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The Effect of Different Drying Temperatures on Antioxidant Activity of Sea Grape (*Caulerpa Lentillifera*)

Tafrozi Muhtar [a], Romadhon [a], Lukita Purnamayati [a], Muhammad Hauzan Arifin [a]*

[a] *Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Semarang, 50275, Indonesia*

A B S T R A C T

Caulerpa lentillifera sea grape is one type of seaweed that has the potentioal as a natural antioxidant. Differences in drying temperature can affect the value of phytochemical compounds and antioxidant activity of the resulting extract. The purpose of this study was to determine the effect of drying temperature on the bioactive content and antioxidant activity of C. Lentillifera extract and to determine the best drying temperature for C. Lentillifera seaweed. The material used in this study was Caulerpa lentillifera sea grape obtained from Karimun Jawa Beach, Jepara. The extraction of Caulerpa lentillifera sea grape in this study used the sonication method using methanol as a solvent with a ratio sample weight and solvent volume of 1:7 for 1 hour. This study used a Completely Randomized Design (CRD) with different temperature treatments, 30°C, 40°C, 50°C, with three repetitions each treatment. The phytochemical compounds in Caulerpa lentillifera sea grape are flavonoids, alkaloids, phenols, tannins, steroids, and triterpenoids. The difference in drying temperature of the sample to the extract of C Lentillifera had a significant effect on the phenolic, flavonoid and IC_{50} values. The highest value of total phenol, flavonoid and IC_{50} content was found in C Lentillifera extract with a drying temperature of 40°C. IC_{50} value of 8774.20 ppm which is above 200 ppm so that it can be classified as a weak antioxidant.

Keywords: Antioxidant activity, Drying temperature, Phenolic content, Sea grapes

1. Main text

Sea grapes (C. lentillifera) are one of Indonesia's abundant marine biological resources. According to KKP (2017), Caulerpa sp seaweed has penetrated export markets such as Japan, China, Korea, and the Philippines. Over a five-year period (2011-2015), seaweed production increased by 22.25%. The national seaweed production volume in 2015 reached ± 11.2 million tons, an increase of 9.8% from the previous year. Sea grapes (Caulerpa sp.) are a type of seaweed that contains fiber, vitamins, minerals, and natural antioxidants, making seaweed a potential natural resource. Sea grapes contain several active compounds. There are antioxidant compounds, namely saponins and flavonoids, which can be used as antifungal components. Fresh Caulerpa lentillifera seaweed has a very high-water content, namely 94.84% (Tapotubun, 2016). This causes this seaweed to easily deteriorate in quality. Fresh Caulerpa lentillifera seaweed has a very short shelf life. Fresh Caulerpa lentillifera is very susceptible to morphological changes such as a change in texture to a withered appearance, a change in color to a faded appearance, and the release of fluid/mucus from the seaweed. Post-harvest handling in the form of drying can reduce the water content in the seaweed, thereby inhibiting the deterioration process and allowing it to be developed into food ingredients.

Drying is carried out to reduce the water content of the material to the desired level and eliminate enzyme activity that can further break down the active ingredients. There are several drying methods that can be used, namely natural drying using sunlight, the cabinet dryer method, and the oven method. Traditional drying using solar heat is a drying method that has the advantage of being inexpensive and easy to do. However, this method has several disadvantages, namely unstable drying temperatures, drying will be disrupted during the rainy season, and the seaweed produced will have low organoleptic value (Rofik et al., 2021). The drying method using energy during the drying process makes oven drying relatively more expensive compared to traditional drying methods. Oven drying has the advantage of controllable drying temperatures, ensuring stable temperatures throughout the drying process. Drying using oven will produce seaweed with high organoleptic value. Extraction of dried sea grapes is carried out using the sonication method. The sonication

2. Methodology

This study consisted of three stages: preparation and drying of C. lentillifera samples, extraction of dried C. lentillifera, and sample analysis.

Preparation and drying of C. lentillifera

Sampling of C. Lentillifera was conducted in Karimun Jawa, Jepara. C. Lentillifera was then cleaned of any dirt using fresh water to remove any salt deposits. Next, C. Lentillifera sea grapes were dried according to (Indayani et al., 2019) with modifications, namely drying using an oven at temperatures of 30°C, 40°C, and 50°C for 23 hours. After drying, C. Lentillifera sea grapes were cut using scissors to obtain small pieces like powder.

