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Effect of Ethanol Solvents in the Extraction Process of Bioactive Compounds from Brown Seaweed (*Sargassum* sp.) with the Ultrasound Assisted Extraction Method

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Abstract. *Sargassum* sp., part of brown algae, is traditionally used as animal feed. Nonetheless, *Sargassum* contains phenolic compounds that promise to be the raw material of natural antioxidants. The work is to extract phenolic compounds from *Sargassum* sp. in ethanol and to investigate the effect of the process on phenol yields. Several dried *Sargassum* sp. are milled in a certain size and extraction using ultrasound assisted extraction with ethanol solvent. Process variables are the extraction time (30-50 minutes) and the Ratio of *Sargassum* sp. to ethanol which is 5:100-15:100 (b/v), and power of 170-190 watts. Phenol compounds are quantified using the error acid method. The DPPH method is performed to check the antioxidant activity. To investigate the influence of the process, surface response methods based on central composite designs are applied in this work. 153.334 mgGAE/g in 30-minute extraction conditions, 170 watts of power, and a ratio between masses and solvents of 0.05. The antioxidant activity (IC_{50}) of *Sargassum* sp. extract is 87.57 ppm.

Keywords: *Sargassum sp., phenolic compound, ethanol, ultrasound assisted extraction*

1. Introduction

Indonesia is a country rich in natural wealth that has the potential to be developed. The natural wealth in Indonesia is seaweed. Seaweed production in Indonesia in 2018 reached 10.18 million tons. Production in 2020 the Ministry of Marine Affairs and Fisheries (KKP) targets seaweed production can reach 10.99 million tons and is projected by 2024 seaweed production to reach 12.33 million tons. The utilization of brown algae has not been developed optimally by the community.

Brown seaweed contains many chemical compounds such as carbohydrates 54.3-73.8%, vitamins (vitamins B1, B2, B6, B16, C, and niacin), protein 0.3-5.9%, minerals especially calcium, sodium, magnesium, potassium, iodine, iron, and contain bioactive compounds namely phenolic compounds, natural pigments, fibers, polysaccharides sulfate, and other

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bioactive compounds [1]. Extracts from brown seaweed contain phenols including eckol, phlorotannin, gallic acid, catechins, epicatechin, and phlorotannin [2]. Brown seaweed has bioactive compounds such as polyphenolics, terpenoids, polysaccharides, carotenoids, phenolic acids, chlorophyll, steroids, and glycolipids [3]. Seaweed extraction results show phytochemical components in the form of alkaloid compounds, flavonoids, phenols, and tannins [4].

The biological activity of seaweed bioactive compounds is antioxidant, anticancer, antitumor, anti-inflammatory, antihypertensive, anti-obesity, antidiabetic, antibacterial, antifungal, antiviral, antiallergic (ovalbumin and shrimp), hypocholesterolemia, neuroprotective, skin lightening and intracellular ROS protection [3]. In addition to containing many bioactive substances, seaweed also has antioxidants. One application of antioxidant activity in seaweed is as a sunscreen [5]. Antioxidants are compounds that can neutralize free radicals to inhibit oxidation [6]. Free radicals will unwittingly form continuously in the human body in the form of normal cell metabolic processes, malnutrition, and such as the result of the response to the influence that exists outside the body [7].

2. Materials and Methods

2.1 Materials

The ingredients used are brown seaweed (*Sargassum* sp.), aquades, ethanol p.a (Smartlab), Folin-Ciocalteu reagent, standard antioxidant (gallic acid), and DPPH solution (1,1-diphenyl-2-picrylhydrazyl). Branson sonifer 250, Hitachi CR 21 GIII High-Speed Refrigerated Centrifuge, and Rotary Evaporator Steroglas Strike 300.

2.2 Methods

The research stages consisted of preparation, extraction, and total phenolic and antioxidant tests. The variables used are the ratio, time, and size of the particles.

2.2.1 Preparation

The material used in the study is brown seaweed (*Sargassum* sp.) from Gedong Tataan, Pesawaran Lampung Regency. Brown seaweed is dried aerated and not exposed to sunlight for 3 days, then mashed using a blender, and then scrambled according to the specified size.

