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Original Research

Effects of F/2 media modification on growth and antioxidant activity of microalga *Nannochloropsis oculata*

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ABSTRACT

Nannochloropsis oculata is a common microalga that can be found in marine water. One of the most commonly used media for microalga culture is F/2 media; however, the optimum F/2 media composition for *N. oculata* growth is rarely informed. Therefore, the aims of this study are to determine the effect of F/2 medium modification on the growth of *N. oculata* and the antioxidant activity of *N. oculata*. This study consists of several procedures including; cultivation, growth observation (cell density and biomass), extraction, and antioxidant activity of *N. oculata* extracts. The highest growth (cell density and biomass) of *N. oculata* was found in media F/2 + 0.5 ml, while the lowest was in media F/2 - 0.5 ml. The strongest antioxidant activity of *N. oculata* was found in media F/2 + 1 ml, and the lowest was in the media F/2 - 0.5 ml. Media modification gave different results on cell density, biomass, and antioxidant activity of microalgae *N. oculata*.

1. Introduction

Microalgae are single-celled microscopic plants that live in both marine and freshwater, having 3 – 30 µm in diameter, and classified as algae classes (Abdurrachman *et al.*, 2013). *Nannochloropsis oculata* is common microalga that is found in marine water. Classified as Eustigmatophyceae classes, *N. oculata* has been utilized as food source in aquaculture. *N. oculata* has high protein, fatty acid, and antioxidants pigment.

The uses of microalgae for our life have been significantly growing, such as the source of functional foods, cosmetics, biogas/biodiesel, antioxidant, and antibacterial. Lately, antioxidant activities of various microalgae have been frequently analyzed (Anam *et al.*, 2014; Bariyyah *et al.*, 2013; Hafsa *et al.*, 2017).

Antioxidants are compounds that prevent or slow the damage caused by free radicals, which happened by the oxidation reaction. Antioxidants could be natural compounds, or made by human (synthetic). Common antioxidants that are sold in public are made from synthetic source, which could be dangerous for human`s life (Bariyyah *et al.*, 2013). Therefore, the natural source of antioxidants becomes important.

Cultivation of microalgae requires a constant supply of several inorganic nutrients, such as nitrogen (N), phosphorous (P), and potassium (K) to optimize microalgae yield. One of the most common media used in microalgae cultivation is F/2 media. These media consists of $NaNO_3$, NaH_2PO_4, Na_2SiO_3, vitamin, and trace metal (Reyimu and Özçimen, 2017). However, the optimum F/2 media composition for the growth of *N. oculata* is rarely informed. Therefore, the aims of this study are to determine the effect of F/2 medium modification on the growth of *N. oculata* and determine the effect of F/2 medium modification to the antioxidant activity of *N. oculata*.

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Table 1. Size of each F/2 media components

No.	Component	F/2	F/2 – 0.5 ml	F/2 + 0.5 ml	F/2 + 1 ml
1	$NaNO_3$	1 mL	0.5 mL	1.5 mL	2 mL
2	$NaH_2PO_4H_2O$	1 mL	0.5 mL	1.5 mL	2 mL
3	Na_2SiO_3 $9H_2O$	1 mL	0.5 mL	1.5 mL	2 mL
4	Trace metal solution	1 mL	0.5 mL	1.5 mL	2 mL
5	Vitamin solution	1 mL	0.5 mL	1.5 mL	2 mL

2. Material and Methods

2.1 Materials

Marine microalgae (*N. oculata)* used in this study were the collection of RCO LIPI, Jakarta. EtOH was purchased from Merck while 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma Chemical Co. The other chemicals and reagents used were of analytical grade commercially available.

2.2 Cultivation of *N. oculata*

Sterilized seawater mixed with F/2 media which consists of $NaNO_3$, NaH_2PO_4, Na_2SiO_3, vitamin (B_1, H and B_{12}), and trace metal consists of $ZnSO_4$ and $CuSO_4$ (one ml/components/a liter of seawater) (F/2). In this study, Na_2SiO_3 was not used because the microalgae are not classified as diatom anddiatom needs silicon (Si) to build the outer cell wall. Since *N. oculata* does not have any cell wall, silicon is not needed (Orefice *et al.*, 2019). Three different modifications were applied to the other cultures; reduction and addition from each component of normal F/2 media, $NaNO_3$, NaH_2PO_4, Na_2SiO_3, vitamin solution, and trace metal solution -0.5 ml, +0.5 ml, and +1 ml, respectively).

