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Determination of the Pigment Content and Antioxidant Activity of the Marine Microalga *Tetraselmis suecica*

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Abstract

Marine microalgae or phytoplankton are marine microorganisms that act as producers. In addition to their important role in the sea, microalgae also have potential application in medicine and can be a good source of food. This study aims to determine the content of chlorophyll a and chlorophyll b pigments, carotenoids as well as antioxidant activity in samples of *Tetraselmis suecica* marine microalga using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) method. The research samples were obtained from the Natural Feed Laboratory of the Brackish Water Aquaculture Center (BBPBAP), Jepara. The study included the following stages: sample preparation, sample extraction, and measurements of pigment content and antioxidant activity using DPPH method. The results showed that acetone extract of marine microalga *Tetraselmis suecica* contained 1.688 mg/g of chlorophyll a, 0.713 mg/g of chlorophyll b, and 5.380 µg/g of carotenoid, as well as an IC_{50} value of 37.320 ppm. It was concluded that the antioxidant activity of *the Tetraselmis suecica* was very strong, i.e. the IC_{50} value found was less than 50 ppm.

Keywords: Pigments, Antioxidants, *Tetraselmis suecica*, DPPH

1. Introduction

Bioactive compounds are found in the body of animals and plants. This compound has various important roles in the human body as an antioxidant, antibacterial, anti-inflammatory and anticancer agent. Various studies on bioactive compounds have been carried out for human health (Lordan *et al*., 2011; Cikos *et al*., 2018).

Antioxidants are defined as compounds that can inhibit and prevent lipid oxidation processes by preventing the formation of free radical reactions (peroxides) (Kikuzaki *et al*., 2002). Antioxidants can inhibit and prevent the oxidation process. They are very beneficial for health and play an important role in maintaining the quality of food products. Antioxidant benefits for health can be seen from its contribution in preventing cancer and tumors, vasoconstriction, and premature aging. The presence of antioxidants in food products prevents the oxidation process that can cause damage, such as rancidity, color and aroma changes, and other physical damage (Firdiyani, 2015).

Today, the most commonly used antioxidants are synthetic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), tertbutyl-hydroquinone (TBHQ) dan propyl gallate (PG). Various studies on BHA and BHT found that prolonged use of these synthetic compounds can cause tumors in animals. The use of this type of antioxidant can be very dangerous, so it is necessary to find a source of natural antioxidants that are relatively safer. Natural antioxidants can be found in land and sea. These antioxidants are naturally occurring in many plants and animals and can be developed as potential alternatives for synthetic antioxidants (Vadlapudi *et al*., 2012).

Microalgae shows promising potential to produce various important biochemical compounds for food, medical treatment, research, and other uses. Also, there are still many important chemical compounds that need to be studied in microalgae. This marine species has the potential to become a natural resource of compounds that can be used as food ingredients and improve the nutrition of food consumed by humans and animals. Previous research has been carried out on algal biomass as a source of protein and systematically uses it for active components and drugs (Raja *et al*., 2008).

A study by Maligan *et al*. (2015) found that acetone extract from *Tetraselmis* sp. contains 21.73 mg / g of total chlorophyll, and antioxidant activity of 21.04 ppm; another study by Abdilah *et al.* (2014) found that the total chlorophyll content and antioxidant of acetone extract of the same species of microalgae were 10.603 mg/g and 27.59 ppm respectively.

Green microalgae is rich in chlorophyll and carotenoids. The pigment is part of the compound that acts as a neutralizing free radicals. Based on these facts, the study aims to determine and measure pigment and antioxidant compounds in *Tetraselmis suecica*.

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2. Materials and Methods

2.1. Sample preparation

Tetraselmis suecica microalga was obtained by culture from the Jepara Brackish Water Research Center. The samples were centrifuged for 10 minutes at a speed of 3000 rpm and then separated from sea water. Then, they were dehydrated at room temperature for 24 hours.

