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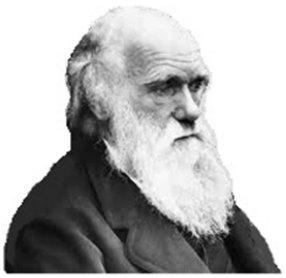

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Early utilization of *Spirulina platensis* cultivation as an antioxidant candidate in laboratory-scale closed reactor system

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ABSTRACT

Spirulina platensis is a microalga that contains a lot of secondary metabolites and is utilized as a dietary supplement. It can inhibit fat peroxidation better compared to the chemical antioxidant. Laboratory-scale closed reactor system was designed for *Spirulina* cultivation. This research aims to analyze identified secondary metabolite (Phytochemical contents) and the antioxidant activity or value of Inhibition Concentration 50% (IC_{50}) of the fresh lab-scale cultivated *Spirulina platensis*. The antioxidant content was evaluated using DPPH method (1,1–diphenyl-2-pikrilhidrazil) on various sample concentrations as well as using Vc (Ascorbic Acid) as a positive control. The principle of hydrogen absorption by free radicals from antioxidants was demonstrated with the absorption value using a spectrophotometer at a wavelength of 517 nm. The results show that *Spirulina platensis* lab-scale cultivation was resulting in 5.0±0.01 g/10.0 L wet basis on the 10th day. The results show that *Spirulina platensis* has positive compounds of flavonoid, steroids, triterpenoids, phenolic, and saponins. Fresh *Spirulina platensis* has a value of IC_{50} 647.045 ppm and Vc as a positive control has the IC_{50} of 2.085 ppm. The potential as a source of natural antioxidants was categorized as a very weak capacity. Therefore this study can be concluded that *Spirulina platensis* cultivated in Laboratory-scale has a potential to act as antioxidant candidate.

Keywords: Antioxidant, DPPH, *Spirulina platensis,* Fresh *Spirulina,* Laboratory-scale

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1. INTRODUCTION

Spirulina platensis is a bluish-green autotrophic microorganism with a column of cells forming twisted filament resembling a spiral or helix. It is also called as blue-green filament algae known as *Cyanobacterium* (Soni *et al.*, 2017). It contains about 20% of phycocyanin pigment of its dry weight. Phycocyanin is a complex protein that has the capability of enhancing the immune system, also acting as anticancer and antioxidant (Nuhu, 2013; Czerwonka *et al*., 18).

Antioxidant has a lot of benefits for health and beauty, for the example to prevent cancer and tumors, narrowing of blood vessels, and premature aging. Besides, it is an instrumental that can be used for maintaining the quality of food products (Santos-Sánchez *et al.*, 2017). An antioxidant is an electron-giver compound that prevents the occurrence of oxidation reaction, preventing the formation of free radicals. It has highly reactive molecules because they have unpaired electrons in their outer orbital. Free radicals in human bodies are hazardous because they tend to do chain reactions and inflict ongoing and continuous damage. The human body has its defense system in counteracting free radical attacks through normal cell metabolic and inflammatory events. Still, with increased levels of pollution, radiation, and stress they caused the ability of existing body defense systems inadequate. Therefore the human body requires additional antioxidants from the outside. Thus, the presence of antioxidants derived from natural ingredients is significant. The results of Karkos *et al.* (2008) study show that *Spirulina platensis* contains antioxidants, which can inhibit fat peroxidation better (65%) compared to the chemical antioxidant example of tocopherol (35%) and BHA (45%).

Due to its benefits, several studies has been explored the cultivation method to obtain optimum biomass and evaluated the antioxidant activity of the *Spirulina platensis*. Moraes *et al.* (2013) proposed luminosity as the main parameter along with different rates of stirring, nitrogen source, and amount of micronutrients. Soni *et al.* (2019) modified organic media and a closed reactor system for better biomass yield. However, the study about the antioxidant activity of the fresh *Spirulina platensis*, that has been cultivated in a glass aquarium is still limited.

Thus, this study was designed as spirulina cultivation methods using the Laboratory-scale closed reactor system. This research aims to analyze identified secondary metabolite (Phytochemical contents) and the antioxidant activity or value of Inhibition Concentration 50% (IC_{50}) of the fresh lab-scale cultivated *Spirulina platensis*.