Table 1 shows the total size of each component that was used for all treatments, Table 2 shows the components of the vitamin solutions, and Table 3 shows the components of the trace metal solutions. The salinity was controlled to 26 ppt, and the temperature was 25°C. The culture was kept for 15 days in a room which controlled to 12 hours of light in average (3.250 lux) and another 12 hours without light.

2.3 *N. oculata* growth measurements

Two variables of growth were measured in this study:cell density and biomass. Measurement was conducted every day from day one until day fifteen. Cell density (cell/ml) was measured with hemocytometer Neuerbauer, and biomass was measured with Whatman filter paper GF/A that weighed on analytical scales (every 30 ml of samples were filtered on Whatman filter paper every day). *N. oculata* has diameter in average ranged from 2.52 to 2.63 µm (Kandilian *et al.*, 2013), which can be filtered by Whatman filter paper GF/A that has filtration size of 1.6 µm.

2.4 Extraction of *N. oculata* culture

N. oculata culture was centrifuged at 3000 RPM for 7 min to get the pellet of the culture. Afterward, the pellet was macerated with two different solvents; 50% acetone, and 70% ethanol. The result of maceration is a dry extract of *N. oculata* which will be used for antioxidants assessment with DPPH method (Goh *et al.*, 2010).

Table 2. Vitamin solution components

No.	Components
1	Thiamine Hcl (B1)
2	Biotine
3	Cyanocobelamine (B12)

Table 3. Trace metal solution components

No.	Component
1	FeCl
2	Na_2EDTA
3	$MnCl_2$
4	$ZnSO_4$
5	$CoCl_2$
6	$CuSO_4$
7	Na_2MoO4

2.5 Antioxidant measurement of *N. oculata*

DPPH (α, α-diphenyl-β-picrylhydrazyl) free radical scavenging method offers the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources (Kedare and Singh, 2011) and it was used in this study. 10 mg of dry *N. oculata* extract was prepared for the assessment. The extract was then diluted into several concentrations (5, 2, 1, 0.5, 0.2, 0.1, and 0.05 mg/ml), and mixed with methanol as their solvent and also the DPPH. The antioxidant value of *N. oculata* was measured by microplate reader at 515 nm two times for each culture (F/2 normal, F/2 – 0.5 ml, F/2 + 0.5 ml, and F/2 + 1 ml). From that, inhibitions were obtained and could be used to determine antioxidant activity at IC_{50} (Inhibitory Concentration 50%) unit. IC_{50} value was determined using a linear regression equation (Y=bX+a) from inhibition graph of each treatment. Y is the dependent variable (on this case, 50 to determine the IC_{50} value), a is the constant (the concentration) while b is the regression slope (the inhibition value).

3. Results

3.1 Effects of media differentiation on *N. oculata* growth (density and biomass)

From the cultivation of N. *oculata* for 15 days, the highest cell density and biomass results were obtained in the treatment of F/2 + 0.5 ml (cell density 19.68 x 10^6 cells/ml and biomass 0.0012433 gr/ml). The treatment of F/2 - 0.5 ml produced the lowest value for cell and biomass density (6.58 x 10^6 cells/ml and 0.0009233 gr/ml). Normal F/2 media produced cell density of 15.18 x 10^6 cells/ml and 0.0011733 gr/ml biomass, while F/2 + 1 ml media produced cell density and biomass higher than normal F/2 but lower than F/2 + 0.5 ml (19.45 x 10^6 cells/ml and 0.00122 gr/ml). Graphs of cell density and biomass respectively can be seen in Figures 1 and Figure 2.

One way ANOVA was conducted to determine the effect of media differences in cell density and biomass. The results of statistical tests show that media differences do not give a significant effect on cell density and biomass of microalgae *N. oculata* ($P \geq 0.05$).

