Optimization of Phenolic Compound Extraction *Ulva lactuca* Using the Ultrasound Assisted Hot Water Method (UAHW)

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Abstract. *Ulva lactuca* is a macroalgae rich in bioactive compounds, including phenolic compounds with potential in cosmetics as antioxidants. Phenolic compounds have antioxidant activity, which can help protect cells against oxidative damage caused by free radicals from ultraviolet radiation. The method used is Ultrasound Assisted Hot Water. Under optimal conditions, it will produce a high total of phenolics. This research was conducted to determine the effect of extraction parameters, namely temperature (40, 60, and 80°C), time (5, 10, and 15 minutes), and the ratio of *Ulva lactuca* to solvent (0.05, 0.10, and 0.15 g/ml). The experimental design was carried out using Design Expert 13 software with the Response Surface Methodology (Box Behnken Design). Based on the research results, it was concluded that the extraction parameters affected the total phenolic content. The highest total phenolic content in *Ulva lactuca* was obtained at 0.1605 mg GAE/g at a temperature of 80°C, time of 15 minutes, and a ratio of *Ulva lactuca* to solvent of 0.10 g/ml.

Keywords: *Ulva lactuca*, Ultrasound Assisted Hot Water, Total Phenolic Content

1. Introduction

Ulva lactuca can be found in the Americas, Europe, Africa, the Caribbean Islands, the Indian Ocean Islands, East Asia, South Asia, Australia, and New Zealand. One of the areas in Indonesia, namely Pathek Beach, Situbondo, East Java, is famous for the growth of *Ulva lactuca,* which is very fertile. *Ulva lactuca* is a macroalgae phylum *Chlorophyta,* which lives in shallow waters worldwide, especially on rocky beaches. *Ulva lactuca* is a polymorphic species with a morphology that depends on the level of salinity of water or symbiosis with bacteria [1]. In Indonesia, *Ulva lactuca* is widely used as a material food because its nutrition consists of carbohydrates, proteins, vitamins, fiber, and minerals. However, *Ulva lactuca* is not utilized as a commercial product like cosmetics. *Ulva lactuca* also contains antioxidants like alkaloids, flavonoids, triterpenoids, phenols, tannins, steroids, and carotene, which can be used as an active compound in the preparation of cosmetics [2].

Phenolic compounds have one or more aromatic rings joined with a hydroxyl group

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Phenolic compounds have one or more aromatic rings joined with a hydroxyl group [3,4]. Phenolic compounds are found in fruit, seed, leaf, stem, etc [5]. This compound has the activity of an antioxidant and antimicrobial in plants [6]. According to Arbi et al. (2016), *Ulva lactuca* extract contains 4.59% phenolic compounds [7]. Phenolic compounds protect cells against oxidative damage caused by free radicals from light radiation ultraviolet [8]. The extraction compound phenolic *Ulva sp* is shown in Table 1.

Table 1 . Extraction compound *Ulva* phenolics *sp.* with various methods

Material	Method	Type of Solvent	Condition Operation	Phenol Content	Reference
Ulva lactuca	Soxhlet	Ethanol	T=70℃, ratio material to solvent = 1:20 g/ml, t = 5 minutes	694.57 mg GAE/g	[2]
Ulva lactuca	UAE	Ethanol	T= 50-60℃, ratio material to solvent = 1:3.5 g/ml, t = 15 minutes	60.0±7. mg GAE/g	[9]
Ulva lactuca	UAE	Aquades	T=60℃, ratio material to solvent = 1:20 g/ml, t = 15 minutes	60.6 mg GAE/g	[10]
Ulva lactuca	UAE	Ethanol	T= 50-60℃, ratio material to solvent = 1:3.5 g/ml, t = 15 minutes	60.0±7.9mg GAE/g	[9]
Ulva lactuca	UAE	Aquades	T=60℃, ratio material to solvent = 1:20 g/ml, t = 15 minutes	60.6 mg GAE/g	[10]
Ulva lactuca	Maceration	Ethanol	T=26℃, ratio material to solvent = 1:4 g/ml, t = 48 hours	4.59 ± 0.0042 mg GAE/g	[11]
Ulva lactuca	UAE	Ethanol	T=60℃, ratio material to solvent = 1:10 g/ml,t = 10 minutes	1.857 mg GAE/g	[12]
Ulva lactuca	Maceration	Methanol	T=27℃, ratio material to solvent = 1:6 g/ml, t = 24 hours	17.79 ± 0.061 mg GAE/g	[13]
Ulva lactuca	Maceration	Ethanol	T=26℃, ratio material to solvent = 1:4 g/ml, t = 12 hours	23.93 ± 1.16 mg GAE/g	[14]

