Potential Of Seaweed Padina Sp. As A Source Of Antioxidant

Beni Setha, Febe F. Gaspersz, Andi Puspa S. Idris, Samsul Rahman, Meigy Nelce Mailoa

Abstract : The aim of the research was to study the antioxidant activity of crude extract of seaweed *Padina* sp. The process of extraction of seaweed *Padina* sp. was done by using maceration stages namely the solvent n-hexane, and then using solvent extraction followed by ethyl acetate and methanol. Solvents that are still left in the third crude extract was evaporated by using a rotary vacuum evaporator, and then tested for DPPH free radical activity (1,1-diphenyl-2-picrylhydrazyl) by UV-VIS spectrophotometer at a wave length of 517 nm. Measurement of antioxidant activity of crude extract of seaweed *Padina* sp. performed at a concentration of 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm. Data from tested for DPPH free radical were analyzed using linear regression, than followed by determination of IC_{50}. The test results of all three antioxidant activity of crude extract of seaweed *Padina* sp. indicated that the higher concentration of solvent, the higher the percentage of arrests of radical activity or percentage inhibition. The antioxidant activity of methanol crude extract of seaweed *Padina* sp. to give IC_{50} value of 200.88 mg/L, crude extract of ethyl acetate to give IC_{50} value of 483.09 mg/L and extract crude n-hexane gave IC_{50} value of 900.00 mg/L. This means that the antioxidant activity of methanol crude extract of seaweed *Padina* sp. was stronger than that of crude extract of ethyl acetate and n-hexane.

Indeks Terms : sea grass, *Padina* sp., antioxidant

1. INTRODUCTION

Free radicals are atoms or molecules that are unstable and highly reactive because they contain one or more unpaired electrons in its outer orbital. To achieve the stability of atoms or molecules, free radicals will react with surrounding molecules to obtain electron pair. The reaction took place continuously in the human body and if not stopped will lead to various diseases such as cancer, heart disease, cataracts, premature aging and other degenerative diseases [1, 2, 3]. Therefore, the human body requires an antioxidant that can capture these free radicals so as not to induce disease [4]. Antioxidants are compounds that can neutralize or reduce free radicals and inhibit the occurrence of oxidation in body cells that can prevent or reduce the occurrence of cell damage [5]. The human body does not have excessive amounts of antioxidant reserve, thus requiring exogenous antioxidant, which is a natural antioxidant derived from fruits and vegetables [6, 7]. Seaweed is one of fishery resources that have important economic value to humans. Several years ago, the plant is only used as human food that is as a vegetable and medicine but in line with advances in science and technology, utilization of seaweed has expanded to various fields, including agriculture, animal husbandry, pharmaceutical, and medical.

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Recently, the study of seaweed as a main incredent to produce bioenergy as bioethanol which using as a substitute for crude oil inventories are running low. In Maluku waters there are some species of seaweed that some important economic value such as *Eucheuma, Gracilaria, Gellidium, Sargassum* and *Hypnea. Gracilaria* and *Gelidium,* both known as a producer for agar or better known as the group agarofit, while *Eucheuma* and *Hypnea* is a producer of carrageenan and *Sargassum* as a producer of alginate. In addition to the species of economically valuable seaweeds, there are many species of seaweed that has been used by communities for generations as both a vegetable and medicine, one of which is to *Padina* sp. Unlike other brown seaweeds such as *Sargassum* which has been widely explored as a potential source of alginate, *Padina* sp. not been widely explored as a abundant source of alginate. Though the population of *Padina* sp. also quite abundant and widespread in Maluku waters. Efficacy of biological and chemical compounds contained in alginate brownn seaweed, including *Padina* sp. can be used as an antibacterial, anticancer, antioxidant and in overcoming the gland disorder and decreasing high blood pressure. Therefore, the potential magnitude of this local species of seaweed to be developed as an ingredient of processed food and medicines will require a study on increasing value added through processing development and exploration of active ingredient to determine the efficacy or other uses as well as food. Exploration and utilization of bioactive contained in local species of seaweed in the waters of Maluku is still very limited especially important source of bioactive that can act as antioxidants. The objective of the research was to study the antioxidant activity of crude extract of seaweed *Padina* sp. The study is expected to provide information about the potential of seaweed extract Padina sp. to be used as an antioxidant as well as reference material for subsequent studies.