2. MATERIALS AND METHODS

2. 1. *Spirulina platensis* cultivation

Spirulina platensis inoculum was obtained from the Hatchery Laboratory of Fisheries and Marine Science Faculty Padjadjaran University (West Java, Indonesia). Spirulina was cultivated in 10 L of water as the media with the addition of feed nutrients (**Table 1**).

Spirulina platensis was cultivated using a glass aquarium with a 40×20×20 cm^3 dimension. The photosynthesis process takes place using the 18 Watt TL lamp light (Philips – Cooldaylight) continuously during the cultivation period. Carbon dioxide (CO_2) was supplied to the water using an aerator, which was connected to the hose. The hose is inserted into the PVC pipe, with small holes on the bottom.

The pipe is attached to the aquarium diagonally (**Figure 1**). The air is to push the water circulating from the bottom to the top of the water column. Thus the water will be mixed so that all parts of *the Spirulina platensis* will get nutrients and light sources evenly.

Table 1. Feed Nutrient Composition for *Spirulina platensis.*

Ingredient	Amount (g)
$NaHCO_3$	84.00
NaCl	5.00
Urea	0.80
TSP	0.30
ZA	0.20
FeCl	0.02

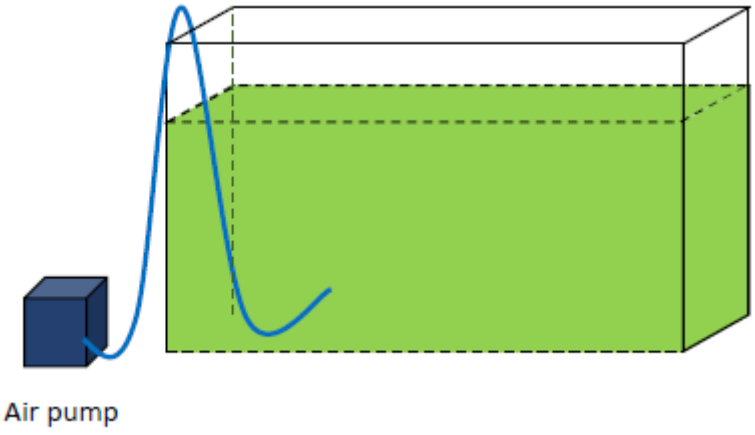


Figure 1. Schematic design of Laboratory-scale open pond and closed reactor system for *Spirulina platensis* culture

2. 2. Harvesting Method

Harvesting was carried out after ten days of culture at the peak of the population density of spirulina, which can be seen under a microscope and marked by changes in the color of the water on the culture media to be thick green (**Figure 2a**). Cultures that have reached the peak population density are harvested by water on culture media filtered using a plankton-net with a mesh size of 20.0 µm (Figure 2**b**). The filtering results are then rinsed and squeezed to obtain spirulina biomass and reported as g wet basis.

(a) **(b)**

Figure 2. Harvesting process for *Spirulina platensis*: **(a)** Cultures that reached the peak population; **(b)** Harvesting process using a 20.0 μm plankton-net

2. 3. Phytochemical Measurement

The secondary metabolites were determined using a qualitative test of phytochemistry. This phytochemical test is used to determine whether or not there are bioactive group compounds in the sample that have the potential as antioxidants. The groups of compounds tested included alkaloid test, flavonoid test, steroid test, triterpenoid test, phenol test, saponin test, and tannin test, describe by Sidi *et al.* (2018).

2. 4. Antioxidant Measurement

The quantitative antioxidant activity of the test *Spirulina platensis* was carried out using the DPPH method expressed in IC_{50} value. According to Teng and Lee (2014), the magnitude of antioxidant activity is indicated by the IC_{50} value, which is the concentration of the sample solution needed to inhibit 50% DPPH free radicals. Vc (Ascorbic Acid) that has been widely used in the community was used as a positive control. Antioxidant activity of the various concentrations of samples and control was indicated by the value of absorbance using the spectrophotometric method (Molyneux, 2004).

The fresh *Spirulina platensis* was dissolved in methanol with concentrations of 100, 200, 400, 600, 800, and 1000 ppm. The Vc solution was prepared by dissolving Ascorbic Acid powder in methanol as a solvent with concentrations of 1, 2, 3, 4, 6, 8, and 10 ppm. Each solution of *Spirulina platensis* and Vc that has been made was taken 2 ml of each concentration and inserted into the vial and added by 1 ml DPPH 0.1 mM. The blanco is made by dissolving 1 ml DPPH 0.1 mM and added by 2 ml of methanol. The solution was then incubated at a temperature of 37 °C for 30 min.