Material	Method	Type of Solvent	Condition Operation	Phenol Content	Reference
Ulva rigida	UAE	Methanol	T=37℃, ratio material to solvent = 1:10 g/ml, t = 2 hours	12.75 ± 0.41 mg GAE/g	[15]

Conventional extraction methods like soxhlet, reflux, and maceration can extract phenolic compounds. The weakness of the traditional way is that it requires a long extraction time and large amounts of sample and solvent [16]. Non-conventional extraction methods like Ultrasound-Assisted Hot Water can also extract phenolic compounds from *Ulva lactuca.* Ultrasound-Assisted Hot Water is a method of extraction that uses ultrasonic waves and hot water. The ultrasound-assisted hot water method is widely applied in the food, pharmacy, and cosmetics processing industries [17]. *Ulva lactuca* is one type of green algae that contains bioactive compounds such as phenols, flavonoids, and carotene, which function as antioxidants. Phenolic compound components can be obtained by Ultrasound- Assisted extraction Hot Water [18]. Ultrasound-Assisted Hot Water extraction is affected by ultrasonic power, temperature, extraction time, solvent concentration, and ratio of material to solvent [16]. Phenolic extraction of compounds is generally carried out at varying temperatures between 40-80℃, whereas a temperature upper than 80℃ causes compound phenolic degradation quickly [19]. Combining hot water and ultrasonic waves can increase the rate of extraction. Therefore, using hot water can also increase the solubility of compounds in solvents to speed up the extraction process. Hot water extraction and ultrasonic waves are modified to extract compound derivative plants, including alkaloids, flavonoids, glycosides, phenolics, and polysaccharides [16,20].

Condition extraction is determined through the software Design Expert to predict experiment results. Using a Design Expert aims to study and choose process conditions from a combination of factors that produce optimal response [21]. *Box-Behnken Design (BBD)* is an RSM design used for optimization that involves three independent variables: material ratio, time, and temperature [22]. Design Experts can provide guidance and direction that can be selected according to the goals of the Design of the Experiment (DOE) [23,24].

2. Research methods

2.1 Materials

The materials used in this research include *Ulva lactuca* purchased from a green algae finder at Pathek Beach Situbondo, distilled water, 96% ethanol, Na_2CO_3, *Folin-Ciocalteu* reagent, and gallic acid.

2.2 Tools

The tools used include an ultrasonic water bath (BAKU BK-1200 1.47L), spectrophotometer UV-Vis (752AP), blender (Philips HR2115), oven, rotary

evaporator, glasswares, analytical balance (OHAUS PX224).

2.3 Method

Sample Preparation and Water Content Analysis

Ulva lactuca was washed thoroughly using water repeatedly to remove impurities that stick like shells and sand. Then, *Ulva lactuca* was dried under the sun for four days [25]. Furthermore, it was ground until powdery and sifted using a 60-*mesh* sieve [2]. Determination of water content was conducted using the previous research procedure[26]:

Extraction of *Ulva lactuca* with Ultrasound Assisted Hot Water

In this procedure, there are three variables of extraction: ratio material to solvent (0.05, 0.10, and 0.15 gram/ml), temperature extraction (40, 60, and 80℃), and extraction time (5, 10, and 15 minutes) [27]. Extraction is carried out based on Table 2.

Table 2. Running point data of box-behnken design

No.	Temperature (C)	Time (Minute)	Material and solvent ratio (g/ml)
1	40	10	0.10
2	60	10	0.15
3	40	15	0.15
4	60	5	0.05
5	60	15	0.05
6	80	10	0.05
7	40	10	0.05
8	60	5	0.15
9	60	15	0.10
10	60	15	0.10
11	80	10	0.10
12	60	10	0.10
13	80	5	0.10
14	40	10	0.10
15	80	10	0.15
16	60	10	0.10
17	60	5	0.10

The extraction result was filtered by filter paper. The filtrate was analyzed using a UV-Vis spectrophotometer to determine the phenolic content[28].