2.2.2 Extraction

Extraction of bioactive compounds on brown seaweed (*Sargassum* sp.) is carried out using ultrasonic extraction methods with ethanol solvents. Experimental design is used in research conducted to facilitate the research process. Based on the experimental design that has

been designed there are 20 treatments for extraction using ultrasonic methods. The variables used are the ratio between samples: solvents are 5:100 to 15:100 (b/v). Then the power used is 170-190 at room temperature. The time used is for 30 to 50 minutes.

2.2.3 Identification of Total Phenols

The standard error acid manufacturing process used in the total test of brown seaweed extraction phenol (*Sargassum* sp.) is a solution of gallic acid stock containing 1000 µg/mL in ethanol. Then the stock solution is diluted to get a working solution with a rate of 10 µg / mL. Then a series of standard solutions are made with levels: 10, 20, 40, 60, 80, 100, and 120 µg/mL. The total phenol test process from the extraction of brown seaweed (Sargassum Sp.) is to dissolve 500 µL residue in 5 mL of 50% ethanol and put it into a test tube. Then added 0.5 ml of Folin-Ciocalteau solution that has been diluted 10 times and left to mix for 2 minutes and then added 2 mL sodium carbonate solution 7.5%. Next, aquades are added up to a volume of 10 mL and incubated at 45ºC for 30 minutes at room temperature. It then measured the uptake at a wavelength of 765 nm.

3. Result and Discussion

To find out the effect of process treatment on *Sargassum* sp. extract, 20 experiments were conducted using the experimental design. The variables used are the ratio (0.05-0.15), the particle size of 80 mesh, the power used are 170-190 watts and the time of 30-50 minutes. The results are presented in Table 1. In experiments, the *Sargassum* extract that had the highest total phenol was about 153.334 mgGAE/g under 30-minute extraction conditions, 170 watts of power, and a ratio between mass and solvent of 0.05.

Table 1. Overview of experiments for extraction of *Sargassum* sp.

Run	Ratio (g/mL)	Power (Watt)	Time (Menit)	Total Phenol (mg GAE/g)
1	0.10	180	23.18	69.026
2	0.15	170	30	78.633
3	0.10	180	40	70.214
4	0.10	180	40	61.493
5	0.15	190	50	122.086
6	0.10	180	40	79.643
7	0.10	180	40	70.962
8	0.01	180	40	142.147
9	0.10	180	40	80.322
10	0.05	190	30	71.453
11	0.05	170	30	153.334
12	0.10	180	40	75.334
13	0.18	180	40	76.028
14	0.10	180	56.81	74.274
15	0.15	190	30	49.247
16	0.05	190	50	95.234

Run	Ratio (g/mL)	Power (Watt)	Time (Menit)	Total Phenol (mg GAE/g)
17	0.10	163.18	40	66.026
18	0.10	196.81	40	83.275
19	0.15	170	50	45.725
20	0.05	170	50	110. 892

The value of R^2 (coefficient of determination) of 0.9344 shows data supporting the model of 93.44%. Model variance analysis is given in Table 2.

Table 2. Results of Variety Analysis (ANOVA) for Total Phenolic Response

Source	F-value	P-value	Description
Model	15.82	< 0.0001	*significant*
A-Ratio	45.10	< 0.0001	
B-Power	0.3413	0.5720	
C-Time	0.6793	0.4290	
AB	24.46	0.0004	
AC	4.34	0.0639	
BC	37.48	0.0001	
A^2	27.88	0.0004	
B^2	0.3882	0.5472	
C^2	0.0472	0.8324	
Residual			
Lack of Fit	2.98	0.1281	*not significant*

Total phenolics are indicators of the efficiency of the brown seaweed extraction process (*Sargassum* sp.). The effect of ratio (g/mL) and power (watt) to total phenolics is expressed in the graph model presented in figure 1.

