

POTENTIAL OF *Navicula salinicola* EXTRACT, A MICROALGAE FROM MALUKU ISLANDS, AS AN ANTI-INFLAMMATORY AGENT USING THE HUMAN RED BLOOD CELLS (HRBC) METHOD

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ABSTRACT

Indonesia is the world's largest archipelagic state, with rich maritime biodiversity that includes microalgae. Microalgae contain various therapeutic components (i.e., anti-inflammatory) that have shown great potential to be used for human health and medicine. *Navicula salinicola* is a new species of microalgae discovered in the Maluku Islands marine environment in Indonesia. There have been no publications yet related to the potential content of compounds from these microalgae for anti-inflammatory purposes. The aim of this paper was to evaluate the anti-inflammatory activity of extracts of the microalgae *Navicula salinicola*. The *Navicula salinicola* was cultivated in optimum conditions after 7 days. The extraction was performed using the hierarchical maceration method with *n*-hexane, chloroform, and 96 percent ethanol. The extract's anti-inflammatory properties were evaluated using the Human Red Blood Cell (HRBC) stability method, and Na-diclofenac was used as a control. The results showed the *n*-hexane, chloroform, and ethanol extracts were able to inhibit red blood cell hemolysis with IC₅₀ values of 154.905 ± 0.056, 80.395 ± 0.372, and 106.915 ± 0.153 %, respectively (IC₅₀ for Na-diclofenac was 54.128 ± 0.033%). Identification of active compounds by using Thin Layer Chromatography (TLC) for the chloroform extract, the highest hemolysis inhibitory activity, showed that its containing alkaloid, phenolic, flavonoid, steroid, and terpenoid. In conclusion, the chloroform extract of *Navicula salinicola* had potent anti-inflammatory activity.

Keywords: *Navicula salinicola*, Microalgae, Indonesia, TLC, HRBC, Anti-inflammatory

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INTRODUCTION

Indonesia, as an archipelagic country, has a great diversity of marine life and has the potential to be developed. Various bioactive materials contained in marine biota have enormous potential for the supply of pharmaceutical industry raw materials. Compounds used for pharmaceuticals and nutraceuticals usually have pharmacological activities. Natural products from the marine-based on chemical structure and characteristics can be used for various purposes, including for nutraceuticals, pharmaceuticals, and as additives in other pharmaceutical industries.¹ Microalgae as one of the aquatic products has enormous potential to be developed, both in the food and pharmaceutical industries. Nature provides an abundant supply of microalgae, and the ease with which they may be grown has contributed to the rapid development of microalgae as raw materials in the pharmaceutical and food industries.² In addition, microalgae are also known to have a variety of complete chemical compositions that are beneficial to the body.³ Microalgae have been identified as a natural source of antioxidants that can be used to treat inflammatory skin illnesses that are becoming increasingly common in today's culture.⁴ The inflammatory process is the body's attempt to neutralize and eliminate harmful chemicals at the site of injury and prepare a situation that can be used for network repair. Tissue injury and infection can cause inflammation. The inflammatory process has symptoms that include rubles (redness), callor (heat), tumors (swelling), dolor (pain), and function loss (changes in function).⁵ Inflammation medications include both steroid anti-inflammatory drugs and non-