Total Phenolic Content

Making Reagent Na_2CO_3 7%

We weighed as much as 3.5 grams of Na_2CO_3, then dissolved with distilled water sterile up to 50 ml [29].

Making Solution Gallic Acid Standard

A 100 ppm gallic acid stock solution was made by weighing 10 mg of gallic acid and dissolved in distilled water to a volume of 100 ml. From 100 ppm gallic acid stock solution pipetted as much 0.1, 0.2, 0.3, 0.4, and 0.5 ml to obtain concentrations of 1, 2, 3, 4, and 5 ppm [29].

Measurement of Gallic Acid Standard

Each concentration of 1, 2, 3, 4, and 5 ppm was added with 0.4 ml of reagent Folin-Ciocalteau and was shaken and left for 8 minutes. Next, 4.0 ml was added to 7% Na_2CO_3 solution and shaken until homogeneous. 10 ml of distilled water was added and let stand at room temperature. The solution was analyzed at wavelength 750 nm. The calibration curve was created for the relationship between gallic acid concentration and absorbance [29].

Determination of Total Phenolic Content

A sample of as much as 1.0 ml was mixed with 0.4 ml of Folin-Ciocalteu. The mixture was shaken until homogeneous. The mixture was left at room temperature for 8 minutes. After that, 4.0 ml solution of Na_2CO_3 7% was poured into the mixture, and distilled water was added until a volume of 10 ml. Absorption was measured with a UV-Vis spectrophotometer at a wavelength of 750 nm. The total phenolic content was expressed as gallic acid equivalents in mg per g weight dry algae (mg GAE/g).

The total phenolic content can be determined [29].

$$TPC = \frac{C \cdot V \cdot Fp}{g}$$

Information :

TPC	= total phenolic content (mg GAE/g)
C	= concentration phenolics (mg/L)
V	= volume of solvent (ml)
Fp	= dilution factor
g	= mass of *Ulva lactuca* (g)

3. Results and Discussion

3.1 Extraction Total Phenolic content from *Ulva lactuca*

The research was carried out from August 2023 to September 2023 at the Natural Materials and Bioactive Component Processing Technology Laboratory, Chemical

Engineering Study Program, Faculty of Engineering, Jember University. The main ingredient used in this research is *Ulva lactuca*. Determination of water content was carried out using the sun drying method for four days. Drying using sunlight was chosen because it can dry *Ulva lactuca* with a large capacity and does not require production costs [30,31].

(a) (b)

Fig. 1. (a) *Ulva lactuca* after drying ; (b) *Ulva lactuca powder*

Figure 1(a) shows *Ulva lactuca* after drying in the sun, then ground 1(b). This process aims to enlarge the sample's surface to make the extraction process more efficacious [32]. Water content influences the extraction results. If the water content value is still high, evaporating will be difficult, which can cause the extraction process to take longer. This analysis was carried out using a temperature of 110ºC for 3 hours and weighed every 1 hour. Re-drying needs to be performed if water content is more than 10%. [26]. The water content of *Ulva lactuca* was 7.33%.

The parameters influencing the *Ulva lactuca* extraction process are temperature, time, and sample-to-solvent ratio. Extraction temperature is the main factor affecting the total phenolic yield. Increasing the temperature during the extraction process can increase total phenolic content. However, when the extraction temperature has reached a specific temperature, The optimum phenolic content will decrease due to the decomposition of phenolic compounds at high temperatures. The decrease in total phenolics occurred because the cell structure was easily damaged due to chemical reactions accompanied by light and oxygen [33]. The time also affects the total phenolic value, where the longer the contact of the material with the solvent can increase the solubility of the extracted material because the solvent will more easily remove phenolic compounds. However, the longer the extra time used can reduce phenolic levels. That matters because the oxidation reaction of phenolic compounds due to exposure to oxygen reduces the extraction effectiveness [34,35]. These two variables have an authentic influence on the total phenolic content. Apart from that, the variable ratio of ingredients to solvent also influences the total phenol content. The more solvent added, the more components are extracted by the solvent. The extracted material will continue to increase until it reaches saturation conditions and there is no longer an increase in the extraction yield [36].