2.2. Assesment of carotenoid and chlorophyll content

0.1 gram of dry sample was extracted with 10 mL of 80% acetone. The filtrate obtained were placed in cuvettes. The content of chlorophyll and carotenoids were measured by a spectrophotometer, at 480 nm, 645 nm, and 663 nm of wavelengths. The absorbance value, carotenoid content, and chlorophyll content were analyzed using the method used in Dere (1998) and Harborne (1984).

2.3. Assesment of antioxidant activity with DPPH method

Crude extract was extracted using technical acetone solvents, with a ratio of 1: 5 to samples and solvents. Maceration was carried out for 24 hours. The results of maceration were filtered using filter paper to separate the filtrate and residue. Extraction with acetone solvent was carried out to obtain a crude extract of microalga, which would be used in the antioxidant test using DPPH. The filtrate from the extraction with acetone solvent was then concentrated using 40^{o}C evaporator rotatory equipment to obtain a crude extract of the sample.

DPPH 0.1 mM solution was made by dissolving DPPH crystals in 100 mL of 95% methanol solvent. Extract samples were grouped into 4 concentration series of 0, 25, 50, 100 and 200 ppm. 4 mL of extract samples from each concentration were added with 1 ml DPPH, incubated for 30 minutes in a dark room. A blank solution was made by dissolving 1 mL of DPPH solution in 4 mL of methanol solution. The absorbance rate is measured at a wavelength of 517 nm using Shimadzu UV1800 spectrophotometer. The measurement of each sample was repeated three times (Badrinath *et al*., 2010).

2.4. Assesment of inhibition concentration (IC50)

Inhibition concentration (IC_{50}) is calculated by the linear regression equation obtained from the correlation between extract concentration and inhibition percentage. The linear regression equation obtained is y = bx + a, whereas x is Inhibition Concentration 50% (IC_{50}). By entering y = 50 into the simple linear regression equation, the value of x can be obtained (Badrinath *et al*., 2010).

3. Results

3.1. Assesment of chlorophyll and carotenoid contents in Tetraselmis suecica

The test results of the chlorophyll and carotenoid pigments in *Tetraselmis suecica* showed that the chlorophyll a content was higher (1.688 mg / g ± 0.266) than the chlorophyll b content (0.713 mg / g ± 0.335). The carotenoid content appeared to be of the highest percentage (5.38 µg / g ± 0.042) compared to that of chlorophyll a and chlorophyll b, as seen in the spectra pattern and absorbance value (Figure 1) and the results of measurement of pigment contents (Table 1).

Table 1. Assesmentof chlorophyll and carotenoid pigments content of *Tetraselmissuecica* acetone extract (n = 3 replications)

No.	Pigment	Content
1.	Chlorophyll a	1.688 mg/g ± 0.266
2.	Chlorophyll b	0.713 mg/g ± 0.335
3.	Carotenoid	5.38 µg/g ± 0.042

Assesment of spectral pattern and absorbance value of *Tetraselmis suecica* extract with acetone solvent showed that it peaked at the approximation of 400 - 450 nm, with lower peaks at absorbance 584.5 nm, 617.5 nm and 662.5 nm.

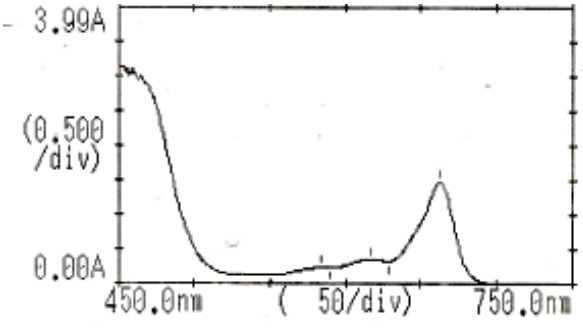

Figure 1. Spectral pattern of chlorophyll and carotenoids of *Tetraselmis suecica* acetone extract

3.2. Extraction yield

By filtering 3 liters of *Tetraselmis suecica* culture, 30 grams of dry biomass was obtained, which was then extracted with 300mL of acetone solvent. This process resulted in 1.9 grams (6.33%) of extract. The resulting extract was in the form of deep green paste.