steroidal drugs. However, the use of these drugs has gastrointestinal side effects that can cause erosion of the gastric mucosa.⁶ Due to the emergence of side effects from these drugs, it is necessary to conduct research to find alternative therapies that have undesirable drug reactions. One of these alternative therapies can be taken from natural materials, both biological and marine, that have efficacious pharmacological activities. *Navicula salinicola*, as one of the brown algae, is rich in fucoxanthin and other photosynthetic pigments, namely chlorophyll a and c⁷, β -carotene, and violaxanthin.⁸ Aside from being a coloring agent, chlorophyll and carotenoid pigments, in general, have been widely studied, and their efficacy has been identified in overcoming various diseases. In numerous studies, it has been demonstrated that chlorophyll derivatives contain a number of biological actions that can be used to prevent and treat cancer, including antioxidant activity, antiproliferative activity, antimutagenic activity, xenobiotic detoxification, and induction of cell death. Chlorophyll and its derivatives are potential photosensitizers that have been developed in an effort to prevent and treat cancer.⁹ Anti-inflammatory and antioxidant action have also been found in carotenoids in the field of health, which have an impact on improving the immune system, preventing cancer, antiobesity, and various other benefits, including provitamin A.¹⁰ According to this description, the purpose of this paper was to undertake an *in vitro* anti-inflammatory evaluation of the marine microalga *Navicula salinicola* using the HRBC method.

EXPERIMENTAL

Materials

This study's equipment was commonplace, namely glass and non-glass equipment, as well as other special equipment. The glassware used includes a measuring cup, beaker, funnel, dropper, stirring rod, saucer, dark glass bottle, and a 1 L capacity glass bottle. The long L-shaped glass pipe in this study is used for aeration and the short one for air out and retrieval of the sample. As for other equipment used, fluorescent lamps, aerators, plastic hoses, micropipettes measuring 10-100 μ L and 100-1,000 μ L, hemocytometers, microscopes, timers, analytical balances, spatulas, ovens, autoclaves, Bunsen, vials, salinity gauges, and UV-Vis Spectrophotometer type UV 1800 by Shimadzu. The microalgae used in this study was an axenic culture of *Navicula salinicola* obtained from the Biochemistry Laboratory, Faculty of Mathematics and Natural Sciences, ITB Bandung. Other materials used in this study include equates, 70% alcohol, salt, filter paper, fat cotton, gauze, aluminum foil, Walne media, Alsever solution from Sigma-Aldrich, *n*-hexane p.a. from Sigma-Aldrich, chloroform p.a. from Sigma-Aldrich, 96% ethanol p.a. from Sigma-Aldrich, phosphate buffer pH 7.4 from Sigma-Aldrich, isosaline solution from Sigma-Aldrich, and human red blood cells. By the Helsinki Declaration, the Health Research Ethics Commission of the Faculty of Medicine at the University of Padjadjaran has approved this project, which has been assigned the registration number 0219010013 and the SK number 208/UN6.KEP/EC/2019. An informed consent form has been completed by each of the subjects.

Cultivation and Biomass Collection

Activation and cultivation of *Navicula salinicola* microalgae were carried out in a simple photo bioreactor filled with seawater with the addition of Walne medium as a 0.1% (v/v) nutrient solution. With aeration for 24 hours at a photoperiod of 12:12 (dark: light) with a light intensity of 10,000 lux and room temperature ($\pm$ 25-27 °C), the salinity of the seawater medium was maintained at a level of 25 parts per million (ppm). The activation procedure itself lasts three days. Microalgae cultivation was carried out by a batch technique with a culture inoculum cell density of 150,000 cells/mL, measured using a hemocytometer under a microscope. Cultivation on the Walne medium was carried out for $\pm$ 7 days, then harvesting was done. A centrifuge was used to harvest at a speed of 6,000 revolutions per minute at 4°C for 20 minutes. The pellet (wet biomass) was collected and weighed after the supernatant was removed.¹¹ The collected wet biomass was dried using a freeze dryer for 24 hours.

Extraction

Twenty grams of dried biomass from *Naviculla salinicola* were extracted by multilevel maceration using *n*-hexane, chloroform, and ethanol (96%). In the first stage, extraction using *n*-hexane was sonicated for 5 $\times$ 10 minutes and then stirred using a stirrer for 1 minute. The maceration process was by replacement of solvents every 1 $\times$ 24 hours and took 3 days to get the amount of extract solution. The extract solution was collected and then evaporated until a viscous extract was obtained.

