4.3 Determination of water soluble essence

Simplicia powder as much as 5 g was macerated for 24 hours in 100 mL of chloroform water (2.5 mL of chloroform in 1000 mL of distilled water) in a corked flask while shaking occasionally for the first 6 hours and then left for 18 hours, then filtered. Evaporated 20 mL of the filtrate to dryness in a flat bottomed shallow dish that has been heated at 105oC and tara. The remainder is heated in the oven at 105oC until a constant weight is obtained, then the water soluble extract content is calculated [15].

Formula:

$$\% \text{ Content of water soluble essence} = \frac{\text{Sari Weight (g)}}{\text{Sample Weight (g)}} \times \frac{100}{20} \times 100\%$$

2.4.4. Determination of ethanol-soluble essence

A total of 5 g of powder which has been dried, macerated for 24 hours with 100 mL of 96% ethanol using a stoppered flask while occasionally shaking for the first 6 hours, then left for 18 hours. Filter, 20 mL of the filtrate is pipetted, evaporated to dryness in heated flat-bottomed shallow dish105°C. The remainder was heated at a temperature of 105°C until a constant weight was obtained, then the ethanol soluble extract content was calculated [15].

Formula:

$$\% \text{ Kadar sari larut dalam etanol} = \frac{\text{Sari Weight (g)}}{\text{Sample Weight (g)}} \times \frac{100}{20} \times 100\ \%$$

2.4.5. Determination of total ash content

As much as 2-3 g of simplicia powder that has been ground and weighed carefully is put into a silicate crucible that has been incandescent and tara, then leveled. The crucible is slowly incandescent until the charcoal runs out, then cooled and weighed until a constant weight is obtained. If the charcoal cannot be removed by this method, add hot water, stir, and filter through ash-free filter paper. Incandescent filter paper along with the rest of the filter in the same crucible. Put the filtrate into the crucible, evaporate and incandescent until the weight remains at a temperature of 800±25º. The total ash content is calculated in percent of the dried material [15].

Formula:

$$\% \text{ Total ash content} = \frac{\text{Sari weight (g)}}{\text{Sample Weight (g)}} \times \frac{100}{20} \times 100\ \%$$

2.4.6. Determination of acid insoluble ash content

The ash obtained in the determination of the total ash content was boiled in 25 mL of 2 N hydrochloric acid for 5 minutes, the part that was not soluble in acid was collected, filtered through ash-free filter paper and then washed with hot water at an exchange rate until the weight remained at a temperature of 800±25º. The ash content that is not soluble in acid is calculated on air-dried materials [15].

Formula:

$$\% \text{ Ash content is not soluble in acid} = \frac{Ash\ Weight(g)}{Sample\ Weight\ (g)} \times 100\ \%$$

2.5 Making red algae ethanol extract

Extracts from red algae were made by soxhletation, using 96% ethanol solvent with the following working procedure: Installed a soxhletizer, then 500 grams of sample was wrapped in filter paper, tied with thread, inserted into a soxhlet apparatus, and put in 96% ethanol solvent. as much as 1L into the soxhlet flask. Perform the socket at 700C until the cycle drops are colorless or for about 5 hours. The liquid extract obtained was then concentrated using a rotary evaporator at a temperature of 30-450C [17].

Formula: % Yield : $\frac{\text{Extract Weight}}{\text{Simplisia Weight}}$ x 100%

2.6 Determination of Total Phenol Content

A total of 10.0 mg of extract was dissolved in 1 ml of ethanol, then made up with distilled water to 10 ml. Take this solution. A total of 0.5 ml is mixed with 1.25 ml of Folin-Ciocalteu reagent 10% in a test tube. The mixture was vortexed for 1 minute and then allowed to stand at room temperature for 5 minutes. As much as 1 ml of 20% Na2CO3 was added to the mixture and allowed to stand again at room temperature for 60 minutes. Measure the absorbance using a visible spectrophotometer at a wavelength of 740 nm 3 times for one measurement and take the average. Measurements were carried out with 3 repetitions.

2.7. Determination of Total Flavonoid Content

Weighed as much as 10 mg of thick extract, dissolved with 10 mL of ethanol solvent to obtain a concentration of 1000 ppm. 2 mL of the solution was pipetted, added with 0.1 mL of AlCl3 and 0.1 mL of CH3COONa and 2.8 mL of distilled water, then incubated for 40 minutes. The absorbance was measured by UV-Vis spectrophotometry at a maximum wavelength of 433 nm.

3. Results and Discussion

The results of the macroscopic examination of red algae showed the shape of a cylindrical thallus with a smooth surface, brownish red in color due to chromatic adaptation. The results of macroscopic examination of the simplicia powder of red algae were obtained as coarse powder, with brown organoleptic color and has a distinctive taste and smell. The chromatic adaptation of red algae is an adjustment between the proportion of pigments with various lighting qualities and can cause various colors in the thallus such as: dark red, pink, blonde, brown, yellow and green. The state of the color cannot always be used to determine its class. Color changes often occur simply due to changing environmental factors. This event is a modification process, namely changes in shape and external characteristics (phenotype) that are not permanent due to environmental influences, including climate and oceanography which are relatively large. Has a dichotomous branching type. Overgrown with nodules (protrusions) and spines [18].

