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Extraction of Antioxidant Compounds from *Sargassum* sp. Using Water and Ultrasound Assisted Extraction Method as A Derivation of Green Chemistry Principles

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Abstract. The content of bioactive compounds in *Sargassum* sp. has already been used in various fields. The Ultrasound Assisted Extraction (UAE) method and water solvent have met the principles of green chemistry, so it is used in this study to extract antioxidant compounds contained in *Sargassum* sp. The principle of green chemistry primarily aims to reduce or eliminate the impact of environmental damage. This research was conducted with several process variables, including the ratio of the sample mass to the volume of solvent (0.05 – 0.15 g/mL), time (30 – 40 minutes), and power (170 – 180 W) using the UAE method and water solvent (*Aquadest*) on the total phenol value of *Sargassum* sp. Analysis of variance was carried out with the help of Design Expert software, Response Surface Method - Central Composite Design, to determine the effect of the process variables carried out in the extraction process on the total phenol yield. The analysis of variance in this study shows the suitability between the research design and the study results, which is indicated by an R^2 value of 0.9785. The highest results were obtained with a variable ratio of sample mass to solvent volume of 0.18 (g/mL), time of 40 minutes, and power of 180 W, with a total phenol yield of 212.8 mg GAE/g and antioxidant activity of 12.3%.

Keywords: *Sargassum sp., green chemistry, antioxidant, total phenol, ultrasound-assisted extraction (UAE), water solvent*

1. Introduction

Indonesia has the second-longest coastline after Canada, so the marine biodiversity in Indonesia is very high [1]. One of the existing species is seaweed, which is commonly known as marine macroalgae. *Sargassum* species are brown macroalgae scattered around tropical oceans [2]. The content of bioactive compounds in *Sargassum* sp. has already been used in various fields, such as food, bioenergy, biofuels, pharmaceuticals, cosmetics, and textiles [3, 4,

5, 6]. *Sargassum* species in Indonesia have pharmacological potential due to the potent antioxidant activity of the phenolic compounds contained [7].

Bioactive compounds can be obtained by extraction. There are several extraction methods, one of which is Ultrasound Assisted Extraction (UAE). The UAE method is categorized as a non-conventional method [8]. The UAE method can extract bioactive compounds quickly and requires lower energy, solvents, and operating temperatures when compared to conventional methods [9]. Based on this effectiveness, the UAE method has a higher probability of application in the chemical and food industries [10, 11, 12, 13].

Extraction methods and solvents that can reduce the impact of environmental damage are needed because the environment has been experiencing a crisis of environmental damage over the last few decades. Green chemistry provides some methods that can overcome this. A method that can reduce the impact of ecological damage is the 'Green Extraction' method, which is based on the design of an extraction process that will reduce or eliminate energy consumption, allow the use of alternative solvents, and ensure safe and quality extracts. It was stated in green chemistry [14]. The UAE method is one of the methods of green extraction [13]. In addition to the extraction method, alternative solvents, called 'Green Solvent', are also used in green chemistry. Solvents indicated as green solvents are non-volatile organic compounds with high solubility, low toxicity, environmentally friendly, obtained from renewable resources at a reasonable price, and easy to recycle [15, 16].

In this study, the extraction of antioxidant compounds from *Sargassum* sp. was performed using the Ultrasound Assisted Extraction (UAE) method and the water solvent (*Aquadest*). The technique used is a derivation of the principle of green chemistry.

2. Materials and Methods

2.1 Materials

The materials needed in this study were *Sargassum* sp., aquadest, gallic acid, 2% Na_2CO_3 solution, $CuSO_4$ solution, sodium solution, potassium tartrate solution, Folin-Ciocalteu reagent, trolox, methanol solution, DPPH (1,1-*diphenyl*-2-*picrylhydrazyl*), and filter paper.

2.2 Sample preparation

The sample used in this study was *Sargassum* sp. obtained from Pesawsaran Regency, Lampung. The sample is then dried until there is no moisture content to prevent fungus growth on the sample. The drying process is carried out indoors so that the sample is not exposed to sunlight, which can cause some damage to the sample. The dried samples were obtained from the constant weight and sieved using an 80-mesh sieve. The sample is weighed based on the value of the variation of the process variable given by the Design Expert.

2.3 Extraction

The prepared sample was then extracted using the UAE method. The mechanism of the UAE method is that ultrasound waves meet a solvent containing a solid sample. Then, cavitation bubbles form, which cause changes in pressure and temperature. This causes an increase in the mass transfer rate of the sample solid to the solvent [18]. Tools for the extraction of the UAE method were prepared, and *Sargassum* sp., which had been weighed, was put into a glass beaker. The solvent in 100 mL of distilled water was put into a glass beaker and mixed with the sample. Extraction with the UAE method was carried out using a variety of treatment variables provided by design experts.