Maximum absorption measurements of gallic acid were carried out at a wavelength of 750 nm. The standard solution curve was prepared with a gallic acid solution

concentration of 1, 2, 3, 4, and 5 ppm. This curve helps determine the total phenolic content in the sample[37].

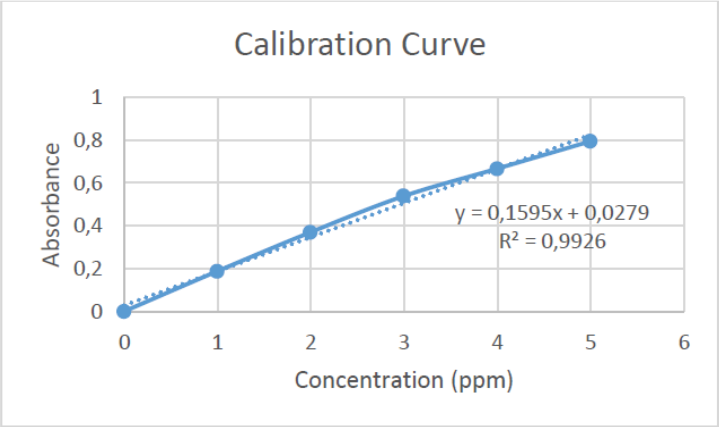


Fig. 2. Calibration curve using gallic acid standard

Figure 2 shows the y regression equation is obtained = 0.1595x + 0.0279, and the R2 is 0.9926. R2 is close to 1, proving the equation is linear [37]. The total phenolic content in Ulva lactuca is shown in Table 3.

Table 3 shows that the highest total phenolic content is 0.1605 mg GAE/g sample with variations in extraction temperature 80ºC, time 15 minutes, and the ratio of sample to solvent 0.10 g/ml. The lowest total phenolic content was 0.024 9 mg GAE/g sample under extraction temperature conditions 40ºC, time 5 minutes, and the ratio of sample to solvent 0.1 gr/ml. High temperature and time will damage cell components in Ulva lactuca and release the phenolic compounds contained therein, resulting in higher total phenolics [35].

Table 3. Total phenolic content in *Ulva lactuca*

Run	Temperature (ºC)	Time (minute)	*Ulva lactuca* ratio (g/ml)	Total Phenolics Content (mg GAE/g sample)
1	40	5	0.10	0.0249
2	60	15	0.15	0.0706
3	40	10	0.15	0.0255
4	60	5	0.05	0.0657
5	60	15	0.05	0.0739
6	80	10	0.05	0.1590
7	40	10	0.05	0.0569
8	60	5	0.10	0.0355
9	60	10	0.10	0.078 8

Run	Temperature (ºC)	Time (minute)	*Ulva lactuca* ratio (g/ml)	Total Phenolics Content (mg GAE/g sample)
10	60	10	0.10	0.072 9
11	80	5	0.10	0.1326
12	60	10	0.10	0.0726
13	80	15	0.10	0.1605
14	40	15	0.10	0.0589
15	80	10	0.15	0.1423
16	60	10	0.10	0.0688
17	60	10	0.10	0.0720

The total phenolic content obtained was smaller compared to previous research on Ulva *lactuca* extraction conducted by Arbi *et al.* (2016), namely 4.59 mg GAE/g, Sofia *et al.* (2022) of 1.875 mg GAE/ g, and Kurniawan *et. Al* (2019) was 23.93 mg GAE/g [7,12,14]. The low total phenolic content can occur due to different extraction conditions in the extraction method used and several other factors that influence the extraction process. This research used *Ulva lactuca* extract without solvent removal with a rotary evaporator. On the other hand, *Ulva lactuca* was not fresh, which affected the total phenolic yield [38].

3.2 ANOVA Analysis

The total phenolics in *Ulva lactuca* were analyzed using *Analysis of Variance* (ANOVA) in Design Expert software. The ANOVA results are shown in Table 4.

