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Antioxidant Activity of Ethanol Extract 96% Gelidium zollingeri with DPPH Method (2,2-diphenyl-1-picryllhydrazyl)

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Abstract

Gelidium zollingeri is a Rhodophyta seaweed that has compounds such as Mycosporine-like amino acids (MAAs) are believed to have a role as antioxidants. Antioxidants are chemical compounds that can inhibit or neutralize free radicals by complementing the electrons possessed by free radicals, thereby breaking the chain reaction of free radical formation. This research was conducted to determine the antioxidant activity of 96% ethanol extract of the red seaweed Gelidium zollingeri. Antioxidant activity testing was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The 96% ethanol extract of the red seaweed Gelidium zollingeri was tested for its antioxidant activity to obtain absorbance using a UV-Vis spectrophotometer at a wavelength of 517 nm. The absorbance results were calculated to obtain the IC50 value. The results of the antioxidant activity test of the 96% ethanol extract of the red seaweed Gelidium zollingeri using a UV-Vis spectrophotometer obtained an IC50 value of 39.2598 ppm, which means that the extract is included in the very strong antioxidant category, so that the red seaweed plant Gelidium zollingeri has the potential as an antioxidant.

Keywords: Antioxidant, DPPH, Gelidum zollingeri, 96% Ethanol Extract, IC50

Introduction

Many people's dietary habits have changed over time, becoming less healthy, and repeated exposure to dangerous chemicals can have an impact on health. Health-related factors include the balance of free radicals and antioxidants in the body. Free radicals are extremely reactive compounds with unpaired electrons (Izazi et al., 2024). Free radicals are useful to health in moderation, such as by killing bacteria and preventing inflammation, but in

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excess, they can induce oxidative stress (Izazi et al., 2024). This situation can cause cell damage, speeding up the aging process and the advent of illnesses (Izazi et al., 2024).

Antioxidants are substances that inhibit or prevent oxidation while also neutralizing free radicals (Izazi et al., 2022). In chemical language, antioxidants are substances that provide electrons. Antioxidants are separated into two groups according to their origin: synthetic antioxidants and natural antioxidants. Synthetic antioxidants at high concentrations in experimental animals can be harmful and carcinogenic (Jun et al., 2006). As a result, natural antioxidants must be used, specifically the red seaweed plant *Gelidium zollingeri.*

Amino acids, alkaloids, saponins, tannins, steroids, and terpenoids are among the secondary metabolites found in *Gelidium zollingeri*, a red seaweed. According to prior research, infrared spectroscopy experiments on Gelidium zollingeri powder revealed that it contains mycosporine-like amino acids (MAAs), which can be utilized as sunscreen (Santoso et al., 2017). Furthermore, the red seaweed *Gelidium zollingeri* has medicinal properties since it includes amino acids, phycobiliproteins, carotenoids, phenols, and fucoidan (Santoso et al., 2017).

Previous research on antioxidant activity in scrub body scrub made from red seaweed *Gelidium sp* extract using three different formulations showed antioxidant activity with IC50 values: formulation 1 at 393.96 µg/mL, formulation 2 at 555.47 µg/mL, formulation 3 at 225.17 µg/mL, and vitamin C (comparison) at 435.79 µg/mL (Sahidi et al., 2015). Previous studies on the antioxidant activity of *Gelidium sp* extract using methanol solvent revealed extremely low antioxidant activity, however n-hexane solvent revealed high antioxidant activity (Sopianti et al., 2022). *Gelidium zollingeri* and *Gelidium sp*. are Rhodophyta seaweeds with similar chemical concentration, so red seaweed *Gelidium zollingeri* is expected to have comparable antioxidant action.

Based on the aforementioned description, a study will be done to determine the antioxidant activity of a 96% ethanol extract of the red seaweed *Gelidium zollingeri* using the DPPH (2,2-diphenyl-1-picrylhydrazyl) technique.

Material and Methods

Tools

Erlenmeyer flask, beaker glass, glass funnel, stir rod, aluminum foil, volumetric flask, graded glass, watch glass, pipette, porcelain crucible, vortex, and UV-Vis spectrophotometer.

E-ISSN : 2715-0518
P-ISSN : 2715-3053

Materials

Extract of the red seaweed *Gelidium zollingeri* with 96% ethanol, distilled water, vitamin C, and DPPH.

Method

This work employed a laboratory experimental method to estimate the IC50 value of a 96% ethanol extract of the red seaweed Gelidium zollingeri.

Preparing test solutions:

1. Sample solution

The extract was weighed at 2 mg in 100 mL of 96% ethanol to make a 200 ppm stock solution. It was diluted into four concentrations: 50, 40, 30, and 10 ppm.