Antioxidant activity tests can be detected by the discoloration of the DPPH solution from violet to yellow. These color changes occur because of the reduction of DPPH free radicals by antioxidant compounds that can provide hydrogen radicals to DPPH radicals and reduced to DPPH-H (1,1-diphenyl-2-pikrilhidrazine). This color change was measured by using a UV-vis spectrophotometer with a wavelength of 517 nm, which is the maximum wavelength for DPPH. To illustrate the magnitude of the concentration of test compounds that can ward off free

radicals by 50% and the right concentration in transferring DPPH free radicals, the IC_{50} values were calculated. The values of IC_{50} were used following the calculations:

$$Inhibition = \frac{Blanco\ Absorbane - Sample\ Absorbance}{Blanco\ Absorbance} \times 100\% \quad \textbf{(Eq. 1)}$$

2. 5. Data analysis

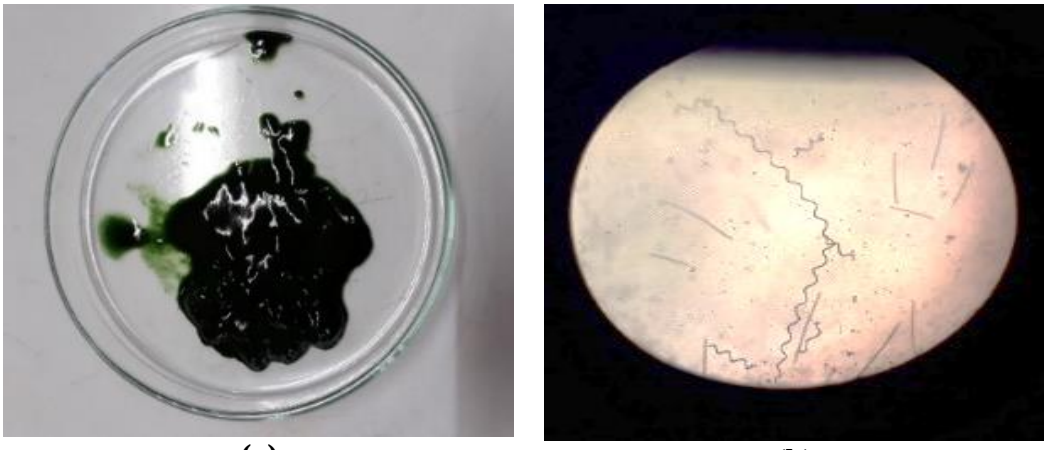
Biomass and phytochemicals data results were analyzed descriptively. As for antioxidant measurement, after measuring the absorbance value, the inhibition percentage value was calculated to make a linear graph. This linear graph can show the relationship of increasing the concentration of samples with Vc to the value of inhibition percentage in counteracting DPPH free radicals. This IC_{50} value is obtained through calculations using linear regression:

$$Y = a + b \quad \textbf{(Eq. 2)}$$

3. RESULTS AND DISCUSSION

3. 1. Cultivation results

The filtering results of spirulina biomass (**Figure 3**) with 10 L of water produced 5.0±0.01 g of spirulina on a wet basis. The implementation of microalgae culture elements N, P, and K is necessary for spirulina growth. The light and temperature are the main factors of growth in a nutrient-operated outdoor pond (N, P, CO_2, etc.) and well-mixed conditions (Huesemann *et al.*, 2016). Pérez-López *et al*. (2017) state that nutrients needed for the growth and development of spirulina are essential to maintain the quantity, quality, and stability of spirulina cell production. Lamp modification in this study was used for the light supply when the indoor culture method does not allow for getting direct sunlight.



(a) **(b)**

Figure 3. Cultured *Spirulina platensis*: **(a)** *Spirulina platensis* crops are ready to be tested; **(b)** Density of spirulina seen under microscope

Wang *et al*. (2007) study shows that photo-period manipulation with different light intensity hase a significant effect on the content of bioactive pigments (chlorophyll-a) in *Spirulina platensis* growth. Manipulation of light spectrum (Ramanna *et al*., 2017) also would allow maximum microalgae utilization and biomass productivity.

3. 2. Secondary metabolite of *Spirulina platensis*

The qualitative phytochemical screening test result is presented in **Table 2**. The result shows that fresh *Spirulina platensis* presents a positive effect on flavonoids, steroids, triterpenoids, phenols, and saponins. This result is in agreement with Firdiyani *et al.* (2015). The results of the phytochemical screening test from *Spirulina platensis* extract using acetone solvents showed positive effects on phenolic, triterpenoid, steroid, flavonoid, and saponin compounds.

