Antioxidant Activity of Ethanolic Extract of Green Algae (*Ulva Lactuca* Linn.) From Sepanjang Beach Gunung Kidul with DPPH Method

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ABSTRACT

Background: Cancer is one of the health problems in the world with the number of sufferers that continues to increase. Based on IARC (International Agencies for Research on Cancer) during 2008 there were 12.7 million cancer cases and 7.6 million cancer deaths. The role of free radicals against damage to human body tissue is known to be a fairly high causative factor. If the number of free radicals increased, the body's defense system against free radicals becomes inadequate. One of the plants that functions as an antioxidant and also as an anti-cancer originates from marine algae, it is green algae (Ulva lactuca L.). Green algae showed the presence of antioxidant compounds that were analyzed qualitatively using thin-layer and quantitative chromatography with DPPH using a UV-VIS spectrophotometer. In the liver of CCl4-induced mice, ethanol extracts of green algae have antioxidant activity by reducing levels of malonylaldehyde (MDA) and increasing the activity of the enzyme superoxid dismutase (SOD).

Objective: Conduct Antioxidant Activity Tests on the ethanolic extracts of green algae (*Ulva lactuca* Linn) obtained from Sepanjang Beach, Gunung Kidul

Method: Antioxidant Activity Test was carried out by DPPH (1,1diphenyl-2-picryl hydrazyl) method

Results: IC_{50} value of Ethanolic Extracts of Green Algae is 17,25 μg/ml and Ascorbic acid is 28,9 μg/ml

Conclusions: Antioxidant activity of the ethanolic extract of green algae is better compared to Ascorbic acid

Keywords: *Green Algae, Antioxidant Activity*

INTRODUCTION

The role of free radicals against damage to human body tissue is known to be a fairly high causative factor. If the number of free radicals increases, the body's defense system against free radicals becomes inadequate (1). There are many types of free radicals, including hydroxyl radicals (-OH), superoxide anions (O2), singlet oxygen and hydrogen peroxyde (H2O2). Free radicals that accumulate in cells cause several pathological reactions such as myocardial infarction, atherosclerosis, rheumatoid arthritis, neurodegenrative disorders, and cancer (2). Cancer is one of the health problems in the world with the number of sufferers that continues to increase. Based on IARC (International Agencies for Research on Cancer) during 2008 there were 12.7 million cancer cases and 7.6 million cancer deaths (3). One of the plant that functions as an antioxidant and also an anti-cancer originates from marine algae, it is green algae (*Ulva lactuca* L.) which is a type of sea lettuce with the species of the genus "ulva". Research conducted by Febriansah et al (4) showed the presence of antioxidant compounds that were analyzed qualitatively using thin-layer and quantitative chromatography with DPPH using a UV-VIS spectrophotometer. In the liver of CCl4-induced mice, ethanolic extracts of green algae have antioxidant activity by reducing levels of malonylaldehyde (MDA) and increasing the activity of the enzyme superoxid dismutase (SOD) (5).

MATERIALS AND METHODS

This research is an experimental research. The material used in this study is green algae from the Special Region of Yogyakarta. The compound 2,2-diphenyl-1- picrylhidrazil (DPPH), a reagent for preliminary tests. The tools used are maceration vessel, rotary evaporator, buchner funnel, electric balance, porcelain cup, separating funnel, filter paper,, oven, flakon, glassware, UV-Vis spectrophotometer.

1. Extraction of Green Algae (*Ulva lactuca Linn*.)

   Extraction of Green Algae with Maceration. 250 gram of green algae powder was added ethanol 96% to the dissertation with Stirring for 3 hours. Maserat is filtered using a Buchner funnel and the maceration process is repeated 2 times. Then the Maserat was evaporated using a rotary evaporator at 40^0C until a thick extract was obtained and the yield was calculated (5).

2. Preliminary Test

   a. Preliminary Test With DPPH method

      As much as 1,0 ml of the sample solution was reacted with 1.0 ml of a 0.15 mM DPPH solution. Color changes occur from purple to yellow (6)

   b. Phytochemical Screening

      Identification of chemical contents in extracts was carried out on alkaloids, flavonoids, steroids, triterpenoids

3. Antioxidant Activity Test

   The antioxidant activity test was carried out by the DPPH method (7). One ml of a 0.3 mM DPPH methanol solution is added to 1 ml of the extract (1000 μg / ml) and allowed to stand at room temperature. After 30 minutes the absorbance value was measured at a wavelength of 517 nm. Methanol solution was used as a blank and DPPH solution as a negative control. Ascorbic acid (1000 μg / ml) as a positive control. The ability to scavenge radical DPPH is calculated :

$$\% \ inhibition = \frac{A.control - A test}{A Control} x100\%$$

RESULTS AND DISCUSSION

1. Preliminary Test

   In the preliminary test using DPPH obtained the results of ethanolic extract of green algae containing compounds that are as antioxidants. This is indicated by the change in DPPH solution from purple to yellow after adding the extract solution. The color change of this solution occurs when DPPH radicals are scavenging by antioxidant compounds that release hydrogen atoms to scavenge stable DPPH (8).