2. MATERIALS AND METHOD

Material

Materials used in this study was seaweed *Padina* sp. collected from the waters of Hative Besar, Ambon, Maluku, Indonesia. Chemicals used in this study include methanol, n-hexane, ethyl acetate and several other chemicals.

Equipment

Equipment used in research to explore the bioactive substances and antioxidants such as seaweed analytical balance, Kjeltech, Soxhlet, rotary vacuum evaporator, a set of glass tools, magnetic stirer, vortev, UV-VIS spectrophotometer and GC-MS.

Research Methods

Sample Preparation

Seaweed *Padina* sp. harvested from the substrate and then taken in to the laboratory of Fisheries Technology, Faculty of Fisheries and Marine Sciences, University of Pattimura, Ambon, Maluku, Indonesia. Furthermore, wind dried seaweed and packed in plastic bags and ready to be extracted bioactive compounds.

Research Procedure

The dried Seaweed *Padina* sp. washed again with clean water, then extraction by maceration. Extraction process was carried out in stages, namely: as much as 50 grams of seaweed that have been mashed macerated with n-hexane of 250 ml for 24 hours. After that, filtered, the filtrate was evaporated using a rotary vacuum evaporator to obtain viscous extract (hexane extract) and stored in bottles. Residues produced wind dried, then macerated using 250 ml ethyl acetate for 24 hours. After that, filtered, the filtrate was evaporated using a rotary vacuum evaporator to obtain viscous extract (ethyl acetate extract) and stored in a bottle. Residues produced wind dried and subsequently re-macerated with 250 ml methanol for 24 hours. After that, filtered, the filtrate evaporated using rotary vacuum evaporator to obtain viscous extract (methanol extract) and stored in a bottle. Third crude seaweed extracts obtained by test activities of free radical DPPH (1,1-diphenyl-2-picrylhydrazyl), [8] with procedure as follows : as much as 0,1 M solution of DPPH in ethanol was prepared, then 2 ml of solution were added to 0.1 ml of seaweed extract. The level of reduction in color of the solution showed the efficiency of radical capture. Five minutes from the last few minutes, absorbance was measured at a wavelength of 517 nm. Measurement antioxidant activity of crude extract of seaweed *Padina* sp. performed at a concentration of 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm. Activity scavenger of free radicals is calculated as a percentage reduction in the color of DPPH with the equation:

Activity scavenger of free radicals =

$$100 \times (1 - \text{abs. samples} / \text{abs. reference})$$

Data Analysis

Observational data capture activity assay of free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) were analyzed using linear regression line equation Y = a + bx [9]. This linear equation showed that the higher the concentration of solvent the higher the percentage of arrests of radical activity or percentage inhibition. Based on the regression line equation is obtained, determine the value of inhibition concentration (IC_{50}) was the concentration of antioxidants that can causes 50% loss of DPPH radical character or concentration of an antioxidant substance that gives percent inhibition by 50%. Determination of IC_{50} was enter the response values (Y) from the regression equation, which is 50, then specify the value of x from the regression equation. The value of x is obtained from the calculation is the value of IC_{50}.

Time and Research Location

The research was carried out from June to October 2010. The process of extraction of bioactive compounds from the seaweed *Padina* sp. performed at the Laboratory of Fisheries Technology, Faculty of Fisheries and Marine Sciences, University of Pattimura, Ambon, Maluku, Indonesia. Separation of bioactive compounds from seaweed extract *Padina* sp. using GC-MS performed at the Laboratory of Instrumentation, Faculty of Science, UGM, Yogyakarta, Indonesia. Testing capture activity of free radicals DPPH (1,1-diphenyl-2-picrylhydrazyl) was in the laboratory of Fisheries Technology, Faculty of Fisheries and Marine Sciences, University of Pattimura, Ambon, Maluku, Indonesia.

3. RESULTS AND DISCUSSION

Antioxidant Activity of Hexane Extract of Seaweed *Padina* sp.

Test result data capture activity of free radicals DPPH hexane extract of seaweed *Padina* sp. obtained in this study could be seen in Table 1.

Table1. Data Analysis of DPPH Hexane Extract of Seaweed *Padina* sp.