2.4 Total Phenol Analysis

The total phenol analysis method used on the sample was the Folin-Ciocalteu method. Calibration was carried out with different concentrations of gallic acid, namely 0.00, 0.25, 0.50, 0.75, and 1 mM. Then, 200 µL extract of *Sargassum* sp. and 2.0 mL of solution A (10 mL of 2% Na_2CO_3 with 0.1 mL of CuSO4 and 0.1 mL of sodium and potassium tartrate) were mixed, and after 4 minutes, 0.4 mL of 0.5 M sodium hydroxide was added. After 10 minutes, 0.2 mL of Folin-Ciocalteu reagent (1:1 v/v with water) was added. The solution was allowed to stand for 30 min, and the absorbance was measured with a UV-Vis Spectrophotometer at 750 nm. Using a gallic acid calibration curve, the total phenol content was calculated as mg gallic acid equivalent (mg GAE) [19].

2.5 Antioxidant Activity Analysis

The DPPH test took 0.4 mL of *Sargassum* sp. extract, gallic acid antioxidant standard, and trolox (50-400 g/mL) mixed with 3.6 mL of DPPH methanol solution (0.1 mM). The amount of methanol (0.4 mL) was used as a blank (control) with 3.6 mL DPPH solution. All samples were vortexed for 1 min and incubated in the dark for 30 min at 37°C. Each sample's absorbance decrease was measured against methanol as a blank on a UV-Visible spectrophotometer at 517 nm. The percentage of DPPH inhibition was calculated using equation 1 [19]:

$$\text{DPPH Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} x100\% \quad (1)$$

When: $A_{control}$: Absorbance control

A_{sample}: Absorbance sample

3. Result and Discussion

3.1 Effect of extraction process variables on total phenol content

The results of the total phenol test on *Sargassum* sp. using the UAE method and water solvent can be seen in Table 1. It shows that the most significant total phenol value is in the ratio of 0.18 (18 g sample: 100 ml solvent), 40 minutes, and power at 180 W is 212.8 mg GAE/g. The smallest total phenol value was found in the 0.016 g/mL ratio, 40 minutes of time and power at 180 W, with 42.7 mg GAE/g.

Table 1. Total Phenol from Treatment Variations Based on Response Surface Methodology Approach - Design Expert

No	Code	Factor 1	Factor 2	Factor 3	Response 1
		A: Ratio (g/mL)	B: Time (menit)	C: Power (W)	Total Phenol (mg GAE/g)
1	C17	0.18	40	180	212.8
2	C20	0.15	50	170	208.7
3	C3	0,.15	30	190	180.4
4	C13	0.15	50	190	177.2
5	C2	0.15	30	170	166.5
6	C6	0.1	57	180	158
7	C1	0.1	40	163	153.9
8	C5	0.1	40	180	151.5
9	C11	0.1	40	197	141.7
10	C9	0.1	40	180	136.7
11	C18	0.1	40	180	135.7
12	C16	0.1	40	180	134.1
13	C7	0.1	40	180	133.1
14	C12	0.1	40	180	131.9
15	C8	0.1	23	180	128.5
16	C4	0.05	50	190	103.3
17	C10	0.05	30	190	92.8
18	C14	0.05	50	170	91.1
19	C15	0.05	30	170	83.1
20	C19	0.016	40	180	42.7

The influence of process variables can be seen in Figures 1 – 3. In Figure 1, the variable that remains is variable C (power) of 180 W, in other words, it shows the relationship between variable A (ratio of sample mass to volume of solvent) and variable B (time) on total phenol yield. Figure 1 shows that the higher the ratio of the mass of the sample to the volume of the solvent, the higher the total phenol, whereas when the extraction time is carried out, the longer it takes, but at a particular time, the total phenol yield does not show a big difference.