2. Vitamin C solution.

Vitamin C was weighed at 0.95 mg and mixed in 10 mL of ethanol to make a stock solution of 100 ppm. Testing was done at four different concentrations: 19; 14.25; 9.5; and 4.75 ppm.

3. DPPH solution.

1 milligram of DPPH was weighed and diluted in 10 mL of 96% ethanol solution, agitated until homogenous to produce a solution with a concentration of 100 ppm, and stored in a dark bottle.

Control Solution Measurement Test

The measurement was performed by pipetting 1 mL of DPPH solution and diluting it with ethanol to a total amount of 10 mL. It was shook until homogeneous, then kept at room temperature for 30 minutes. The absorbance was then measured using a UV-Vis spectrophotometer at wavelengths ranging from 400 nm to 800 nm, and the maximum wavelength of DPPH was determined to be 517 nm.

Antioxidant Activity Test - Sample Solution

Taken Each received 4 mL of a 96% ethanol extract of the red seaweed Gelidium zollingeri at concentrations of 50 ppm, 40 ppm, 30 ppm, and 10 ppm, as well as 1 mL of an ethanol-based DPPH solution, and was then thoroughly mixed. If the solution turns yellow, it means that the 96% ethanol extract of red seaweed Gelidium zollingeri at each concentration has free radical scavenging activity. The samples were then incubated for thirty minutes. Following that, the absorbance of each combined solution was measured with a UV-Vis spectrophotometer at 517 nm. The operation was repeated three times.

Vitamin C Solution

E-ISSN : 2715-0518
P-ISSN : 2715-3053

4 mL of Vitamin C at concentrations of 19 ppm, 14.25 ppm, 9.5 ppm, and 4.75 ppm were combined with 1 mL of DPPH solution in ethanol and stirred until homogenous. The samples were then incubated for thirty minutes. The absorbance of each combined solution was then measured using a UV-Vis spectrophotometer with a wavelength of 517 nm. The operation was repeated three times.

Data analysis

The antioxidant activity was assessed at a single point in time (cross-sectional study). The collected results were then entered into a calculation to determine the percentage inhibition as follows:

$$\frac{\text{(Control absorbance-sample absorbance)}}{\text{Control absorbance}} \times 100\%$$

The antioxidant activity against radicals is assessed by determining the IC50 value using a regression equation with the sample concentration on the x-axis and the percentage inhibition on the y-axis. The lower the IC50 value, the higher the antioxidant activity.

Results and Discussion

The antioxidant activity of a 96% ethanol extract of the red seaweed *Gelidium zollingeri* was measured in this work using the DPPH method. The DPPH method is simple and rapid, and its absorbance can be measured using a UV-Vis spectrophotometer (Susiloningrum et al., 2021). The absorbance of the DPPH control solution was measured using a UV-Vis spectrophotometer at 517 nm, yielding a control absorbance of 1.0809. **Table 1** shows the antioxidant activity test findings for a 96% ethanol extract of the red seaweed *Gelidium zollingeri* performed with a UV-Vis spectrophotometer at concentrations of 50 ppm, 40 ppm, 30 ppm, and 10 ppm. The findings of the antioxidant activity test of vitamin C using a UV-Vis spectrophotometer with concentrations of 19 ppm, 14.25 ppm, 9.5 ppm, and 4.75 ppm are shown in **Table 2.**

The data above reveal that the two solutions at different concentrations were duplicated three times, hence the average of these three replications must be calculated. The inhibition % was then calculated and shown in Tables 3 and 4. The percentage of inhibition of free radical scavenging is one of the criteria that reflect a substance's ability to inhibit free radicals, and it is proportional to the concentration of the examined material. The inhibition % demonstrates that the higher the concentration of the solution, the greater the inhibition percentage. This happens because the higher the concentration of the solution, the stronger the antioxidant activity in the test solution.

E-ISSN : 2715-0518
P-ISSN : 2715-3053

The percent inhibition data are entered into a regression equation with the sample concentration as the x-axis and the percentage inhibition as the y-axis. The IC50 value is obtained by performing regression calculations. The IC50 value represents the concentration of a substance that can block the oxidation process by 50% (Widyasanti et al., 2016). The lower the IC50 value, the greater the antioxidant activity. A compound's antioxidant power is termed very high if its IC50 value is less than 50 ppm, strong if it is between 50 and 100 ppm, moderate if it is between 100 and 150 ppm, and weak if it is between 150 and 200 ppm. If the IC50 is between 200 and 1,000 ppm, the sample is deemed less active yet still possesses antioxidant properties (Salim, 2018). Figures 1 and 2 show graphs of a 96% ethanol extract of the red seaweed *Gelidium zollingeri* vs vitamin C.