Extraction of C. lentillifera samples

Extraction of C. lentillifera sea grapes was carried out using the sonication method with methanol as the solvent. The dry samples were immersed in a sonicator bath with a sample weight to solvent volume ratio of 1:7 (w/v) for 1 hour. The sonication results were then filtered using Whatman No. 42 filter paper and the filtrate was concentrated using a rotary vacuum evaporator at a temperature of 70°C. The extraction results were weighed and stored in sealed bottles in a refrigerator.

3. Sample analysis

Moisture content test (BSN, 2015)

The moisture content test was conducted in accordance with SNI 2353.2:2015 using the gravimetric method. An empty dish was placed in an oven for 2 hours at a temperature of 105°C. It was then transferred to a desiccator for about 30 minutes until it reached room temperature and weighed as the empty weight (A). the ground sample weighing + 2 g into the dish (B). Place the dish containing the sample in an oven at a temperature of 105 o C for 16- 24 hours. Using tongs, transfer the dish into a desiccator for approximately 30 minutes, then weigh it (C). Perform the test at least twice and calculate the results.

Moisture content (%) = (B-C)/(B-A)× 100%

Description:

A: empty cup weight (g)

B: Weight of dish + initial sample (g)

C: Weight of dish + dry sample (g)

Yield test (AOAC, 1995)

Yield analysis is performed by weighing the initial material (dried C. Lentillifera) and the final weight (C. Lentillifera extract). Yield can be calculated using the formula:

Extract Yield = Extract Weight/Sample Weight× 100%

DPPH antioxidant activity test

The highest in vitro SPF test results were weighed and diluted with an ethanol solvent made in a concentration of 106 mg/mL. Then, each sample was pipetted 2 mL, then 1 mL of ethanol solvent and 1 mL of DPPH-ethanol solution were added and vortexed for 20 seconds. The samples were placed in a dark place for 30 minutes, then the absorption of the solution was measured at the maximum wavelength of DPPH that had been optimized previously. The antioxidant activity of the samples was determined by the degree of inhibition of DPPH radical absorption through the calculation of the percentage of DPPH absorption. The regression equation was obtained from the relationship between sample concentration and percentage inhibition of free radical activity. The value of 50% inhibition of free radical activity (IC_{50}) was calculated using the regression equation. The IC_5 o value was obtained by entering Y = 50 and the known values of A and B. The value of x as IC_5 o was calculated using the equation:

$y = A + B\ In(x)$

Explanation:

y: percentage inhibition

A: slope

B: intercept

x: sample concentration (mg/l)

Phytochemical screening test

Phytochemical screening tests are conducted to determine the levels of bioactive compounds present in samples. Quantitative phytochemical tests include alkaloids, steroids and triterpenoids, saponins and tannins, while qualitative phytochemical tests include phenolic compounds and flavonoids.

Data Analysis

The method used in this study was the experimental laboratory method with a completely randomized design (CRD) with three repetitions. The drying temperature variations used were 30°C, 40°C, and 50°C..

Results and Discussions

Water content of C. lentillifera sea grapes

Results of moisture content testing on C. Lentillifera can be viewed in Table 1.

Table 1. Moisture content of C. lentillifera sea grapes

Sample	Fresh sea grape weight before drying (g)	Dry sea grape weight (g)	Average moisture content (%)
Fresh sea grapes	600	-	83.11 ± 0.249
Temperature 30°C	600	397.80	77.36 ± 1.135
Temperature 40°C	600	232.20	67.35 ± 0.550
Temperature 50°C	600	77.20	5.74 ± 0.052

- Data is the result of the average of 3 repetitions ± standard deviation.

Table 1 shows that fresh C. Lentillifera sea grapes contain a fairly high-water content, namely 83.11 ± 0.249. The sea grapes with the highest dry weight are sea grapes dried in an oven at 30°C, weighing 397.8 g, while the lowest dry weight was in sea grapes dried in an oven at 50°C, weighing 77.2 g. This is directly proportional to the water content in dried sea grapes, which shows that drying at a temperature of 30°C has the highest water content of 77.36 ± 1.135, and the lowest water content is found in drying at a temperature of 50°C of 5.74 ± 0.052. Drying has different effects on the yield of extractives, phenolic compound content, and antioxidant activity. Luliana et al. (2016) stated that antioxidant activity has a very strong correlation with the moisture content of the sample. Very high moisture content indicates low antioxidant content. This is because high humidity causes high enzymatic degradation.