Blood Cell Preparation and Anti-Inflammatory Evaluation

Blood was taken from volunteers who had not used NSAIDs for 2 weeks. 10 mL of blood was inserted into a tube that contained 10 mL of alsever solution. For ten minutes at 27°C, the blood and alsever solution mixture were centrifuged at 3000 rpm. A sterile pipette was used to separate the supernatant. Isosaline solution is used to wash the residual blood cells, which are then re-centrifuged. This procedure was repeated four times until the isosaline was free of any trace of color. Red blood cell volume was measured and resuspended in isosaline to produce a 10% (v/v) red blood cell suspension. If the blood cell suspension has not yet been used, it is kept at 4 °C.¹² Evaluation of anti-inflammatory activity *in vitro* was carried out using the red blood cell membrane stabilization method and compared to diclofenac sodium. Before moving on to subsequent steps in the dilution series (e.g., series dilution: 20, 40, 60, 80, 100, and 120 ppm). There were two mL hyposaline solution, 0.15 M buffer (pH 7.4) of sodium phosphate, 0.5% (v/v) of red blood cell suspension, and 1.0 mL of the test sample and standard solution in the mixture. After being centrifuged at 3,000 rpm for 30 minutes, the test mixture was incubated for 30 minutes at 37 °C before being analyzed. Measurement of the solution's absorbance is done at 560 nm. The formula was used to calculate the percentage of hemolysis inhibition.¹³

$$\% \text{ Hemolysis Inhibition} = 100\% \times \left(\frac{A_1 - A_2}{A_1} \right)$$

Where A_1 is the absorbance of the control solution used in the test and A_2 is the absorbance of the test solution or standard test solution used in the test.

Identification of Active Compounds

Extracts with the highest anti-inflammatory activity were identified for their active compounds by using TLC coating with silica gel F₂₅₄ using the appropriate mobile phase. Then the plate was squashed using a spotting visualization, namely 10% H₂SO₄ in methanol, 5% FeCl₃, 5% AlCl₃ in methanol, and cytochrome in ethanol.

RESULTS AND DISCUSSION

Cultivation and Biomass Collection

Cultivation was carried out to collect as much biomass as possible. A Pure microalgae culture of *Navicula salinicola* was obtained from the Biochemical Laboratory of ITB. Early microalgae culture was obtained in an axenic state that only had one type of microalgae in the liquid medium stock. There were no microalgae or other contaminants, such as fungi, bacteria, or protozoa.¹³ The observations of *Navicula salinicola* cells under a microscope with a magnification of 40× can be seen in Figure-1A. *Navicula salinicola* has an oblong shape and a golden color, and it is classified as a Pennale due to its bilateral symmetry.¹⁴

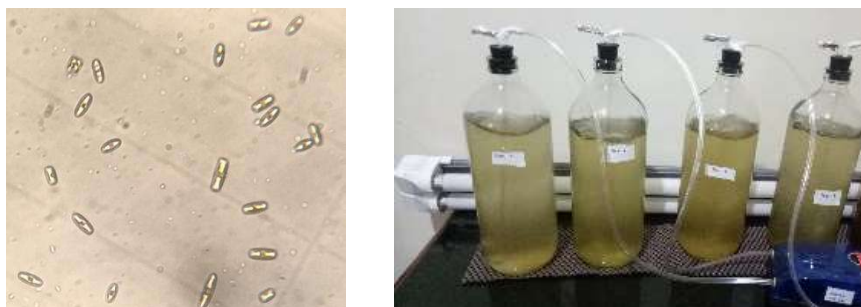


Fig.-1: A. *Navicula salinicola* cells B. A simple photobioreactor was used to cultivate *Navicula salinicola*

Cultivation of *Navicula salinicola* was carried out in a simple photobioreactor (Fig.-1B). The lighting comes from lamps that are adjusted to the optimum conditions for *Navicula salinicola* to grow well. This lamp light was used as a substitute for sunlight, which is the source of photons in the photosynthesis process of microalgae cells. Aeration given to the photobioreactor was used as a source of CO₂ for microalgae to carry out photosynthesis. In addition, the aeration also functions as a homogenizer of nutrients and light in the photobioreactor, preventing cell precipitation. The harvest time was determined from the results of the growth curve, which indicated the optimum growth time on the 7th day. Growth curves were performed by

microalgae cell calculation methods using a haemocytometer. The growth curve was generated in 15 days because, after the 15th day, the *Navicula salinicola* started to enter the death phase. Fig.-2 shows the data growth curves for *Navicula salinicola*.