Table 4. Results *of Analysis of variance* (ANOVA)

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	0.0288	9	0.0032	167.45	< 0.0001	*significant*
A-temperature	0.0229	1	0.0229	1198.55	< 0.0001	
B-time	0.0014	1	0.0014	72.34	< 0.0001	
C-sample to volume ratio	0.0008	1	0.0008	43.53	0.0003	
AB	9.302E-06	1	9.302E-06	0.4865	0.5080	
AC	0.0001	1	0.0001	2.83	0.1367	
BC	0.0002	1	0.0002	9.43	0.0181	
A^2	0.0033	1	0.0033	170.81	< 0.0001	
B^2	0.0002	1	0.0002	9.73	0.0169	
C^2	0.0001	1	0.0001	5.39	0.0533	
Residual	0.0001	7	0.0000			
Lack of Fit	0.0001	3	0.0000	2.07	0.2469	*Not significant*
Pure Error	0.0001	4	0.0000			
Cor Total	0.0290	16				

Parameters are to be significant if they fulfill two conditions: probability (*p-value*)

≤ 0,05 and mismatch value (*Lack of fit*) ≥0.05 [39]. Based on table 4 obtained mark *p-value* ≤0.0001, this shows that the model has a significant influence on the response of total phenolic content. Meanwhile, the non-conformity (Lack of Fit) value in the response temperature, time, and ratio is insignificant, with a value of 0.2469. It indicates that the model selection is appropriate because the lack of fit value is inversely proportional to the p-value [40].

Table 5. Fit statistics

R^2	*Adjusted* R^2	*Predicted* R^2	*AdeqPrecision*
0.9954	0.9894	0.9522	39.7598

The R^2 is 0.9954, which shows the model's suitability with the research results. [41,42]. The adjusted R^2 of 0.9894 shows a strong relationship between temperature, time, and the ratio of *Ulva lactuca* to solvent on the response of total phenolic content [43,44]. By mathematics, equation models for total phenolic content as the response from variable extraction can be modeled with the equation below.

$$Y = 0.150638 – 0.005895A + 0.006173B – 0.297700C – 0.000015AB + 0.003675AC + 0.026900BC + 0.000070A^2 – 0.000266B^2 – 1.97900C^2$$

Where:
Y = Total phenolic content (mgGAE /g)
A = Temperature (℃)
B = Time (minutes)
C = *Ulva lactuca* to solvent (g/ml)

A negative coefficient indicates a decrease in the value of total phenolic content and vice versa [44]. Based on the equation, all variable extraction in a way statistics influential and significant to response total phenolic content Because own mark *p-value* ≤ 0,05. A graph of the relationship between model data (*predicted*) and experimental data *(actual*) is presented in Figure 3.

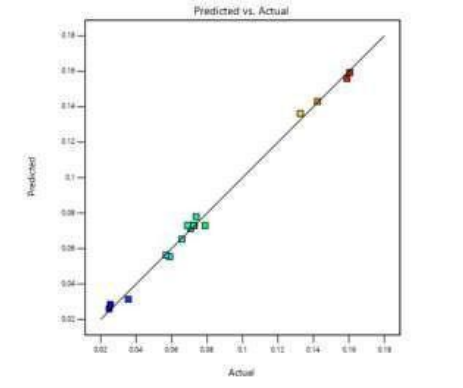
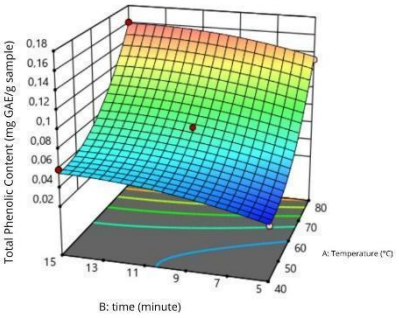


Fig. 3. Chart relationship between model data (*predicted*) and experimental data (*actual)*

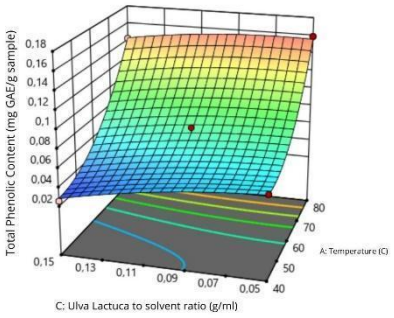
Figure 3 shows that the graph of the relationship between experimental data and predicted data from the model has a relatively high level of accuracy. The distance between the data points and the trendline indicates the accuracy of the two data. The closer the data is to the trendline, the more accurate the data [43].