Extract Yield of C. lentillifera Sea Grapes

Yield is the percentage ratio between the weight of the material produced and the total weight of the material. The yield of the extract of C. dried in an oven at 30°C yielded 49.07%, while drying in an oven at 40°C yielded 87.26%, and drying in an oven at 50°C yielded the lowest result at 122.00%. The extraction yield of Caulerpa sp. with a dry weight of 8% in Nursandi's (2014) study was 14.30%. The extraction yield of the sea grape C. Lentillifera is dark green and liquid in form.

Phytochemical screening test

The results of phytochemical screening tests aimed to determine the bioactive components in C. Lentillifera sea grape crude methanol extract, which include alkaloids, steroids and triterpenoids, flavonoids, phenols, tannins, and saponins, showed the presence of bioactive components in C. Lentillifera sea grape crude extract. Based on the qualitative phytochemical screening results, the C. Lentillifera sea grape extract is presented in Table 2.

Table 2. Results of Phytochemical Screening Tests of C. Lentillifera Sea Grape Extract

Phytochemical Screening	Reagent	Indicator	Results		
			30°C	40°C	50°C
Flavonoids	Concentrated HCl	Red/purple	+	+	+
Phenol	$FeCl_3$	Blue	+	+	+
Saponin	H_2O	Foaming	+	+	+
Steroid/ Triterpenoid	Anhydrous acetate + $CHCl_3$ + H_2SO_4	Violet/red-brown ring	+	+	+
Tannin	$FeCl_3$	White precipitate Blue black	-	-	-
		Brownish green	-	-	-
			+	+	+

Alkaloid	Dragendorf reagent Mayer reagent	Orange precipitate Yellow precipitate	+ +	+ +	+ +

Description: - : not detected

\+ : detected

Based on Table 2 shows the results of qualitative phytochemical testing, which found that sea grape (C. *lentillifera*) extracts dried at three different oven temperatures contained flavonoids, phenols, saponins, steroids/triterpenoids, tannins, and alkaloids.

Flavonoid compound analysis

Flavonoid test results on sea grape extract C. Lentillifera can be viewed at in Table 3. Table 3 shows the results of quantitative flavonoid testing of C. Lentillifera sea grape extract, indicating that C. Lentillifera sea grape extract dried in an oven at 40°C had the highest flavonoid content at 0.460 ± 0.005 ppm, followed by sea grape extract dried in an oven at 30°C at 0.095 ± 0.003 ppm, and the lowest content was found in sea grape extract dried in an oven at 50°C at 0.045 ± 0.001 ppm. The highest flavonoid content based on the three oven temperatures was obtained from sea grape extract dried in an oven at 40°C. Drying at 50°C yielded the lowest results because high temperatures can reduce the flavonoid content. According to Syafarina et al. (2017), flavonoids are a group of polyphenols with a phenolic structure phenolic compounds that are easily oxidized and sensitive to heat treatment, so that the drying temperature affects the flavonoid content. The mechanism of flavonoid compound reduction due to drying temperature is caused by changes in flavonoid compound decomposition. The reduction in flavonoid compounds can be caused by changes in the chemical composition of phenolic compounds due to high drying temperatures.

Table 3. Flavonoid Test Results of *C. Lentillifera* Sea Grape Extract

Drying Method	Flavonoid Content Total (% QE/g)
Oven at 30°C	0.10 ± 0.003^{a}
Oven temperature 40°C	0.46 ± 0.005^{b}
Oven temperature 50°C	0.05 ± 0.001^{c}

Note:

Data is the average result of three repetitions

Data followed by different superscript letters indicate significant differences between treatments (P<5%).

Analysis of phenolic compounds

Phenolic compounds are compounds that have one or more hydroxyl groups directly attached to an aromatic ring. The results of the phenol test on C. Lentillifera extract are presented in Table 4.