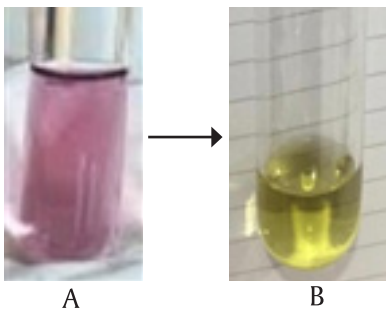

Figure 1. Preliminary tes with dpph (a) before and (b) after added ethanolic extract of green algae (*ulva lactuca* linn.)

From the results of phytochemical tests, ethanolic extracts of green algae showed positive presence of alkaloids and saponins.

While flavonoids, terpenoids, steroids were not detected.

Table 1. Phytochemical screening ethanolic extract of green algae (*ulva lactuca* linn.)

Test	Result
Alkaloid (pereaksi mayer)	+
Alkaloid (pereaksi dragendorf)	+
Flavonoid	-
Saponin	+
Steroid	-
Terpenoid	-

Ket : (+) Terdeteksi , (-) tidak terdeteksi

2. Antioxidant Activity of Ethanolic Extract Green Algae (*Ulva lactuca* Linn) with DPPH method

In this study, The method used to determine the antioxidant activity of ethanolic extracts of green algae (*Ulva lactuca* Linn.) is DPPH (2,2 Diphenyl-1 picrylhydrazil) radical scavenging In this study was the method of scavenging DPPH free radicals (2,2 Diphenyl-1 picrylhydrazil). This method was chosen because it is fast, simple and easy and does not require a large fee (9). The results of the antioxidant activity test of green algae extract compared to the standard (Ascorbic acid) can be seen in table 2 and figure 2. To get the amount of inhibition concentration (IC_{50}) from the extract or standard (Ascorbic acid), the percentage inhibition was calculated first. Percentage of inhibition shows how much the ability of a substance to free radicals scavenging. In table 2 it can be seen that the ability to free radicals scavenging will be greater along with the increasing concentration of both ascorbic acid or ethanolic extracts of green algae. This is in accordance with the pharmacodynamic principle where the dose / concentration is directly proportional to the response of the drug (10).

The IC_{50} value of the ethanolic extract of green algae is 17.25 μg / ml and the standard (ascorbic acid) is 28.9 μg / ml. Antioxidant activity is strong if the IC_{50} value is less than 200 μg / ml, less active if the IC_{50} is 200-1000 μg / ml, and very weak if the IC_{50} value is more than 1000 μg / ml (11). Based on these results, both green algae and standard ethanolic extracts have very strong antioxidant activity. But Ethanolic Extract of Green algae is better than Ascorbic acid. This result is also supported by a statistical test using a different independent sample T-test obtained a significance value of 0.042 which means that there is a significant difference in the IC_{50} value of ethanolic extracts of green algae compared with the standard.

The ability to scavenge free radical activity from the ethanolic extracts of green algae is associated with compounds contained in this plant. One of them is melatonin which is an alkaloid. Based on research conducted by Rodriguez et al (11) melatonin can affect antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase and also affect cellular mRNA levels. In addition, ethanolic extracts of green algae may have phenolic hydroxyl groups that can scavenge free radicals such as hydroxyl radicals (6).

Tabel 2. Free radical scavenging activity of ethanolic extract of green algae (ulva lactuca linn.) and standard (ascrobic acid)

Standard (Ascorbic acid)				Ethanolic Extract of Green Algae (*Ulva lactuca* Linn)			
No	Concentration (μg/ml)	Percentage of Inhibition (%)	IC_{50}	No	Concentration (μg/ml)	Percentage of Inhibition (%)	IC_{50}
1	4	17,88	28,9 μg/ml	1	4	29,52	17,25 μg/ml
2	8	20,89		2	8	32,22	
3	12	26,3		3	12	45,43	
4	16	31,19		4	16	48,96	
5	20	39,71		5	20	52,59	

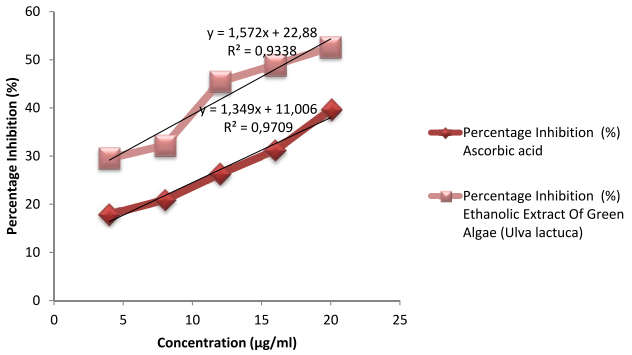

Fig. 2. Graphic of Percentage Inhibition Ethanolic Extract of Green Algae (Ulva lactuca Linn) compare with standard (Ascorbic acid)

CONCLUSION AND RECOMMENDATION

Ethanolic Extract of Ganggang Hijau (*Ulva lactuca* Linn.) from Sepanjang Beach Gunung Kidul Has ability DPPH free radical scavenging better than standard (Ascorbic acid) with IC_{50} values respectively 17,25 µg/ml and 28,9 µg/ml

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