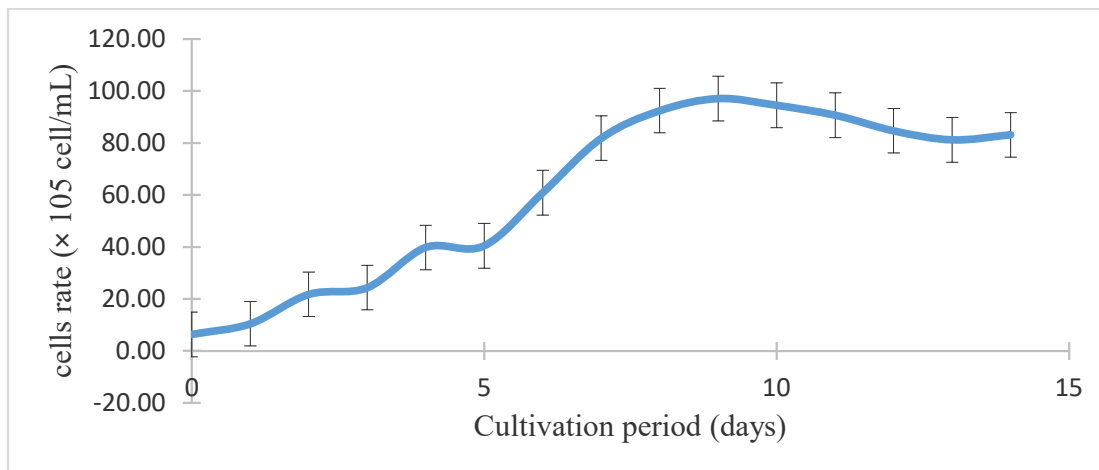


Fig.-2: Growth Curve for Microalgae *Navicula salinicola*

Microalgae cultivation was carried out using the Walne medium, also known as a rich medium that contains all the micro, macro, and vitamin minerals needed for the growth of marine microalgae. Harvesting is done by the 5,000-rpm speed method for 20 minutes at 4°C. The centrifugation technique is a cell harvesting technique that is considered more effective and efficient to be carried out for harvesting small cells. Except for the fact that centrifuged cells were intact, there was no difference in cell composition between centrifuged and non-centrifuged cells. To eliminate water from the microalgae sample, the microalgae were freeze-dried, resulting in dry biomass (Fig.-3). Microalgal biomass samples can be preserved for a long period without being overrun by fungi by lowering the water concentration in the sample.



Fig.-3: Typical of Dried Biomass

Extraction

Navicula salinicola dried microalgae were extracted using a multi-level maceration process using *n*-hexane, chloroform, and ethanol (96 percent). To avoid the breakdown of active compounds, present in the sample by temperature, the maceration method was chosen, as this approach does not require heating. With multilevel maceration, vast amounts of polarity-varying yield can likewise be produced in large quantities. Before maceration, cell splitting was carried out in a physical way, namely with ultrasonic waves using a sonicator. The sound waves produced can cause cavitation, which is the formation of gas bubbles that move at high speeds so that mechanical energy is formed. The cavitation effect is widely used to disrupt microalgae cells and mechanical energy that causes cell wall rupture.^{15,16}

Anti-Inflammatory Evaluation

In vitro Anti-inflammatory activity of marine microalgae, *Navicula salinicola* can be performed *in vitro*, one of them using the method of red blood cell membrane stability. In this anti-inflammatory test, using red blood cell membranes, because red blood cell membranes are similar to lysosomal membranes, which can

influence the inflammatory process.¹⁷ Lysosome stability is critical in reducing the inflammatory response, namely by blocking the release of enzymes from within the lysosomes throughout the inflammatory process. In the anti-inflammatory test of marine microalgae, *Navicula salinicola* extract was first made into a solution with a concentration of 1000 ppm dissolved with an isosalin solution.