The antioxidant activity tests in this investigation revealed that the 96% ethanol extract of the red seaweed Gelidium zollingeri yielded a regression equation of y = -45.4094 + 25.9955x with a r value of 0.9943 and an IC50 value of 39.2598 ppm. The antioxidant activity test employed vitamin C as a reference. The results showed that vitamin C may scavenge DPPH free radicals and has very significant antioxidant activity. Based on the IC50 value, this is characterized as very strong antioxidant activity, with an IC50 value less than 50 ppm (Yuslianti et al., 2018). This suggests that the 96% ethanol extract of the red seaweed Gelidium zollingeri has high antioxidant activity. The regression equation for vitamin C was likewise determined as y = -31.8510 + 37.3207x, with a r value of 0.9919 and an IC50 value of 8.9639 ppm.

Conclusion

Based on the results obtained in this study, the antioxidant activity test on the 96% ethanol extract of the red seaweed *Gelidium zollingeri* revealed very strong antioxidant activity with an IC_{50} value of 39.26 ppm.

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Appendix.

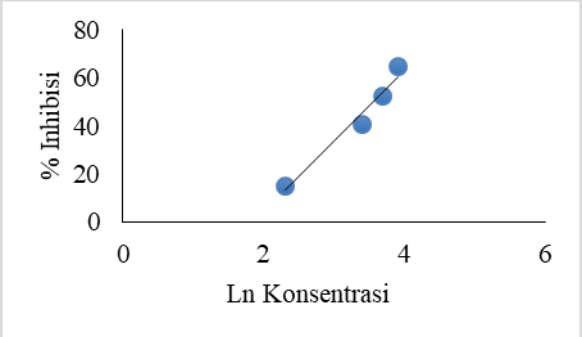

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Figure 1 IC_{50} of 96% Extract Ethanol of Red Seaweed *Gelidium zollingeri*

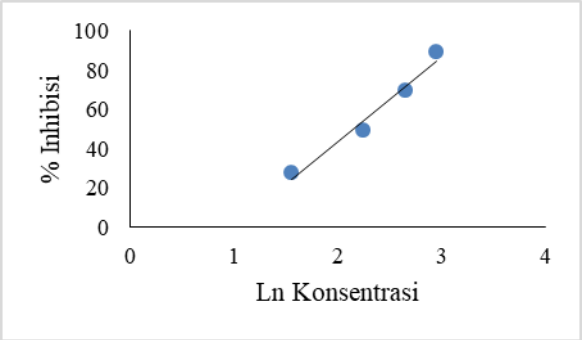

Figure 2 IC_{50} of Vitamin C

Tabel 1 Absorbance Results of Antioxidant Activity Test of 96% Ethanol Extract of Red Seaweed *Gelidium zollingeri* on a UV-Vis Spectrophotometer at a Wavelength of 517 nm

Concentration (ppm)	Replikasi			Mean
	1	2	3	
50	0,3879	0,3811	0,3752	0,3814
40	0,5223	0,5177	0,5086	0,5162
30	0,6389	0,6389	0,6424	0,6401
10	0,9270	0,9196	0,9126	0,9197

Tabel 2 Absorbance Results of Antioxidant Activity Test of Vitamin C on a UV-Vis Spectrophotometer at a Wavelength of 517 nm

Concentration (ppm)	Replikasi			Rata-rata
	1	2	3	
19	0,1048	0,1116	0,1259	0,1141
14,25	0,3236	0,3319	0,3425	0,3327
9,5	0,5443	0,5470	0,5582	0,5498
4,75	0,7840	0,7813	0,7881	0,7845

Tabel 3 % Inhibition Result of 96% Ethanol Extract of Red Seawed *Gelidium zollingeri*

Concentration (ppm)	Ln Concentration	Mean	% Inhibition
50	3,9120	0,3814	64,65
40	3,6889	0,5162	52,25
30	3,4012	0,6401	40,78
10	2,3026	0,9197	14,91

Tabel 4 % Inhibition Result of Vitamin C

Concentration (ppm)	Ln Concentration	Mean	% Inhibition
19	2,9444	0,1141	89,44
14,25	2,6568	0,3327	69,22
9,5	2,2513	0,5498	49,13
4,75	1,5581	0,7845	27,42

Tabel 5 IC_{50}Result of 96% Ethanol Extract of Red Seawed *Gelidium zollingeri*

Sampel	Linier Regression	IC_{50}
96%Ethanol Extract	y = -45,4094+ 25,9955x r = 0,9943	39,2598

Tabel 6 IC_{50} Result of Vitamin C

E-ISSN : 2715-0518
P-ISSN : 2715-3053

Sampel	Linier Regression	IC_{50}
Vitamin C	y = -31,8510 + 37,3207x r = 0,9919	8,9639

E-ISSN : 2715-0518
P-ISSN : 2715-3053