Concentration	Replication		Total	Average
	I	II		
50	23.49	23,01	46,50	23,25
100	23.96	24.04	48.00	24.00
150	25.16	25.96	51.12	25.56
200	28.11	28.34	56.45	28.23
250	29.14	29.30	58.44	29.22

Based on data obtained in Table 1, linear regression analysis showed that the line equation Y = 21.20 + 0.032 x (R^2 = 0.965). From the regression equation, the IC_{50} values obtained for the hexane extract of seaweed *Padina* sp. amounted to 900.00 mg/L respectively (Figure 1).

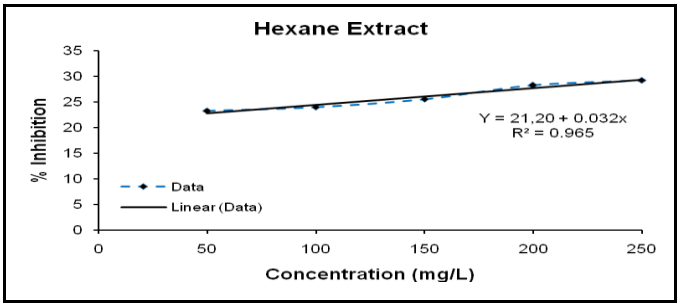

Figure 1. Antioxidant Activity Hexane Extract of Seaweed *Padina* sp.

Regression analysis showed that the higher the concentration of hexane extract of seaweed *Padina* sp., the higher the percentage of arrests of radical activity or percentage inhibition. This is due to the higher the concentration of hexane extract of seaweed *Padina* sp., the higher the antioxidant content. From the regression equation obtained, explaining that the arrest of free radical activity or percentage inhibition of free radicals increased by 0.032% with the increase of single unit concentration of hexane extract of seaweed *Padina* sp. Tested antioxidant activity using DPPH method at the hexane extract of seaweed *Padina* sp. was obtained IC_{50} value of 900.00 mg/L respectively. The smaller the IC_{50} value the higher the antioxidant activity (Blois *in* [10]). Specifically, a compound said to be a very powerful antioxidant if the value of EC_{50} or IC_{50} less than 50, strong antioxidant if the value of EC_{50} or IC_{50} of 50 -100, moderate antioxidant if the value of EC_{50} or IC_{50} of 100-150 and weak antioxidant if the value of EC_{50} or IC_{50} of 151-200. Based on IC_{50} values obtained, the hexane extract of seaweed *Padina* sp. have antioxidant activity as very weak. This is due to the hexane extract of seaweed *Padina* sp. not a pure compound, but still contains other compounds that probably may not have antioxidant activity.

Antioxidant activity of Ethyl Acetate Extracts of Seaweed *Padina* sp.

Test result data capture activity of free radicals DPPH ethyl acetate extract of seaweed *Padina* sp. obtained in this study could be seen in Table 2.

Table 2. Data Analysis of DPPH Ethyl Acetate Extract of Seaweed *Padina* sp.

Concentration	Replication		Total	Average
	I	II		
50	25.16	26.75	51.91	25.96
100	28.98	30.02	59.00	29.50
150	31.13	31.69	62.82	31.41
200	34.47	35.03	69.50	34.75
250	37.90	36.54	74.44	37.22

Based on data obtained in Table 2, linear regression analysis indicated that the line equation Y = 23.43 + 0.055 x (R^2 = 0.993). From the regression equation, the IC_{50} values obtained for the ethyl acetate extract of seaweed *Padina* sp. amounted to 483.09 mg/L respectively (Figure 2).

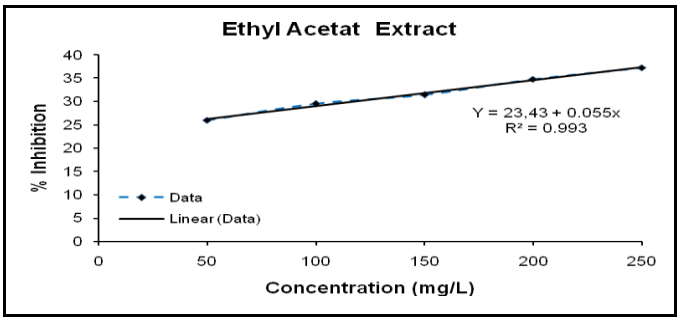

Figure 2. Antioxidant Activity Ethyl Acetate Extract of Seaweed *Padina* sp.