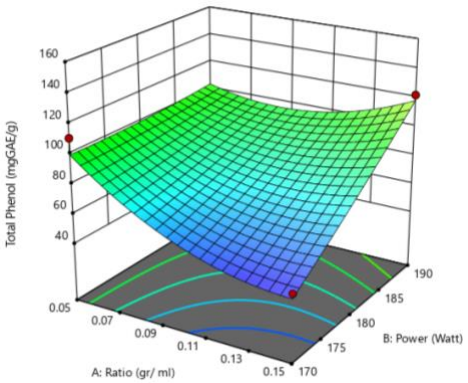

Figure 1. Surface model graphic to total phenolic ratio (gr/ml) to power (watt) at the constant time (50 minutes)

From figure 1 it is seen that the graph model's acquisition of the total phenolic expressed in mg gallic acid equivalent (GAE) per gram of brown seaweed (*Sargassum* sp.) is seen to be affected by the ratio between weight per solvent volume and the power used at a constant time. The greater the ratio and power used at a constant time of 50 minutes can be seen the total acquisition of phenolics will also be greater. However, there is an optimum point that slows the

total value of phenolics to decrease. So that if it has reached the optimum value or power above 170 watts will cause the total value of phenol to decrease.

The effect ratio (g/ml) and time (minutes) to the total phenolic are expressed in the graph model presented in figure 2.

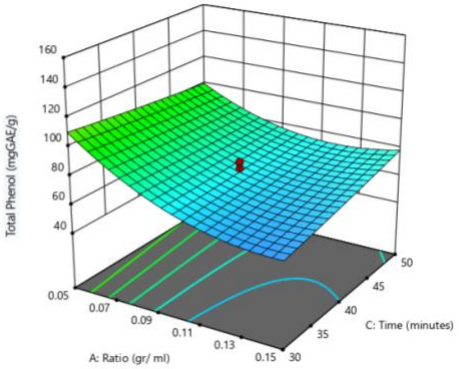

Figure 2. The surface model graph to the total phenolic ratio (gr/ml) with time at constant power (180 watts)

From figure 2 it is seen that the graph model's acquisition of the total phenolic expressed in mg gallic acid equivalent (GAE) per gram of brown seaweed (*Sargassum* sp.) is seen to be affected by the ratio between weight per solvent volume and time used at constant power. This is supported by his research [8] that the longer the time, the more bioactive compounds are obtained but, there is an optimum point that increases the total value of phenolics to decrease. So that if it has reached an optimum value above 30 minutes will result in the total value of phenols decreased.

The effect of power (watt) and time (minutes) on the total phenolic are expressed in the graph model presented in figure 3

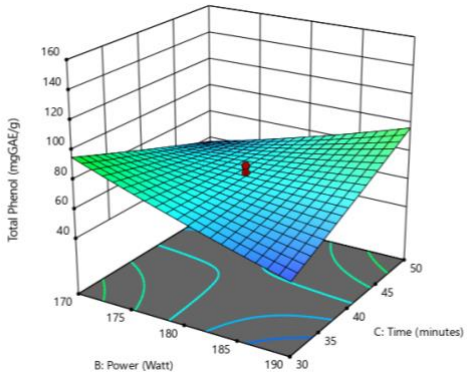

Figure 3. The surface model graph to total phenolic between power (watt) and time (minutes) at a ratio (0.1)

From figure 3 it is seen that the greater the ratio and power used can be seen the total acquisition of phenolics will also be greater as well. However, there is an optimum point that slows the total value of phenolics to decrease. So that if it has reached the optimum value or power above 180 watts will result in the total value of the phenol decreased. In addition, the longer the extraction time, the more levels of extracted compounds.

To check the antioxidant activity of the extracted *Sargassum* sp., a DPPH test was carried out. The results imply that fucoxanthin extracted from *Sargassum* sp. has radical cleaning activity. This is indicated by the IC_{50} value. IC_{50} value (50% inhibitory concentration) indicates the concentration of the sample needed to find 50% of DPPH free radicals. IC_{50} value is inversely proportional to antioxidant activity. The IC_{50} value calculated in this work was 87.57 ppm.

4. Conclusions

The effect of variables on the total phenolic is the greater the ratio of weight: volume, power, and time used then the total phenolic obtained will be greater but, there is an optimum point on the power that causes the total value of phenolics to decrease. The total phenolic test results found that the highest value was located at A11 which was 153.334 mgGAE /g in the operating conditions of 30 minutes, power of 170 watts, and the ratio between mass and solvent of 0.05. The results of antioxidant activity testing obtained an IC_{50} value of 87.57 ppm.

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