Boosting Antioxidant Activity and Phenolic Content in *Scenedesmus dimorphus*

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Abstract. *Scenedesmus dimorphus*, a microalgae from the Chlorophyceae class, possesses compounds with antioxidant potential. This study focuses on determining the antioxidant activity of the methanol extract of **Scenedesmus dimorphus** cultured in Bold Basal Medium (BBM) with NaCl concentrations of 10 g/L, 12.5 g/L, and 15 g/L. NaCl was added after 3, 6, and 9 days of cultivation to evaluate the impact on antioxidant activity. The antioxidant activity was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The results showed that the highest antioxidant activity occurred in cultures with 15 g/L NaCl, with DPPH inhibition of 13.71% and a biomass yield of 0.1914 g in 500 mL of medium. The microalgae demonstrated the ability to withstand NaCl stress, showing improved antioxidant activity over time. On day 9, with the addition of 15 g/L NaCl, the antioxidant activity increased to 26.82%, the IC50 value was 183.63 mg/L, and the biomass weight reached 0.2568 g in 500 mL of medium. Additionally, the total phenolic content increased to 70.10 mg GAE/g extract on day 9. These findings suggest that NaCl stress at specific intervals enhances both antioxidant activity and phenolic content in *Scenedesmus dimorphus*.

1 Introduction

Age is growing from time to time, so a lot of positive things that can be enjoyed by humans. However, not only the positive effects which can be enjoyed, but there are also negative effects such as increased its various chronic diseases due to his existing mutant cells in the body. The mutant cells are formed due to his existing free radicals in the body. Free radicals are atoms or molecules that have no electrons pairs on orbitals outer, which will react with other atoms to achieve stability, so that it will produce a reaction that continues over time and will damage vital cells in the body. How that can be taken in addition to exercise is with the use of antioxidants [1-5].

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Antioxidants are compounds that can counteract free radicals in a way his right donate electrons called a reductant. In the presence of an antioxidant the body can be protected from a variety of degenerative diseases and cancer. Good sources of antioxidants can be derived from plants, bacteria and microalgae [6]. Microalgae is one of the most interesting phytoplankton because it has so many benefits for human life. Microalgae have active components that are useful as an antioxidant, in addition to the microalgae can be used in the food industry, cosmetics and pharmaceuticals. One species of microalgae which has antioxidant activity is of class Chlorophyta, that is *Scenedesmus dimorphus*. Microalgae *Scenedesmus dimorphus* has active compound carotenoids, riboflavin, and the phenol can be used as an antioxidant [7-11].

Due to the rise of chronic diseases caused by free radicals in the body height, the more requests to develop the benefits of microalgae, then for it to do cultivation to increase the biomass of microalgae [12-14]. In the process of cultivation will be no environmental factors that influence the growth of microalgae, such as temperature, salinity and light. High salinity can provide influence microalgae growth due to salinity in the culture media is dominated by Na^+ and Cl^- which can disrupt the osmotic balance between the inside of the cell to the outside environment. The increasing concentration of salt will increase also the concentration of Reactive Oxygen Species (ROS) that would cause oxidative stress. To protect themselves from oxidative stress microalgae cells would secrete compounds antioxidants to maintain life [15-18].

This research aimed to study the effects of salt stress on the growth and antioxidant activity of the microalgae *Scenedesmus dimorphus*, grown in Bold Basal Medium (BBM) with added NaCl. The active compounds were extracted using methanol through the maceration method, and the antioxidant activity was tested using the DPPH assay.

2 Material and Methods

2.1 Chemicals

Materials used in this research that microalgae *Scenedesmus dimorphus* derived from stocks already in Biochemistry laboratory, BBM medium, NaCl, methanol, gallic acid, Na_2CO_3 Folin-Ciocalteu reagent, reagent 1,1diphenyl-2-picrylhydrazyl (DPPH) as well as other supporting material.

2.2 Equipments

Equipment used culture tubes, pump tank, hose (diameter 10 mm), autoclaves, centrifuges (Health HC-12), analytical balance (Kern ABJ220-4M), uv-vis spectrophotometer (Genesys 20), and its other glass.