3.3. Antioxidant activity test

The antioxidant activity of *Tetraselmis suecica* extract was determined based on the percentage of inhibition and DPPH value used. Data obtained from the results of antioxidant activity test was analyzed using a linear regression test. The result of the analysis was a linear regression equation used to calculate the IC_{50} value obtained. The results of the calculation of the percentage of inhibition and inhibition of the concentration of IC_{50} extract of *Tetraselmis suecica* were IC_{50} 38.26 ppm, as seen in Table 2 and Figure 2.

Table 2. Inhibition percentage and IC_{50} of *Tetraselmis suecia* acetone extract.

Concentration (ppm)	Mean of Inhibition Percentage	IC_{50} (ppm)
0	0	
25	62.771	
50	76.046	38.26
100	83.405	
200	88.745	

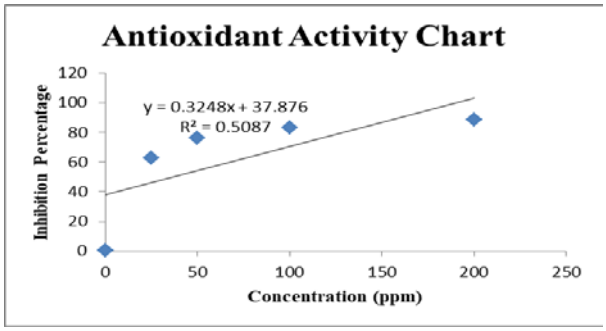

Figure 2. Regression test result chart.

4. Discussions

4.1. Assesment of chlorophyll and carotenoids

Measurement of pigment content in this study produced chlorophyll a value of 1.6885 mg/g and chlorophyll b value of 0.7139 mg/g. Previous research conducted by Ginting (2018) measured the values of chlorophyll a and chlorophyll b at 48 µg/mL and 40.88 µg/mL respectively, and Sani *et al*. (2014) measured of total chlorophyll between 3.65-19.20 mg/g. According to Harborne (1984), the spectral characteristics of chlorophyll pigments show major peak around 400 nm, a number of small peaks at 500 - 600 nm and other major peaks between 630 - 688 nm.

Chlorophyll measurement is one of the initial tests in determining antioxidant activity. Chlorophyll is a compound with free electrons structure from nitrogen atoms. The principle of free radicals is compounds that lack electrons. To complement the lack of electrons, free radicals will take electrons from human cells, which can eventually trigger cancer. Chlorophyll acts as an antioxidant by donating free electrons to free radicals, which stabilize the free radical structure (Firdiyani, 2015). Chlorophyll is an important antioxidant in food. The results of the research of Hsu *et al*. (2013) proved that chlorophyll and its derivatives (pheophytins) can prevent DNA damage with the same mechanism of antioxidant action against radical DPPH. Chlorophyll have the role of increased antioxidant activity.

This puts carotenoids as the prominent antioxidant agents in plants. By calculating the absorbance value of *Tetraselmis suecica* extract, a carotenoid value of 5.38 µg / g was obtained. Previous studies conducted on *Tetraselmis chuii* measured its carotenoid value at 6.70 µg / mL (Ginting, 2018). Carotenoids neutralize free radicals in three ways, namely electron transfer, hydrogen abstraction and the addition of radical species (Martinez *et al*., 2010). The process produces a carotenoid molecule that is radical, after which the electrons will be relocated so that it is spread throughout the carotenoid structure. This way, radical compounds can be stabilized in a short time by releasing heat (Dimara and Yenusi, 2011).