3.3 Influence of Extraction Parameters on Total Phenolic Content from *Ulva lactuca*

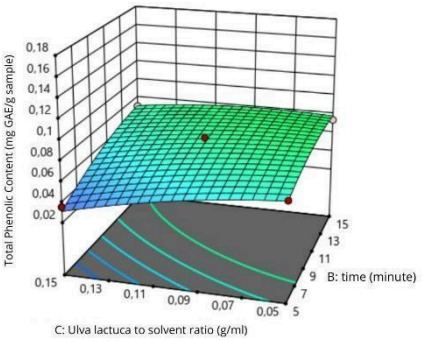
The total phenolic content is strongly influenced by temperature extraction, shown in figures 4(a) and (b), where total phenolic content is enhanced from temperature 40 - 80 ℃ ℃. Extraction with a temperature of 40 ℃ for 15 minutes produced total phenolics of 0.589 mg GAE/g sample. On temperature extraction of 60 ℃ with the same operating conditions, total phenolics increased to 0.0739 mg GAE/g sample. Total phenolic content was increased to 0.1605 mg GAE/g sample until temperature 80 ℃ with an extraction time of 15 minutes. The results of this research follow those of Sitoresmi *et al.* The phenolic content will keep increasing until the temperature is 90 ℃ and then decrease [45]. Based on a study, Novianti (2021) stated that total phenolic content will experience enhancement along with increasing temperature extraction and a decrease in specific temperature [46]. This matters because the temperature can increase solvent effectiveness in extracting phenolic compounds from a material. Phenolic compounds can be released from the cell walls of *Ulva lactuca* due to damage to cell elements. More phenolic compounds will be extracted if the extraction process is carried out at high temperatures [47,48].



(a)



(b)



(c)

Fig. 4. Relationship between variable to total phenolic content (a) time (minutes) and temperature (℃); (b) ratio of material to solvent (g/ml) and temperature (ºC); (c) ratio of material to solvent (g/ml) and time (minutes).

In Figure 4(a) and (c), it can be seen that forever time extraction can influence the total phenolic content produced. Total phenolics of 0.1326 mg GAE/g sample were produced at 5-minute extraction with a temperature of 80 ℃. At an extraction time of 10 minutes at the same temperature, total phenolics were produced at 0.1423 mg GAE/g sample. The largest total phenolic content was produced at an extraction time of 15 minutes and a temperature of 80 ℃, namely 0.1605 mg GAE/g sample. This matter, under research conducted by Yunita et al., uses *Ulva lactuca,* which states that the longer the extraction time, the higher the total phenolics produced. The longer the extraction time, the higher the temperature, the more excellent the solubility of phenolic compounds in the solvent. Too short an extraction time means that phenolic compounds are not thoroughly extracted. Therefore, the extraction time variable must be considered because if additional time is continuously added, it can reduce the phenolic content produced. After all, phenolics are sensitive to heat [34,45].

Based on Figure 4(b) and (c), the Ulva lactuca to solvent ratio passes optimum conditions and can lower the rate of phenolics produced. A ratio of *Ulva lactuca* to solvent 0.05 g/ml with a temperature of 80 ℃ produced total phenolics of 0.1589 mg GAE/g sample. At a 0.1 g/ml ratio with the same operating conditions, the largest total phenolics were 0.1605 mg GAE/g sample .

4. Conclusion

This research was carried out to determine the effect of temperature, time, and Ulva lactuca to solvent ratio on the total phenolic content present in the extract using the ultrasound-assisted hot water extraction method. The research results show that extraction parameters significantly influence the total phenolic content in *Ulva* lactuca. The most influential parameters are temperature extraction at 80ºC, time at 15 minutes, and *Ulva lactuca* to solvent ratio of 0.10 gr/ml, producing the largest total phenolics, 0.165 mg GAE/g. This research can help develop commercial products,

such as cosmetics derived from natural ingredients.

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