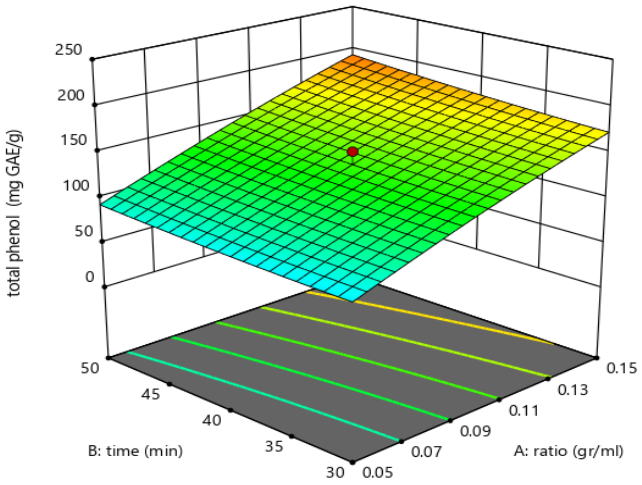

Figure 1. Relationship between total phenol with the ratio of sample mass to solvent volume (A) and time (B) at power (C): 180 W

The variable mass ratio of the sample to the solvent volume has a significant impact. The larger the ratio value or the more samples contained in the extracted solution, the higher the total phenol yield. This happens because the difference in the concentration of a higher solute will increase the diffusivity and dissolution of the solute in the solvent, which will increase during the extraction process. At a high ratio, the ultrasonic intensity applied to the sample is higher, causing more effects of fragmentation, erosion, and pore formation, thereby increasing the yield. A high ratio also increases the contact area between the material and the solvent, increasing the yield [9].

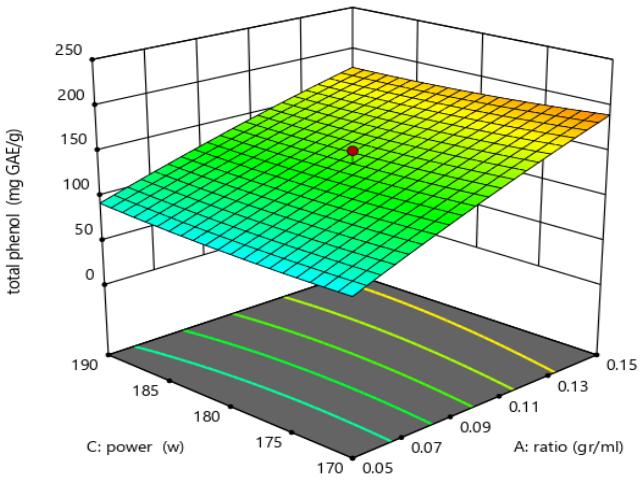

Figure 2. The relationship between total phenol with power (C) and the ratio of sample mass to solvent volume (A) at time (B): 40 minutes

In Figure 2, the variable that remains is variable B (time) for 40 minutes; in other words, it shows the relationship between variable A (ratio of sample mass to solvent volume) and

variable C (power). Figure 2 shows that the higher the sample mass ratio to the solvent volume, the higher the total phenol yield. At the same time, the ultrasound power increases with increasing power, giving a low total phenol yield but not a significant difference in the total phenol value. The time variable is influential but does not significantly impact the total phenol value. An increase in total phenol yield was also reported in the study [20], which states that the longer the extraction time, the higher the total phenol yield will be. However, several treatments with a longer extraction time in this study gave smaller results. This is because increasing the ultrasound time initially increases the results; after that, the results decrease, or there is no increase in the results for a longer time. As the time increases, the exposure of the solute and the extraction medium will aid their release into the solvent. Giving ultrasound waves of a very long duration will cause structural damage to the solute, reducing the extraction yield [9]. High total phenol values can be obtained in a short time, and if carried out for a long time, will also allow damage to the extracted compounds.

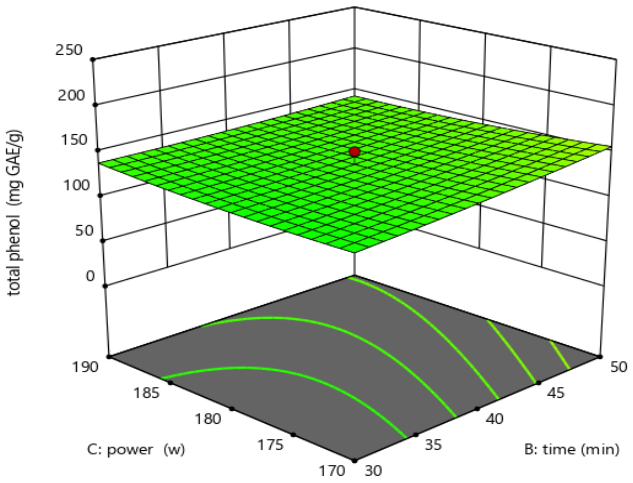

Figure 3. The relationship between total phenol with time (B) and power (C) on the ratio of sample mass to solvent volume (A): 0.1 g/mL

In Figure 3, the constant variable is variable A (sample mass ratio to solvent volume) of 0.1 g/mL, in other words, showing the relationship between variable B (time) and variable C (power). Figure 3 shows that the variable treatment does not significantly affect the total phenol value. The influence of the power variable is less influential in this study; it is intended that when the sample is given an increase in extraction power, the yield increases and then decreases. This is explained by the effect of cavitation bubble collapse, which increases with increasing power, causing fragmentation, pore formation, and mixing in the tissue to increase diffusivity and increase extraction yield [21]. Very high ultrasound intensity can reduce bioactive compounds. Very high power causes an increase in the number of bubbles formed. The layer

of cavitation bubbles around the probe tip (physical device used to connect electronic test equipment to the sample being tested) blocks energy transmission to the extraction medium (saturation effect), thereby reducing yield [9].