4.2. Antioxidant activity test

Antioxidants are compounds that can delay, inhibit and prevent molecular oxidation by preventing the formation of free radicals (Rohman, 2016). One test of antioxidant activity in a sample is by using a measurement of sample solution concentration which can reduce DPPH activity by 50% or IC_{50}. The IC_{50} value is derived from linear regression equation for the correlation of extract concentration to the value of DPPH inhibition. DPPH is a free radical that can react to compounds that can donate hydrogen atoms, which can be useful for testing the antioxidant activity of certain components in extracts. IC_{50} denotes the concentration needed by the sample to reduce DPPH by 50%, the smaller the IC_{50} value the higher the value of its antioxidant activity (Badrinath *et al*., 2010).

Antioxidant compounds will react with DPPH free radicals through the mechanism of donating hydrogen atoms and causing DPPH color to shift from purple to pale yellow, which has been measured with a wavelength of 517 nm (Rohman, 2016). The Lambert-Beer law states that the higher the extract concentration, the higher the absorbance value is. The parameter used to show antioxidant activity is the value of inhibitory concentration (IC_{50}). Inhibitory concentration is the concentration of an antioxidant which causes DPPH to lose 50% of its radical character (Molyneux, 2004).

Based on the IC_{50} calculation results in this study, *Tetraselmis suecica* extract has a value of 37.32 ppm. This finding proved that the antioxidants in *Tetraselmis suecica* are strong antioxidants, since the value of the measured IC_{50} was under 50 ppm (Molyneux, 2004). Several studies proved that certain types of microalga produce natural antioxidant content. A study by Ridlo *et al*. (2017) found that chlorophyll and carotenoid contents had positive correlation with antioxidant activity. This finding affirmed a previous study by Pramesti (2013) which stated that the structure of chlorophyll has an important role in antioxidant activity. Chlorophyll is able to capture radical compounds with the presence of a main structure in the form of tetrapyrrole and conjugated polyene. Ferruzzi *et al*. (2002) complement this finding by stating that the structure of the porphyrin ring and magnesium, well known in chlorophyll, also play an important role in inhibiting free radicals. The explanation by Dutta *et al*. (2011), Young and Lowe (2018) stated that carotenoids are pigments that act as antioxidants. The compound can counteract the oxygen singlet (singlet oxygen quenching) via conjugation C = C bond in the carbon chain of the polyene. Carotenoid pigments can also scavenge peroxyl radical, transformed into carotenoids peroxide radical and easily broken down, so it is not harmful to live cells.

5. Conclusion

Based on the results of the study, it was concluded that the extract of *Tetraselmis suecica* showed the potential as a source of strong natural antioxidants. Its acetone extract had pigment content 1.689 mg/g of chlorophyll a, 0.714 mg/g of chlorophyll b, and 5.38 µg/g of carotenoids, but IC_{50} value 37.32 ppm, which meant significant antioxidant activity. The antioxidant activity of *the Tetraselmis suecica* extract was very strong, i.e. the IC_{50} value found was less than 50 ppm.

References

Abdillah F, Raya I and Ahmad A. 2014.Antioxidant activity and toxicity of Co(II) chlorophyll derivative extract . *Journal of Chemistry FMIPA Universitas Hasanudin*, **18(3)**: 1-10.

Badarinath AV, Rao KM, Chetty CMS, Ramkanth S, Rajan TVS and Gnanaprakash K. 2010. A review on in-vitro antioxidant

methods : comparisons, correlations and considerations. *Inter Jl Pharma Technol Res.*, **2(2)**: 1276-1285.

Cikos AM, Jokic S, Subaric D and Jerkovic I. 2018. Overview on the application of modern methods for the extraction of bioactive compounds from marine macroalgae. *Mar. Drugs*, **16(10)**: 348-368.

Dere S, Gunes T and Sivaci R. 1998. Spectrophotometric determination of chlorophyll-a, b, and total carotenoid content of some algae species using different solvent. *J of Botany*, **22**:13-17.