Table 2. Secondary metabolite of *Spirulina platensis.*

Secondary Metabolite Compounds	Result
Alkaloids	–
Flavonoids	+
Steroids	+
Triterpenoids	+
Phenols	+
Saponins	+
Tannins	–

Alkaloid compounds in the phytochemical screening test showed negative results, namely by not forming white or brown deposits. Tannin compounds also show negative results marked by no change in color to green or reddish. Secondary metabolites that showed positive results on the phytochemical screening test were thought to support the antioxidant activity of *Spirulina platensis*. These compounds had a potential as natural antioxidants that can counteract free radicals. Hirata *et al.* (2000) stated that compounds that have antioxidant activity are zeaxanthin, α-tocopherol, and phycocyanin, for example, linoleic acid.

3. 2. Antioxidant activity of *Spirulina platensis*

The antioxidant activity of fresh *Spirulina platensis* with the DPPH method performed at various concentrations showed different inhibition percentage values. Absorbance reading of fresh Spirulina with various concentrations of free radicals using spectrophotometer results in the curve, as presented in **Figure 4**, while the absorbance reading of Vc with multiple

concentrations of DPPH free radicals obtained is shown in **Figure 5**. The results showed that with increasing concentration, the inhibitory ability also increased in the deterrence of free radicals.

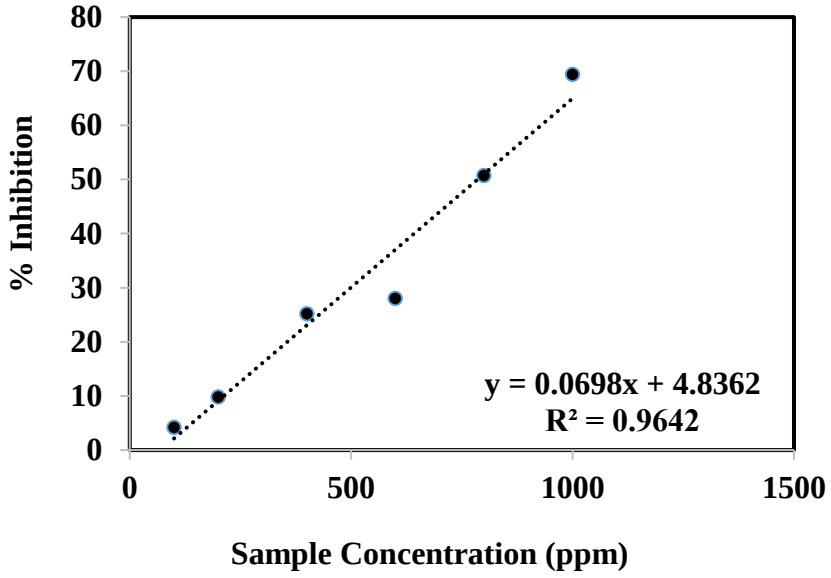


Figure 4. Antioxidant Potential Curves for *Spirulina platensis* Against Free Radicals DPPH

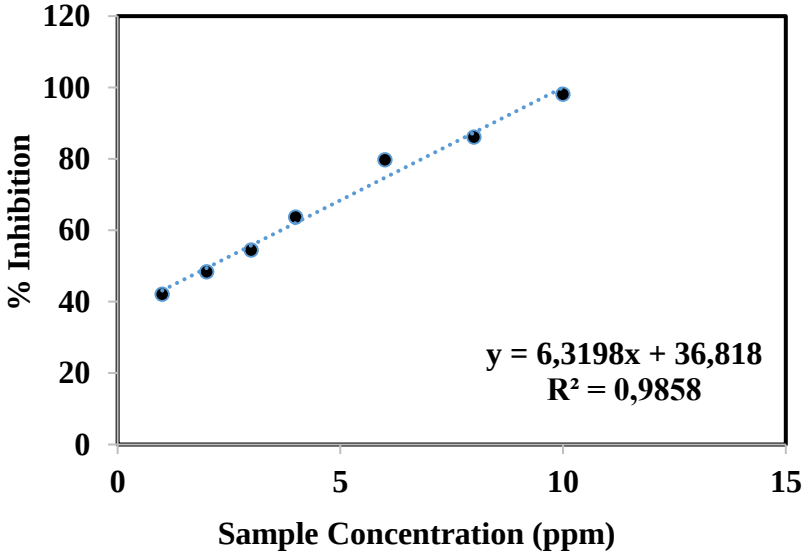


Figure 5. Vc Potential Antioxidant Curves Against Free Radicals DPPH

The result shows that the higher the level of fresh *Spirulina platensis*, the higher the inhibition percentage value. These phenomena are due to the increasing number of samples.