3.2 Analysis of Variance

The total phenol results from each variable variation were then analyzed using analysis of variance to determine the equation model that links the independent variables to the response variables. The analysis of variance model in this study is Central Composite Design – Quadratic. The results of the variance analysis for the total phenol response can be seen in Table 2. The F-value of the model is 50.52, which implies that the model is significant, meaning that the variables used substantially affect the total phenol yield of *Sargassum* sp.

Table 2. Results of analysis of total phenol variance from treatment variations based on the response surface methodology approach - Design Expert

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	32588.00	9	3620.89	50.52	< 0.0001	significant
A-ratio	30801.16	1	30801.16	429.74	< 0.0001	
B-time	840.10	1	840.10	11.72	0.0065	
C-power	19.26	1	19.26	0.2687	0.6155	
AB	52.53	1	52.53	0.7329	0.4120	
AC	195.03	1	195.03	2.72	0.1300	
BC	230.05	1	230.05	3.21	0.1035	
A^2	199.62	1	199.62	2.79	0.1261	
B^2	44.55	1	44.55	0.6216	0.4487	
C^2	163.37	1	163.37	2.28	0.1620	
Residual	716.74	10	71.67			
Lack of Fit	455.25	5	91.05	1.74	0.2788	not significant
Pure Error	261.49	5	52.30			
Cor Total	33304.75	19				

From Table 2, the p-value of the model is < 0.0001, which indicates that the quadratic model used is influential or significant. The p-value less than 0.05 indicates a considerable model term. A value greater than 0.1 indicates an insignificant model term, so it can be said that variables A and B (< 0.0001 and 0.0065) are significant models (provide a significant effect). This shows that the quadratic model can predict the optimum response conditions for

total phenol from *Sargassum* sp. using the UAE method and water as a solvent with a variable ratio and time. The R^2 value obtained in this study is 0.9785.

Analysis of variance also provides a quadratic model equation that can be seen in equation 2 of the process variable to total phenol. The total phenol value is directly proportional to the variable mass ratio of the sample to the solvent volume, and the extraction time is indicated by a positive constant, which suggests that when the variable value of the sample mass ratio to the solvent volume and the extraction time increases, the total phenol value produced will also increase. The inversely proportional power variable is indicated by a negative constant, which suggests that when the value of the power variable increases, the total phenol yield will decrease.

$$Y = 11.71 + 2.14\,A + 0.3264\,B - 0.023\,C + 0.058\,AB - 0.2179\,A - 0.1951\,BC - 0.4011\,A^2 + 0.091\,B^2 + 0.1624\,C^2 \quad (2)$$

When Y: total phenol (mg GAE/g)

A: ratio (g sample: mL solvent)

B: time (minutes)

C: power (W)

The statistical comparison between the actual and predicted data can be seen in the graph in Figure 4. This graph aims to determine the suitability of the given model and the actual data.

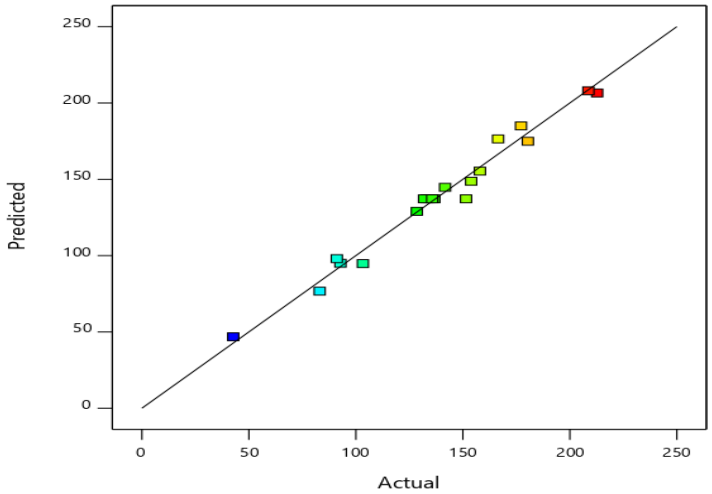

Figure 4. Prediction data plot vs. actual data

From Table 1, the analysis results of the total phenol contained in the extract of *Sargassum* sp. give the highest value to the highest ratio variable of all ratio variations. Apart from Table 1, the response between the total phenol value and the extraction process variable can be seen in the p-value in Table 2 and the response visualization (3D Surface) in Figures 1