2.3 Research Procedures

2.3.1 Cultivation of microalgae Scenedesmus dimorphus on the BBM medium with the addition of NaCl

Culture *Scenedesmus dimorphus* cultured in a jar volume of 500 mL which already contain the BBM medium with the addition of different NaCl concentration, namely Control (BBM without addition NaCl); (BBM + NaCl 10 g/L medium); (BBM + NaCl 12.5 g/L medium); (BBM + NaCl 15 g/L medium). Then observed and measured the growth of its antioxidant activity. Observation of the growth phase *Scenedesmus dimorphus* performed every 2 days.

Measured by its cell density (Optical Density) with UV-Vis spectrophotometer at a wavelength of 680 nm [19].

2.3.2 Cultivation Scenedesmus dimorphus on BBM medium with the addition of NaCl at different times

Scenedesmus dimorphus which provides the highest antioxidant activity in the culture medium back on BBM and added NaCl on days 3, 6, and 9 of 15 g/L. Microalgae biomass harvested at stationary phase in this way with a speed of 3000 rpm centrifuge for 10 minutes at room temperature, then the wind dried to obtain dry biomass [19-20].

2.3.3 Preparation Microalgae Extract

Dry biomass *Scenedesmus dimorphus s* crushed until smooth, then soaked in methanol with a ratio of 1:5 for 2 days were placed in dark conditions. To obtain the filtrate concentrated done centrifuges until the filtrate produced pale. The filtrate obtained in the dry wind that dried extract obtained methanol *Scenedesmus dimorphus*.

2.3.4 Antioxidant Activity Test

Testing of antioxidant activity against methanol extract microalgae *Scenedesmus dimorphus* of each treatment the addition of NaCl with DPPH method, by adding 2 ml of 0.1 mM DPPH into 2 mL of each extract solutions were prepared. The mixture was allowed to stand for 30 minutes, then measured the absorbance of the test solution, the positive control and the negative control at a wavelength of 517 nm. The percentage inhibition of each extract was calculated using the formula [21].

$$\% \text{ inhibition} = \frac{Ac \times A}{Ac} \text{x}100\%$$

Description:
Ac = control absorbance value
A = Sample absorbance value

2.3.5 Determination of total phenolic content

Determination contents phenolic total was conducted by Folin-Ciocalteu in each treatment and addition of NaCl days 3, 6, and 9. 0.5 mL sample was taken and put in a 10 mL volumetric flask and then added with 0.5 mL of Folin-Ciocalteu reagent and let stand for 5 minutes. Then add 1 mL of 20% sodium carbonate solution and diluted with distilled water to mark boundaries. The mixture let stand for two hours, then measured the absorbance at a wavelength of 765 nm.

3 Result and Discussion

3.1 Effect of the addition of NaCl to the growth variation *Scenedesmus dimorphus*

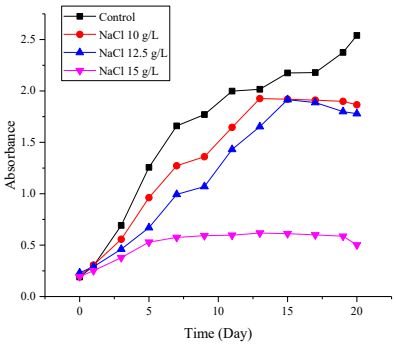

Fig. 1. Growth curve *Scenedesmus dimorphus* at Different Salinity Medium

Figure 1 can be seen the difference in growth of microalgae *Scenedesmus dimorphus* of each treatment. Increase salt in the culture medium can suppress the growth of microalgae cells, so that the growth of microalgae slowed because inhibition of enlargement and cell division. Inhibition of cell growth of microalgae this due concentration salt very high resulting in salt stress. Research has been carried out by Ji Xiang et al. (2018) against microalgae Scenedesmus obliquus XJ002 with the addition of NaCl into the Scenedesmus obliquus XJ002 with the addition of NaCl into the growing medium of 0.01 M to 0.2 M obtained microalgae growth decreases as concentration NaCl. According to NaCl Stress can damage oxygen evolving complex (OEC) and photosystem II and disrupt the process of electron transport [21-22].