3.2 Effects of media differentiation on antioxidant activities of *N. oculata*

From all of the treatments, the F/2 + 1 ml medium had the strongest antioxidant activity (inhibition of 55.03% and IC_{50} 4.53 mg / ml). Reduction of F/2 (F/2 - 0.5 ml) media had the lowest antioxidant activity with inhibition of 18.94% and IC_{50} 14.99 mg/ml. Normal F/2 media had an inhibition value of 23.66% and IC_{50} 14.46 mg/ml, and for F/2 + 0.5 ml the inhibition and IC_{50} values were 21.26% and 13.88 mg/ml, respectively. All inhibition percentage values were obtained from the highest dilution concentration (5 mg/ml). Figure 3 and Figure 4 show the antioxidant activities of *N. oculata* from different concentration of F/2 media.

Factorial ANOVA was conducted to determine the effect of media differences on the antioxidant activity of *N. oculata*. The results showed that the media differences had no significant effect on the antioxidant activity of *N. oculata* ($P \geq 0.05$). It can be concluded that the differences in F/2 media concentration on the antioxidant activity of *N. oculata* is still normal (not significant).

4. Discussions

Microalgae cultivation requires a constant supply of several nutrients, such as nitrogen (N), phosphates (P), and potassium (K) to obtained optimum yields. (Brahmantara *et al.*, 2015). Macronutrient such as nitrate is used for the metabolism of microalgae, while phosphate is used for reproduction and energy transfer. Not only macronutrients, microalgae also need other media constituents (i.e micronutrients) for their life nutrition (Sari *et al.*, 2012). However, because the function of nitrate