– 3. Table 2 shows that the p-value of variable A (ratio of sample mass to solvent volume) and variable B (time) is said to be significant. In contrast, variable C (power) is not substantial. Likewise, the response visualization (3D Surface) shown in Figures 1 – 3 shows that the response variable A (ratio) is said to be significant, B (time) is quite significant, and C (power) is not significant. The three considerations (result table, p-value, and 3D surface) on the total phenol value of *Sargassum* sp. show that the most influential variable is the ratio treatment variable, followed by the time variable, which is quite influential. At the same time, the power variable is less influential.

3.3 Antioxidant activity

The results of the antioxidant activity test were carried out on the samples with the highest total phenol test results. This was done because there was a relationship between total phenol content and antioxidant activity. The greater the total phenol content, the higher the antioxidant activity. The antioxidant activity test in this study was the DPPH method. This method is based on the ability of the tested extract to donate hydrogen, which reacts with the DPPH radical, thereby neutralizing the free radical character and giving rise to the reduced form of DPPH (non-radical). Phenol compounds have a hydroxyl group, and the function of the hydroxyl group is to act as a contributor to hydrogen atoms that react with free radicals through an electron transfer mechanism [7]. These conditions were obtained at the ratio variable 0.18 (18 g sample: 100 ml solvent), time 40 minutes, and power at 180 W with a value of 212.8 mg GAE/gr. In the antioxidant test carried out on this sample, the results of DPPH inhibition were 12.3%.

3.4 Comparison with Other Methods

The total phenol yield of *Sargassum* sp. was the highest in this study, at 212.8 mg GAE/g. In another study regarding the total phenol yield of *Sargassum* sp., values obtained with the maceration method and water solvent gave higher total phenol yield, namely 669.33 mg GAE/g with heating for 20 minutes and 352.5 mg GAE/g without heating for 24 hours [23]. Study of *Sargassum muticum* using the maceration method with water solvent also gave a high total phenol yield of 275.8 ± 4.98 5 mg GAE/g. There are variables such as the ratio of the sample to the solvent volume of 0.04 g/mL and 24 hours [22]. The difference in the total phenol yield can be seen from the differences in the extraction method used, the ratio of the sample to the solvent, and the extraction time.

Another study [7] produced a lower total phenol of as much as 45 mg GAE/g compared to this study. The study used the maceration method with heating and the help of a magnetic stirrer, and the ratio of the mass of the sample to the volume of water solvent is 1:20. Comparisons can be made between the study [7] and this study, which uses the same extraction method. In this study, using the ultrasound-assisted extraction (UAE) method, there was a treatment with a ratio of 0.05 (g/mL) and a time of 30 minutes, and the highest total phenol yield was 83.1 mg GAE/g, while in [7] using the maceration method under the same conditions gave 45 mg GAE/g.

Study [20] also showed that the extraction results of macroalgae (one of them *Sargassum* sp.) using the UAE method gave higher yields when compared to conventional methods. Some of these studies show that the UAE method is more efficient than the maceration method. This is also in accordance with [24], which states that the UAE method provides higher polyphenol extraction results when compared to the conventional maceration method. The UAE method is more efficient and gives a higher total phenol yield when compared to the maceration method because, in the UAE method, ultrasound waves will provide effective interference on the sample cell wall, so that it can facilitate the release of more polar bioactive compounds [20]. Study [9] also stated that the UAE method can extract bioactive compounds in a very short time, at a low temperature, and requires lower energy and solvents when compared to conventional methods.

Based on the studies conducted on *Sargassum* sp., the variables that affect the total phenol value are extraction method, ratio, time, power (UAE method), temperature, and type of *Sargassum*.

4. Conclusion

The influence of the variable ratio of sample to solvent on the extraction is very influential; it is shown that the total phenol yield in a high ratio variable will also give a high total yield. As for the time variable is quite influential; with increasing time, the total phenol yield shows an increase. And for the power variable, it is less influential as the intensity of the total phenol yield tends to decrease. The highest value of total phenol from *Sargassum* sp. was obtained in the variable ratio 0.18 (g/mL), time of 40 minutes, and power of 180 W, with 212.8 mg GAE/g and antioxidant activity of 12.3%.

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