Regression analysis showed that the higher concentration of ethyl acetate extract of seaweed *Padina* sp., the higher the percentage of arrests of radical activity or percentage inhibition. This is due to the higher the concentration of ethyl acetate extract of seaweed *Padina* sp., the higher the antioxidant content. From the regression equation obtained, explaining that the arrest of free radical activity or percentage inhibition of free radicals increased by 0.055% with the increase in single unit concentration of ethyl acetate extract of seaweed *Padina* sp. Tested antioxidant activity using DPPH method in ethyl acetate extract of seaweed *Padina* sp. was obtained IC_{50} value of 483.09 mg/L respectively. The IC_{50} values ethyl acetate extract of seaweed *Padina* sp. was smaller than that of IC_{50} values hexane. It showed that the antioxidant activity of ethyl acetate extract of seaweed *Padina* sp. was strong than the hexane extract with antioxidant activity.

Antioxidant Activity of Methanol Extract of Seaweed *Padina* sp.

Test result data capture activity of free radicals DPPH methanol extract of seaweed *Padina* sp. obtained in this study could be seen in Table 3.

Table 3. Data Analysis of DPPH Methanol Extract of Seaweed *Padina* sp.

Concentration	Replication		Total	Average
	I	II		
50	37.10	37.90	75.00	37.50
100	41.88	41.08	82.96	41.48
150	49.04	45.86	94.90	47.45
200	51.04	50.64	101.68	50.84
250	53.03	53.03	106.06	53.03

Based on data obtained in Table 3, linear regression analysis showed that the line equation Y = 33.93 + 0.080 x (R^2 = 0.974). From the regression equation, the IC_{50} values obtained for the methanol extract of seaweed *Padina* sp. amounted to 200.88 mg/L respectively (Figure 3).

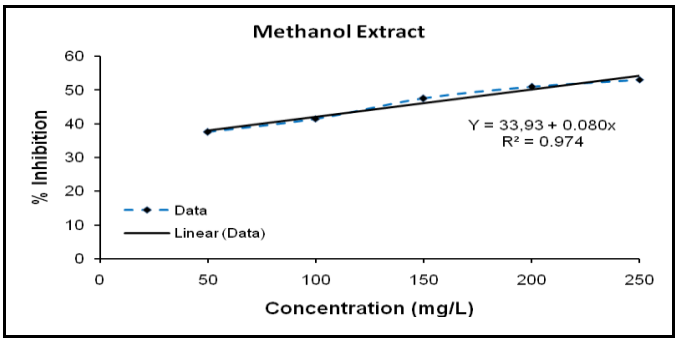

Figure 2. Antioxidant Activity Methanol Extract of Seaweed *Padina* sp.

Regression analysis showed that the higher concentration of methanol extract of seaweed *Padina* sp., the higher the percentage of arrests of radical activity or percentage inhibition. This is due to the higher concentration of methanol extract of seaweed *Padina* sp., the higher the antioxidant content. From the regression equation obtained, explaining that the arrest of free radical activity or inhibition of free radicals increased by 0.080% with the increase of single unit concentration of the methanol extract of seaweed *Padina* sp. From the linear regression equation obtained, tested antioxidant activity using DPPH method in methanol extracts of seaweed *Padina* sp. was obtained IC_{50} value of 200.88 mg/L repectively. The IC_{50} value of methanol extract of seaweed *Padina* sp. was smaller than that of IC_{50} value of hexane and ethyl acetate. It showed that the antioxidant activity of methanol extract of seaweed *Padina* sp. was strong compared to the antioxidant activity of hexane and ethyl acetate. Antioxidant extracted with ethanol and methanol from rosemary and sage have the best activity [11].

4. CONCLUSION

Based on the results of this study, it could be concluded that the value of IC_{50} crude hexane extract of seaweed *Padina* sp. (900,00 mg/L) was higher compared to IC_{50} values of ethyl acetate extract (483.09 mg/L) and methanol (200.88 mg/L). This means that the antioxidant activity of methanol extract of seaweed *Padina* sp. more powerful than that of hexane and ethyl acetate. Nevertheles, overall antioxidant activities of three extracts of hexane, ethyl acetate and methanol of seaweed *Padina* sp. were relatively weak.

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