The results of the examination of the characteristics of red algae simplicia consisting of determination of water content, water soluble extract and ethanol content, total ash content and acid insoluble ash content of red algae simplicia can be seen in Table 1.

Table 1 Results of simplicia characterization of red algae

No	Characterization	Results
1	Determination of water level	7.21%
2	Determination of water soluble juice content	31.54%
3	Determination of ethanol soluble extract content	17.27%
4	Determination of total ash content	33.96%
5	Determination of acid insoluble ash content	11.42%

Determination of the water content in the simplicia was carried out to determine the amount of water contained in the simplicia used. The results of the determination of the water content obtained are less than 10%, namely 7.21%. Water content that exceeds 10% can be a good medium for microbial growth and the presence of fungi, as well as encourage damage to the quality of simplicia [19].

The determination of the juice content was carried out using two solvents, namely water and ethanol. The determination of water-soluble and ethanol-soluble extracts was aimed to determine the concentration of active compounds extracted in the solvent from a number of simpislia powder. Result of determination of water soluble extract content from red algae 31.54%, while the content of the soluble extract in ethanol is 17.27%.

Determination of total ash content and acid insoluble ash content aims to provide assurance that simplicia does not contain certain heavy metals exceeding the specified value for plant simplicia because it can be harmful (toxic) to health. Determination of total ash content states the amount of inorganic compound content in simplicia such as Mg, Ca, Na, Zn and K. Ash content is not soluble in acid to determine the levels of inorganic compounds that are insoluble in acid such as silicates. Total ash is divided into two, namely physiological ash and non-physiological ash. Physiological ash is ash that comes from the plant tissue itself while non-physiological ash is the

residue after combustion that comes from external materials found on the surface of the simplicia [19]. 33.96% and acid insoluble ash content of 11.42%. Determination of the total ash content of red algae plant simplicia was obtained at33.96%,high ash content in algae plant simpliciaThis is because red algae plants contain the minerals potassium, sodium, calcium, and magnesium [20].

A total of 500 grams of red algae simplicia powder was wrapped in filter paper adjusted to the size of the soxhletizer and then put into the soxhletizer. 96% ethanol solvent as much as 1L was put into a soxhletation flask and soxhletated at a temperature of 700C until the cycle drops were almost colorless. The results of 500 grams of simplicia powder obtained a thick extract of 68.92 grams (13.78% yield). Yield is a comparison between the results of the number of metabolites obtained after the extraction process and the weight of the sample used. The yield is said to be good if the value is more than 10%. Therefore, the extract yield obtained was declared good because the yield was > 10% [21].

Results Determination of total phenol content in the ethanol extract of Dayak onion can be seen in Table 2.

Table 2 Total phenol content in ethanol extract of red algae

No.	Aample weight (g)	Total phenol content (mg GAE/ g sample)
1.	0,0109	5,0478
2.	0,0137	4,0519
3.	0,0139	3,9938
Average content of total phenol		4,3645

Measurement of the total phenol content of the ethanolic red algae extract was based on a standard curve using gallic acid. The total phenolic content of the red algae ethanol extract was 4.3645 GAE/g. Phenol compounds are a large and diverse group of molecules, which differ from the aromatic secondary metabolites in plants. It was concluded that some of the biological activities included antioxidant properties. Based on previous research, the content of phenolic compounds in the ethanolic extract of red algae was 4.64 GAE/g, this indicates that the ethanolic extract of red algae, especially its polyphenols, has antioxidant activity. The ability of antioxidants as free radical scavengers is associated with the ability of these antioxidants as proton and electron donors. Various phenolic compounds can play a role in scavenging free radicals with different capacities.

The total flavonoid content was expressed in mg quercetin equivalent per g sample (mg EQ/g sample). The results of determining the total flavonoid content of the ethanol extract of Dayak onion can be seen in Table 3.

Table 3 Total flavonoid content in ethanol extract of red algae

Sample	Absorbance	Total flavonoid content (mg QE/g sampel)	Average content of total flavonoid (mg QE/g sample)
Ekstrak Etanol Ganggang Merah	0,606	9,0494	11,0941
	0,607	10,4374	
	0,607	13,7955	

The result of determining the flavonoid content of the red algae extract was 11.0941 mg QE/g extract. Based on previous research, the content of phenolic compounds in the ethanolic extract of red algae was 16.47 GAE/g, this indicates that the ethanolic extract of red algae, especially its flavonoids, has antioxidant activity (Lee, 2013). Flavonoids are the largest group consisting of several different structures so that they have different levels of solubility, but generally flavonoid compounds are soluble in semi-polar to polar solvents, due to the low levels of flavonoids in microalgae, their overall contribution as antioxidants is considered small.

4. Conclusion

Simplicia characterization of red algae meets the requirements and has a large yield and could antioxidant substances in the red algae.

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