The higher the antioxidant content so that it also affects the level of inhibition of free radicals was carried out by these antioxidant substances, Teng and Lee (2014).

The quantitative antioxidant activity of the test *Spirulina platensis* was carried out using the DPPH method expressed in IC_{50} value. Test results of Value of IC_{50} shows that *Spirulina platensis* has a very weak level of antioxidant abilities compared to Vc (**Table 3**). Each type of *Spirulina platensis* has different antioxidant abilities. This is due to the varied the content of secondary metabolites contained, especially in the content of phenolic compounds and alkaloids, which are responsible for antioxidants. Antioxidant activity of *Spirulina platensis* fresh is classified as very weak, presumably because it is a fresh preparation wherein the antioxidant compounds are not yet pure.

The results in following Wikanta *et al.* (2005) study show, the low antioxidant activity can be due to the presence of impurities found in the sample. Although its antioxidants include a very weak category of spirulina, it has been classified as having antioxidant potential. Scale up production of spirulina cultivated by an open pond with freshwater shows that this method was environmentally friendly as a part of spirulina tablet production (Ye *et al.*, 2018)

Table 3. Value of IC_{50} *Spirulina platensis* and Vc.

Name of Sample	**Sample IC_{50} Concentration (ppm)**	**Antioxidant Capacity** (Molyneux, 2004).		
		Category	**IC_{50} Concentration (ppm)**	**Description**
Vc (Ascorbic Acid)	2.085	1	< 50	Very Strong
		2	50 - 100	Strong
		3	100 - 150	Moderate
		4	150 - 200	Weak
Spirulina platensis	647.045	5	>200	Very Weak

4. CONCLUSIONS

This study demonstrates the utilization of *Spirulina platensis* cultivation using the Laboratory-scale closed reactor system. The filtering results of spirulina biomass in the from 10.0 L of water produced 5.0±0.01 g of spirulina on a wet basis. Secondary metabolite contents found in the fresh *Spirulina platensis* are Flavonoids, Steroids, Triterpenoids, Phenols, and Saponins. The antioxidant activity of *Spirulina platensis* has an IC_{50} value of 647.045 ppm.

The potential as a source of natural antioxidants was categorized as a very weak capacity. Therefore this study can be concluded that *Spirulina platensis* cultivated in Laboratory-scale has a potential to act as antioxidant candidate. More research on improving biomass and antioxidant content in the sample extract is worth to be conducted in the future.

References

[1] Czerwonka, A., Kaławaj, K., Sławińska-Brych, A., Lemieszek, M.K., Bartnik, M., Wojtanowski, K.K., Zdzisińska, B., Rzeski, W. Anticancer effect of the water extract of a commercial Spirulina (Arthrospira platensis) product on the human lung cancer A549 cell line. *Biomedicine and Pharmacotherapy*, 106 (2018) 292–302. https://doi.org/10.1016/j.biopha.2018.06.1

[2] Firdiyani F., Agustini T.W., Ma'ruf W.F. Extraction of bioactive compounds as natural antioxidants from fresh spirulina platensis using different solvents. (Ekstraksi Senyawa BIoaktif sebagai Antioksidan Alami Spirulina Platensis Segar dengan Pelarut yang Berbeda). *Jurnal Pengolahan Hasil Perikanan Indonesia*, 18(1) (2015) 28–37. https://doi.org/10.17844/jphpi.2015.18.1.28 (In Indonesian Language).