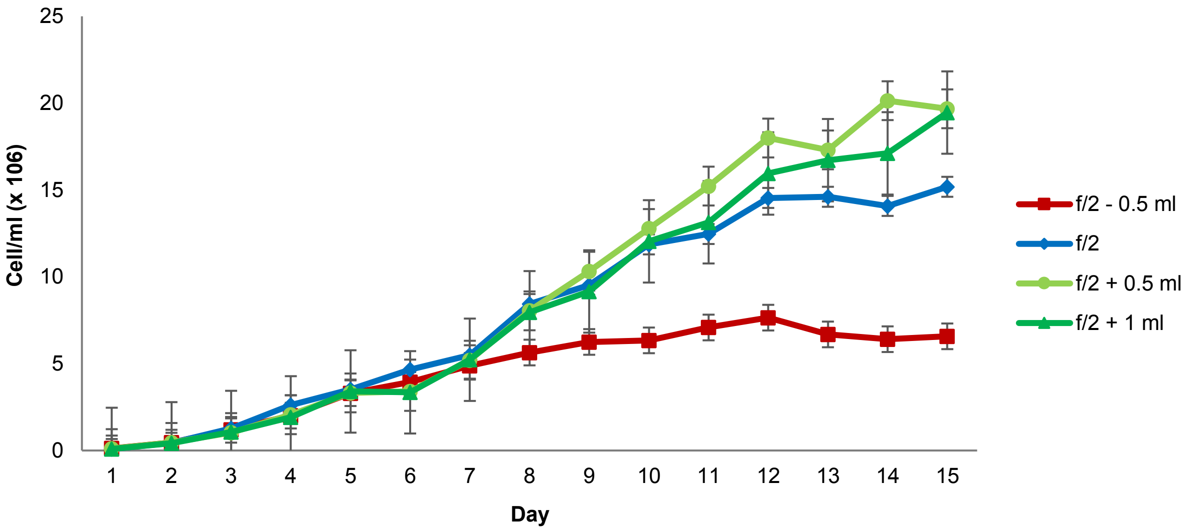

Figure 1. Cell density of cultured *N. oculata* in controlled environtment

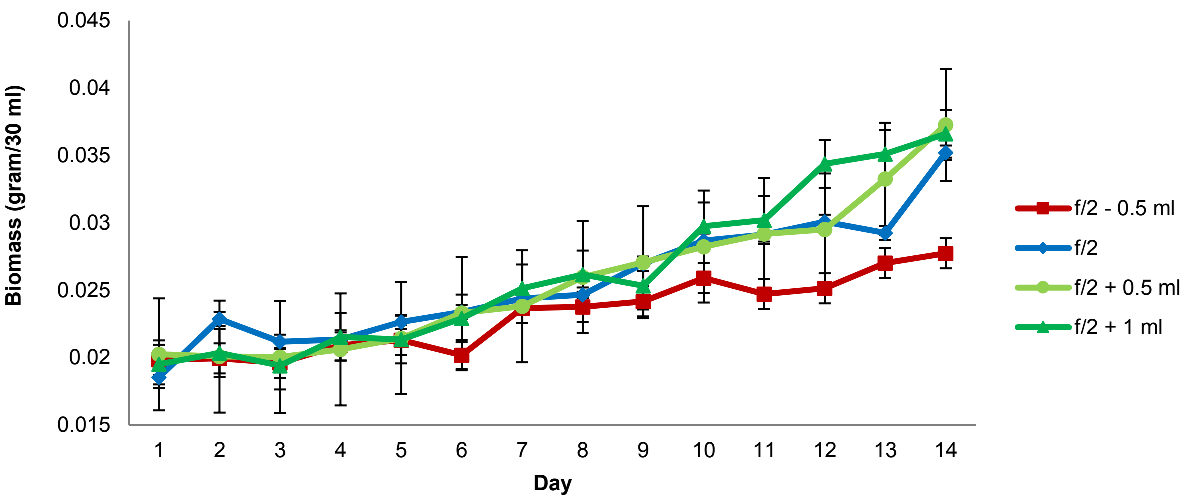

Figure 2. Biomass of cultured *N. oculata* in controlled environtment

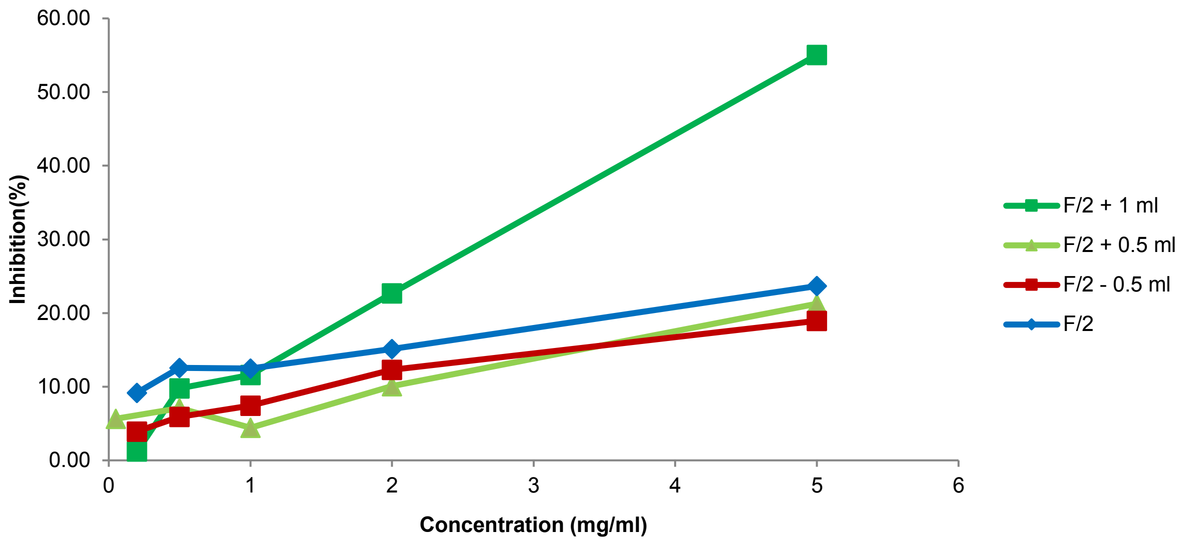

Figure 3. Antioxidant inhibitions from each concentration conducted with DPPH method