Table-1: Yield of *Navicula salinicola* by using Various Solvents

Solvent	Extract weights (gram)	Yield (%)
<i>n</i> -Hexane	5.066	25.330
Chloroform	4.295	21.475
Ethanol	3.282	16.410

The anti-inflammatory activity of the sample was indicated by the decrease in absorbance of the test sample. The greater the concentration used, the smaller the absorbance, so the stability of the membrane is greater. The lower the absorption measured in the test solution mixture, the more stable the red blood cell membrane and the less lysis.¹⁸ The absorbance data acquired from the measurement result were as used to calculate the percent inhibition of hemolysis of red blood cells. This percentage of inhibition represents a sample's ability to stabilize red blood cells and was calculated by dividing the absorbance of the test control by the absorbance of the test solution. The results of the tests revealed that the highest concentration in the stabilization of the red blood cell membrane or suppression of hemolysis in chloroform extract with a concentration of 120 ppm was 75.602 percent, compared to diclofenac sodium 100 ppm by 71.268 percent. Table-2 shows the results of assessing the anti-inflammatory activity of *Navicula salinicola* extract.

Table-2: Hemolysis Inhibition by *Navicula salinicola* Extract

Concentration (ppm)	Inhibition extract (%)		
	<i>n</i> -Hexane	Chloroform	Ethanol
20	10.032 ± 0.303	6.300 ± 0.069	14.286 ± 0.139
40	22.632 ± 0.069	21.429 ± 0.139	19.944 ± 0.139
60	27.408 ± 0.208	37.119 ± 0.069	30.899 ± 0.208
80	30.778 ± 0.120	53.371 ± 0.069	36.356 ± 0.069
100	33.186 ± 0.120	62.480 ± 0.069	45.506 ± 0.139
120	40.289 ± 0.120	75.602 ± 0.358	57.945 ± 0.069
$IC_{50} = 154.905 \pm 0.056$ $IC_{50} = 80.295 \pm 0.372$ $IC_{50} = 106.915 \pm 0.153$			

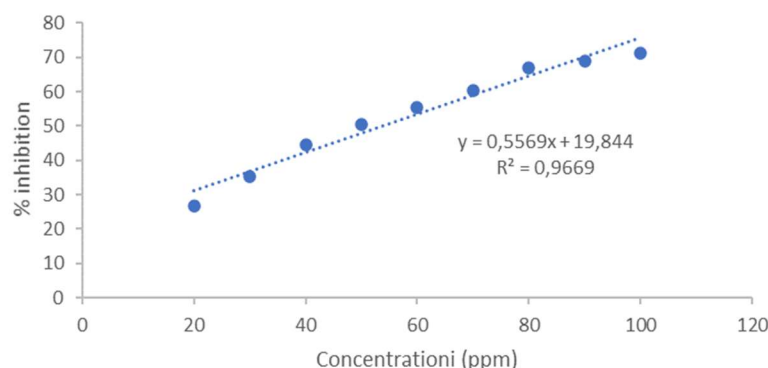


Fig.-4: Standard Calibration Curves For Hemolysis Inhibition by Using Diclofenac Sodium