3.2 Effect of the addition of NaCl at a time that different to growth *Scenedesmus dimorphus*

In this treatment *Scenedesmus dimorphus* which has been in cultivation in medium BBM the addition of NaCl 15 g/L, cultivated back on medium BBM, with the addition of NaCl 15 g/L on day 3, 6 and 9 (Figure 2).

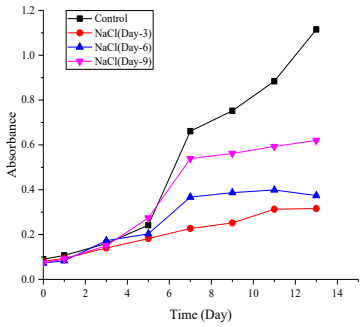

Fig. 2. The growth curve *Scenedesmus dimorphus*

Overall with each treatment can be seen on day 3, *Scenedesmus dimorphus* begins the exponential phase, day 6 *Scenedesmus dimorphus* are in the exponential phase and exponential, day 6 *Scenedesmus dimorphus* are in the exponential phase and 9 is still in the

exponential phase to the stationary phase. The addition of NaCl on days 3, 6 and 9, seen microalgae are still experiencing growth of cells and able to maintain viability. This is caused by *Scenedesmus dimorphus* used is *Scenedesmus dimorphus* maintain viability. This is caused by *Scenedesmus dimorphus* used is *Scenedesmus dimorphus* which has been cultivated in advance in a medium containing NaCl. This in line with research conducted by Pancha et al (2015) microalgae that has been cultivated in the salt concentration of 200 mM cultivated back in the medium supplemented with the same salt concentration, still survive. According microalgae capable of sustaining life on salt levels high because microalgae produce antioxidative enzymes and compound antioxidants. Microalgae too produce proline that could balancing pH, water content in cells and protect cells from stress salinity, the presence of proline microalgae can grow well even in extreme circumstances [19].

3.3 Effect of addition of NaCl at different times of the total phenolic content

In determining the total phenolic compound. Total phenolic content be used gallic acid as a standard solution. Total phenolic content of the extracts of microalgae *Scenedesmus dimorphus* can be seen in Table 1.

Table 1. Content of total phenolic extract methanol *Scenedesmus dimorphus* with the addition of NaCl on different days.

Adding NaCl	Extract (mg GAE/g)
Control	50.48
Day 3	57.45
Day 6	60.63
Day 9	70.10

Table 1 it can be seen that the highest total phenolic cultivation *Scenedesmus dimorphus* with the addition of salt treatment on day 9, the value 70.10 mg GAE/g extract, salt addition on day 9 can trigger microalgae cells secrete secondary metabolites more phenolic compounds, because on day 9 of growth *Scenedesmus dimorphus* closer to the stationary compared with day 3 and day 6. The amount of phenolic compounds in an extract highly influential to test activity antioxidants [14-17].

3 Conclusions

Based on research that has been done can be concluded that with increasing number of levels of NaCl in the medium growth microalgae *Scenedesmus dimorphus* getting lower and higher antioxidant activity. From the addition of NaCl treatment variation obtained enhancement activity most antioxidants in the addition of NaCl 15 g/L medium with DPPH and had a low dry weight of most cells. *Scenedesmus dimorphus* survive in the medium BBM added NaCl 15 g/L, grown again with the addition of NaCl 15 g/L at different times, day 3, 6 and 9. The addition of NaCl 15 g/L on day 9 produces antioxidant activity and highest cell dry weight. The methanol extract *Scenedesmus dimorphus* has antioxidant classified as moderate by inhibition value IC_{50} 183.63% mg/L. Total phenolic content of the extract *Scenedesmus dimorphus* an increase in the addition of NaCl treatment day 9 obtained 70.10 mg GAE/g extract.

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