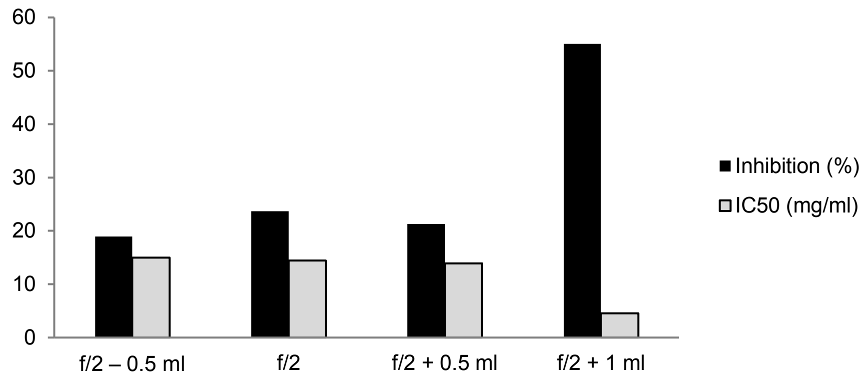

Figure 4. Antioxidant activity of *N. oculata* conducted With DPPH method

and phosphate is very important for microalgae growth, according to Dinata *et al.* (2017), both macronutrients are needed in optimum amount.

In this study, we found that the modification of nutrients in the media (reduced or added), affect the cell density and biomass of *N. oculata*. When the F/2 media is reduced to 0.5 ml per component per liter (F/2 – 0.5 ml), the cell density and biomass of *N. oculata* were lower compared to the normal F/2 media (1 ml per component per L); however, the tdifference in the media concentration does not give a significant effect on the growth of microalgae *N. oculata*.

The addition of F/2 media (1.5 and 2 ml per component per L) showed higher cell density and biomass compared to the normal F/2 media. From this study, it can be concluded that the addition of F/2 + 0.5 ml per component per L is better than the addition of 1 ml per component per L due to the results of cell density and biomass after cultivation. The addition of 0.5 ml gives the highest cell density and biomass of *N. oculata*. The results of this studyindicated that*N. oculata* have optimum nutrient levels for its growth (cell density and biomass).

There are no previous studies that examined the effect of F/2 media modification on the growth of *N. oculata*, but several studies have conducted at the effect of different levels of nitrate and phosphate on microalgae growth. Hu and Gao (2006), Dinata *et al.* (2017), and Mahat *et al.* (2015) studied the effect of different levels of nitrate and phosphate on the growth of *N. oculata* and *Nannochloropsis* sp., with various range of modification at the nitrate and phosphate composition. The studies show that different levels of nitrate and phosphate on the culture media will affect the growth of microalgae *N. oculata* and *Nannochloropsis* sp. Furthermore, using a linear regression correlation, Brahmantara *et al.* (2015) found the most optimum amount of nitrate and phosphate at F/2 media for the growth of *Nannochloropsis* sp., are 178.14 gr/l and 13.34 gr/l, respectively.

Antioxidant ability of algae is affected by the molecular weight of polysaccharides, monosaccharide composition, and sulfate content (Hafsa *et al.*, 2017). From this study, it can be confirmed that the sulfate content affects the antioxidant ability of *N. oculata*. The F/2 had sulfate content in forms of $ZnSO_4$ and $CuSO_4$ compounds which are the components of the trace metal solutions. The lowest antioxidant activity of *N. oculata* (both inhibition and IC_{50}) was found in the reduction of F/2 media (0.5 ml per component per L) which had the lowest sulfate content compared to the other treatments. The highest antioxidant activity was found in the addition of F/2 media (2 ml per component per liter) where the sulfate content was also higher than the other treatments.