Dimara L and Yenusi TNB. 2011. Antibacterial and antioxidant activity of chlorophyll pigments extracts of seaweed *Caulerpa racemosa* (Forsskal) J.Agardh. *Papua J Biol*., **3(2)**: 53-58.

Dutta D, Chaudhuri UR and Chakraborty R. 2011. Structure, health benefits, antioxidant property and processing and storage of carotenoids. *African J Food, Agricul, Nutrition and Develop.*, **4(13)**:1510–20.

Ferruzzi MG, Bohm V, Courtney PD and Schwartz SJ. 2002. Antioxidant and antimutagenic activity of dietary chlorophyll derivatives determined by radical scavenging and bacterial reverse mutagenesis assays. *J Food Sci*., **67(7)**:2589- 2595.

Firdiyanti F, Agustini TW and Ma'ruf WF. 2015. Extraction of bioactive compounds as natural antioxidants from fresh spirulina platensis using diffrent solvents. *JPHPI*., **18(1)**: 28-37.

Ginting NK, Sedjati S, Supriyantini E and Ridlo A. 2018. Effect of lighting on pigment content *Tetraselmis chuii* as a source of natural antioxidants. *Bulletin Oceanografi Marina*, **7(2)**: 91-97.

Harborne JB. 1984**. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis**. second ed. Chapman and Hall, London.

Hsu C, Chao P, Hu S and Yang C. 2013. The antioxidant and free radical scavenging activities of chlorophyll and pheophytins. *Food and Nutrition Sci.*, **4**:1–8.

Kikuzaki H, Hisamoto M, Hirose K, Akiyama K and Taniguchi H. 2002. Antioxidant properties of ferulic acid and its related compounds. *J Agricul Food Chem.*, **50(7)**: 2161-2168.

Lordan S, Ross SP and Stanton C. 2011. Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Mar. Drugs*, **9:** 1056-1100

Maligan JM, Marditia AP, and Putri WDR. 2015. Analysis of marine microalgae *Tetraselmis chuii* extract bioactive compounds as a ntural antioxidants source. *Rekapangan J*., **9(2)**: 1-10.

Martinez A, Vargas R and Galano A. 2010. Theoretical study on the chemical fate of adducts formed through free radical addition reactions to carotenoids. *Theor Chem Acc.*, **127**: 595–603.

Molyneux P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (dpph) for estimating antioxidant activity. *Songklanakarin J Sci. Technol*., **26(2)**: 211-219.

Pramesti R. 2013. Antioxidant activity of seaweed extract Caulerpa serrulata used DPPH (1,1 difenil 2 pikrilhidrazil) method. *Bulletin Oseanografi Marina*, **2(2)**: 7-15.

Raja R, Hermaiswarva S, Kumar NA, Sridhar S and Rengasamy R. 2008. A perspective on the biotechnological potential of microalgae. *Crit Revi Microbiol*., **34 (2)**: 77–88.

Ridlo A., Pramesti R., Koesoemadji, Supriyantini E dan Soenardjo N. 2017. Antioxidant activity of mangrove leaves extracts Rhizopora mucronata. *Bulletin Oseanografi Marina*, **6(2)**: 110-116.

Rohman A. 2016. **Lipid: Chemical Physics Properties and Its Analysis**. Pustaka Pelajar, Yogyakarta.

Sani RN, Nisa FC, Andriani RD and Maligan JM. 2014. Analysis of yield and phytochemical screening ethanol extracts of marine microalga *Tetraselmis* sp. *J Food and Agroindustries*, **2(2)**: 121-126.

Vadlapudi V, Kaladhar DSVGK, Paul MJ, Kumar SVNS and Behara M. 2012. Antioxidant activities of marine algae: a review. *Inter J Recent Sci Res.*, **3(7)**: 574-580.

Young A and Lowe G. 2018. Carotenoids-Antioxidant Properties. *Antioxidants*,**7 (2)**: 28-31.