[3] Hirata T., Mikiya T., Masaki O., Teppei T., Morihiko S. Antioxidant activities of phycocyanobilin prepared from Spirulina platensis. *Journal of Applied Phycology*, 12(3–5) (2000) 435–439. https://doi.org/10.1023/a:1008175217194

[4] Huesemann, M., Crowe, B., Waller, P., Chavis, A., Hobbs, S., Edmundson, S., Wigmosta, M. A validated model to predict microalgae growth in outdoor pond cultures subjected to fluctuating light intensities and water temperatures. *Algal Research*, 13 (2016) 195–206. https://doi.org/10.1016/j.algal.2015.11.008

[5] Karkos, P.D., Leong, S.C., Karkos, C.D., Sivaji N., Assimakopoulos D.A. Spirulina in clinical practice: Evidence-based human ap.plications. *Evidence-Based Complementary and Alternative Medicine*, (2011). Volume 2011, Article ID 531053. https://doi.org/10.1093/ecam/nen058

[6] Molyneux, P. The Use of the Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity. *Songklanakarin Journal of Science and Technology*, 26 (2004) 211–219. https://doi.org/10.1287/isre.6.2.144

[7] P D Karkos, S C Leong, C D Karkos, N Sivaji, D A Assimakopoulos. Spirulina in clinical practice: evidence-based human applications. *Evid Based Complement Alternat Med.* 2011; 2011: 531053. doi: 10.1093/ecam/nen058

[8] Nuhu, A.A. Spirulina (Arthrospira): An Important Source of Nutritional and Medicinal Compounds. *Journal of Marine Biology*, (2013), Article ID 325636, 1–8. https://doi.org/10.1155/2013/325636

[9] Pérez-López, P., Ledda, F.D., Bisio, A., Feijoo, G., Perino, E., Pronzato, R., Manconi, R., and Moreira, M.T. Life cycle assessment of in situ mariculture in the Mediterranean Sea for the production of bioactive compounds from the sponge Sarcotragus spinosulus. *Journal of Cleaner Production*, 142 (2017) 4356–4368. https://doi.org/10.1016/j.jclepro.2016.11.137

[10] Ramanna, L., Rawat, I., Bux, F. Light enhancement strategies improve microalgal biomass productivity. *Renewable and Sustainable Energy Reviews*, 80 (2017) 765–773. https://doi.org/10.1016/j.rser.2017.05.202

[11] Santos-sánchez, N.F., Salas-coronado, R., Valadez-blanco, R., Hernández-carlos, B., Guadarrama-mendoza, P.C. Natural antioxidant extracts as food preservatives. *Acta*

Scientiarum Polonorum Technologia Alimentaria, 16(4) (2017) 361–370. http://dx.doi.org/10.17306/J.AFS.2017.0530

[12] Sidi, N., Aris, A.Z., Mohamat, F., Looi, L.J. Tape seagrass (*Enhalus acoroides*) as a bioindicator of trace metal contamination in Merambong shoal, Johor Strait, Malaysia. *Marine Pollution Bulletin* Volume 126, January 2018, Pages 113-118. https://doi.org/10.1016/j.marpolbul.2017.10.041

[13] Soni, A.R., Sudhakar, K., Rana, R.S. Spirulina - From growth to nutritional product : A review. *Trends in Food Science and Technology*, 69 (2017) 157–171. https://doi.org/10.1016/j.tifs.2017.09.010

[14] Soni, E.A., Sudhakar, K., Rana, R.S. Comparative study on the growth performance of Spirulina platensis on modifying culture media. *Energy Reports*, 5 (2019) 327–336. https://doi.org/10.1016/j.egyr.2019.02.009

[15] Teng, H., Lee, W.Y. Antibacterial and antioxidant activities and chemical compositions of volatile oils extracted from Schisandra chinensis Baill. seeds using simultaneous distillation extraction method , and comparison with Soxhlet and microwave-assisted extraction. *Bioscience, Biotechnology and Biochemistry*, 78(1) (2014) 79–85. https://doi.org/10.1080/09168451.2014.877815

[16] Wang, C., Fu, C., Liu, Y. Effects of using light-emitting diodes on the cultivation of Spirulina platensis. *Biochemical Engineering Journal* Volume 37, Issue 1, 15 October 2007, Pages 21-25. https://doi.org/10.1016/j.bej.2007.03.004

[17] Wikanta, T., Januar, H.I., and Nursid, M. Assay activity antioxidant, toxicity and cytotoxicity red algae extract *Rhodymenia palmate* *Jurnal Penelitian Perikanan Indonesia*, 11(4) (2017) 41. https://doi.org/10.15578/jppi.11.4.2005.41-49

[18] Ye, C., Mu, D., Horowitz, N., Xue, Z., Chen, J., Xue, M. Life cycle assessment of industrial scale production of spirulina tablets. *Algal Research*, 34 (2018) 154–163. https://doi.org/10.1016/j.algal.2018.07.013