Antioxidant activity is classified as very active at IC_{50} values <10 µg/ml, active at IC_{50} values 10 - 100 µg/ml, and

Table 4. Comparison of this study to other previous study of *N. oculata* antioxidant activity

No.	Author	Media	Solvent	Sample concentration	Inhibition (%)
1	Sangeetha *et al.* (2018)	-	Methanol	500 µg/ml	64.71
2	Feller *et al.* (2018)	Conway	Supercritical CO_2	-	33.8
3	Feller *et al.* (2018)	Conway	Subcritical n-butane	-	19
4	Ebrahimzadeh *et al.* (2018)	Walne	Methanol	400 µg/ml	21.68
5	Ebrahimzadeh *et al.* (2018)	Walne	Ethyl acetate	400 µg/ml	39.03
6	Hafsa *et al.* (2017)	f/2	Distilled Water	10 mg/ml	59.28
7	Hafsa *et al.* (2017)	f/2	Distilled Water	1 mg/ml	24.79
8	This study	f/2 – 0.5 ml	Methanol	5 mg/ml	18.94
9	This study	f/2	Methanol	5 mg/ml	23.66
10	This study	f/2 + 0.5 ml	Methanol	5 mg/ml	21.26
11	This study	f/2 + 1 ml	Methanol	5 mg/ml	55.03

inactive at IC_{50} values > 100 µg/ml (Muharni *et al.*, 2013). The strongest antioxidant activity in this study had an IC_{50} value of 4.53 mg/ml (4,530 µg/ml), and it can be concluded that antioxidant activity of *N. oculata* was very weak.

There are no previous studies that examined antioxidant activity of *N. oculata* with modified F/2 media treatments. From this study, it can be assumed that the optimal addition of nutrient levels can increase antioxidant activity of *N. oculata*, since the results of antioxidant assessment shows differences from each media used in this study.

Several studies had examined antioxidant activity of *N. oculata* with various treatments (as can be seen in Table 4.). Sangeetha *et al.,* (2018) found a higher antioxidant activity of *N. oculata* compared to this study The results might be different due to the cultivation process and sample concentration. Sangeetha *et al.,* (2018) did not cultivate the microalgae and used less sample concentration (500 µg/ml). Therefore, it can be assumed that cultivation process might affect the antioxidant activity of *N. oculata*.

Another study carried by Hafsa *et al.,* (2017), who cultivate the *N. oculata* with F/2 media for 11 days and extracted with distilled water. Hafsa *et al.,* (2017) found the antioxidant activity was 24.79 % (from 1 mg/ml extract) while antioxidant activity found in this study was 23.66 % (from 5 mg/ml extract). Hence, it can be assumed that the extraction process would effect the antioxidant activity of microalga.

5. Conclusions

The modification of F/2 media in this study did not give a significant effect on growth (cell density and biomass) and antioxidant activity of *N. oculata*. Therefore, further studies on the composition of F/2 media to obtain the optimum growth and antioxidant activity of *N. oculata* is required.

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References

Abdurrachman, O., Mutiara, M., Buchori, L., 2013. Pengikatan Karbon Dioksida Dengan Mikroalga (*Chlorella vulgaris, Chlamydomonas* sp., *Spirullina* sp.) Dalam Upaya Untuk Meningkatkan Kemurnian Biogas. J. Teknol. Kim. Dan Ind. 2, 5.

Anam, C., Agustini, T.W., Romadhon, 2014. Pengaruh Pelarut Yang Berbeda Pada Ekstraksi *Spirulina platensis* Serbuk Sebagai Antioksidan Dengan Metode Soxhletasi. J. Pengolah. Dan Bioteknol. Has. Perikan. 3, 7.

Bariyyah, S.K., Hanapi, A., Fasya, A.G., Abidin, M., 2013. Uji Aktivitas Antioksidan Terhadap Dpph dan Identifikasi Golongan Senyawa Aktif Ekstrak Kasar Mikroalga *Chlorella* sp. Hasil Kultivasi Dalam Medium Ekstrak Tauge. Alchemy. https://doi.org/10.18860/al.v0i0.2890.

Brahmantara, I.B.G., Anggreni, A.A.M.D., Gunam, I.B.W., 2015. Pengaruh Konsentrasi Penambahan Sodium Nitrat Dan Sodium Fosfat Pada Media Guillard Terhadap Konsentrasi Biomassa Mikroalga *Nannochloropsis* sp. J. Rekayasa Dan Manaj. Ind. 3, 9.