Table 4. Phenol Test Results of C. Lentillifera Sea Grape Extract

Drying Method	Phenol Content Total (% GAE/g)
Oven at 30°C	0.03 ± 0.000^{a}
Oven temperature 40°C	0.16 ± 0.001^{b}
Oven temperature 50°C	0.01 ± 0.000^{c}

Note:

-Data is the average result of three repetitions

- Data followed by different superscript letters indicate significant differences between treatments (P<5%)

Table 4 shows the results of quantitative phenol testing of C. Lentillifera sea grape extract, indicating that C. Lentillifera sea grape extract dried in an oven at 40°C had the highest phenol content, namely 0.164 ± 0.001 ppm, followed by extract oven drying at 30°C at 0.028 ± 0.000 ppm, and the lowest content of sea grape extract with oven drying at 50°C was 0.010 ± 0.000 ppm. The highest phenolic content based on the three oven temperatures was obtained from sea grape extract with oven drying at 40°C. Drying at 50°C yielded the lowest results because high temperatures can reduce the phenolic compound content. The higher the drying temperature used, the higher the phenolic content in the sample. This occurs because the temperature can gradually reduce the water content without degrading or reducing the bioactive compound content due to the heat on the phenolic compounds. According to Sidoretno and Annisa (2018), compounds polyphenols are mentioned can be damaged during high-temperature drying, and carotenoids undergo degradation with the presence of large amounts of oxygen. This phenolic compound is thought to influence the antioxidant content in sea grapes. C.

Lentillifera, based on the statement by Sidoretno and Anisa (2018), natural antioxidants are influenced by the composition of secondary metabolites they contain. One group of these secondary metabolites is polyphenols. The mechanism of phenolic compounds as antioxidants, according to Puspitasari et al. (2016), is through the ability of phenolic groups to scavenge oxygen and alkyl radicals by donating electrons, thereby forming relatively stable phenoxyl radicals. The total number of hydroxyl groups is a key factor influencing their antioxidant activity mechanism.

Analysis of antioxidant

Antioxidants are substances or molecules that can inhibit cell damage caused by free radicals. The test results of C. Lentillifera extract are expressed as a percentage of inhibition against DPPH radicals. This percentage is obtained from the difference in absorption between the DPPH absorbancy and the sample absorbancy. The antioxidant activity is indicated by the IC$_5$ o value, which is the concentration of the sample solution required to inhibit 50% of the DPPH free radicals. The IC$_5$ o values of the sea grape extract ( ) C. Lentillifera are presented at on Table 5. Based on Table 5, there are known differences in the IC$_5$ o or 50% inhibition concentration values of each sample extract. Sea grapes C. Lentillifera had the best IC$_5$ o value at 40°C oven treatment, which was 8774.20 ppm, followed by ex tracts dried at 30°C oven at 9593.64 ppm and extracts dried at 50°C oven at 10164.20 ppm. The IC$_5$o value of sea grape extract dried at 50°C was lower than the other two drying methods. The low antioxidant activity at higher temperatures is thought to be due to the antioxidant compounds being easily damaged or oxidized. Dewi (2017) states that antioxidant activity will decrease if the drying temperature is too high. This is because higher heating temperatures cause the secondary metabolites that act as antioxidants to be damaged. The IC$_5$ o value in the best C. Lentillifera sea grape extract study with oven drying at 40°C was above 200 ppm, so it can be classified as a weak antioxidant. According to Tristantini (2016), a compound is considered a very strong antioxidant if the IC50 value is less than 50, strong (50-100), moderate (100-150), and weak (151-200). The smaller the IC50 value, the higher the antioxidant activity (1)

Table 5. IC$_5$ values of C. Lentillifera sea grape extract

Drying Method	**Concentration (ppm)**	**Graphic Equation**	**IC$_5$ Value o**
Temperature 30°C	1000	y = 0.005x + 2.0318	9593.64
	2000		
	3000		
	4000		
	5000		
Temperature 40°C	1000	y = 0.0054x + 2.6193	8774.20
	2000		
	3000		
	4000		
	5000		
Temperature 50°C	1000	y = 0.0048x + 1.2118	10164.20
	2000		
	3000		
	4000		
	5000		

4. Conclusions

The conclusion that can be drawn from the study of the antioxidant activity of C. *Lentillifera* sea grape extract dried at different temperatures is that phytochemical compounds were found in *C. Lentillifera* sea grape extract dried at different temperatures, namely compounds Flavonoids, alkaloids, saponins, sterols + triterpenoids, tannins, and phenols. The difference in sample drying temperature on the extract of C. *lentillifera* sea grapes had a significant effect on the phenolic, flavonoid, and IC$_5$ o values. The highest total phenolic, flavonoid, and IC$_5$ o values were found in sea grape extracts dried at 40°C. The IC$_5$ o value of 8774.20 ppm is above 200 ppm, classifying it as a weak antioxidant. Therefore, further research is needed on the bioactive compounds of C. *lentillifera* sea grapes that have other functions, such as antibacterial properties.

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