Dinata, K.D.W., Anggreni, A.A.M.D., Antara, N.S., 2017. Pengaruh Konsentrasi Natrium Nitrat dan Natrium Dehidrogen Fosfat pada Media Walne Terhadap Konsentrasi Biomassa dan Protein *Nannochloropsis oculata*. J. Rekayasa Dan Manaj. Agroindustri 5, 9.

Ebrahimzadeh, M.A., Khalili, M., Dehpour, A.A., 2018. Antioxidant activity of ethyl acetate and methanolic extracts of two marine algae, *Nannochloropsis oculata* and *Gracilaria gracilis* - an in vitro assay. Braz. J. Pharm. Sci. 54. https://doi.org/10.1590/s2175-97902018000117280.

Feller, R., Matos, Â.P., Mazzutti, S., Moecke, E.H.S., Tres, M.V., Derner, R.B., Oliveira, J.V., Junior, A.F., 2018. Polyunsaturated ω-3 and ω-6 fatty acids, total carotenoids and antioxidant activity of three marine microalgae extracts obtained by supercritical CO2 and subcritical n-butane. J. Supercrit. Fluids 133, 437–443. https://doi.org/10.1016/j.supflu.2017.11.015.

Goh, Su-Hua, Yusoff, F. M., Loh, S., 2010, A comparison of the antioxidant properties and total phenolic content in a diatom, *Chaetaros* sp. and a green microalga, *Nannochloropsis* sp. Journal of Agricultural Science, 2:3.

Hafsa, M.B., Ben, I., Garrab, M., Aly, R., Gagnon, J., Naghmouchi, K., 2017. Antimicrobial, antioxidant, cytotoxic and anticholinesterase activities of water-soluble polysaccharides extracted from microalgae *Isochrysis galbana* and *Nannochloropsis oculata*. J. Serbian Chem. Soc. 82, 509–522. https://doi.org/10.2298/JSC161016036B.

Hu, H., Gao, K., 2006. Response of Growth and Fatty Acid Compositions of *Nannochloropsis* sp. to Environmental

Factors Under Elevated CO2 Concentration. Biotechnol. Lett. 28, 987–992. https://doi.org/10.1007/s10529-006-9026-6.

Kedare, S. B., Singh, R. P., 2011. Genesis and development of DPPH method of antioxidant assay. J. Food Sci Technol. 48(4): 412-422.

Mahat, K., Jamaluddin, H., Mohd. Zain, N.A., 2015. The Effect Of Different Phosphate Concentration On Growth, Lipid Productivity And Methyl Palmitate Methyl Ester Production By *Nannochloropsis oculata*. J. Teknol. 77. https://doi.org/10.11113/jt.v77.6915.

Muharni, Elfita, Amanda, 2013. Aktivitas Antioksidan Senyawa (+) Morelloflavon Dari Kulit Batang Tumbuhan Gamboge (*Garcinia xanthochymus*). Pros. Semirata FMIPA Univ. Lampung.

Orefice, Ida, Musella, M., Smerilli, A., Sansone, C., Chandrasekaran, R., Corato, F., Brunet, C., 2019. Role of nutrient concentrations and water movement on diatom's productivity in culture. Sci Rep. 9:1479.

Reyimu, Z., Özçimen, D., 2017. Batch cultivation of marine microalgae *Nannochloropsis oculata* and *Tetraselmis suecica* in treated municipal wastewater toward bioethanol production. J. Clean. Prod. 150, 40–46. https://doi.org/10.1016/j.jclepro.2017.02.189.

Sangeetha, P., Anuradha, V., Suganya, V., Bhuvana, P., 2018. In Vitro Antioxidant And Radical Scavenging Activity Of Marine Microalga *Nannochloropsis oculata*. World J. Pharm. Pharm. Sci. 7, 15.

Sari, A.S.P., Ratnasari, E., Wisanti, 2012. Pengaruh Pemberian Jenis Pupuk yang Berbeda terhadap Laju Pertumbuhan Populasi dan Kadar Lemak *Nannochloropsis oculata*. Lentera Biol. 1, 7.

Declarations

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Conflict of interest

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