An IC_{50} value was obtained from a linear regression equation (Fig.-4). Based on the linear regression equation, the IC_{50} values of diclofenac sodium, *n*-hexane, chloroform, and ethanol extract were 54.128, 154.905, 80.295, and 106.915 ppm, respectively. The smaller the IC_{50} value, the greater the anti-inflammatory activity. Based on the results obtained, IC_{50} values of diclofenac sodium, chloroform extract,

and ethanol extract are classified as having anti-inflammatory activity. Classification of an anti-inflammatory activity has a potential IC_{50} value when its less than 50 ppm, classified as active when the IC_{50} value is 50-100 ppm, and classified as a moderate activity if the IC_{50} values are 101-250 ppm. ppm, and is classified as weak if the IC_{50} value is 250-500 ppm, and is classified as inactive if the IC_{50} value is greater than 500 ppm.¹⁹

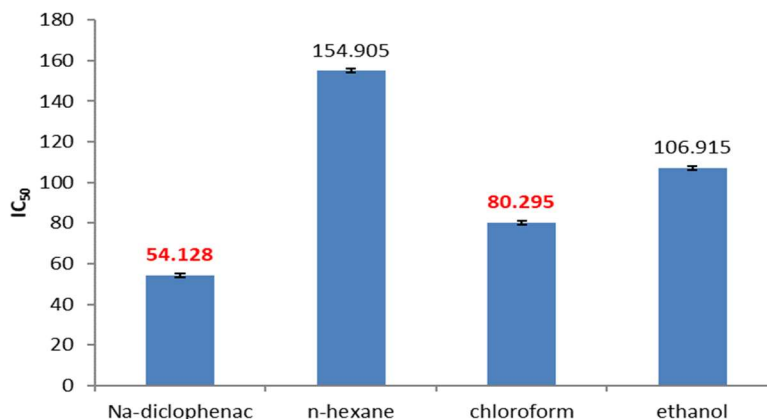


Fig.-5: Anti-inflammatory Properties of *Navicula salinicola* Extracts

Identification of Active Compounds

The results of the anti-inflammatory test on the *Navicula salinicola* extract showed that the chloroform extract had the highest hemolysis inhibitory activity compared to the *n*-hexane and ethanol extracts. According to the results of TLC monitoring, chloroform extract sprayed with Dragendroff spot reagents produces orange-brown spots that indicate the presence of alkaloid compounds. On the detection of compounds with $FeCl_3$ patches, reagents showed positive results with a change from orange to black or dark with a yellow background, stating that the sample contained phenol compounds. Furthermore, detection with $AlCl_3$ spotting appearance reagents showed positive results by causing yellow to orange patches, indicating that the sample contained a class of flavonoid compounds. For the monitoring of steroid and triterpenoid compounds, the detection of compounds using the Lieberman-Bouchard's spot marking reagent shows green spots, indicating that they contain steroid compound groups. Further detection using Lieberman Bouchard's spotting reagent showed green spots in *n*-hexane and chloroform extracts that contained positive-containing compounds, and we found purple spots on chloroform extracts that contained steroids and triterpenoids. The result of staining can be seen in Fig.-5 and Table-4.

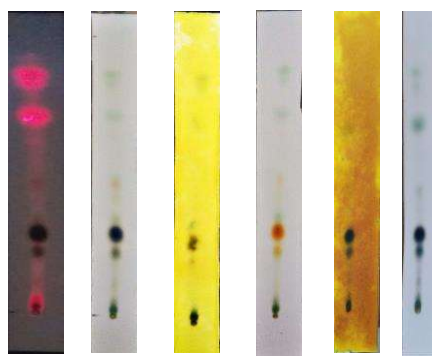


Fig.-6: The Results of Monitoring of Chloroform Extract With The Mobile Phase of *n*-hexane: Ethyl Acetate: Chloroform (5: 4: 1, v/v). The Spots Were Visualized Using The Following Techniques: (i) UV 365 nm, (ii) H_2SO_4 , (iii) $FeCl_3$, (iv) $AlCl_3$, (v) Dragendroff and (vi) Lieberman-Bouchard

According to the results of the study, the analysis of the extracts, shows that the extract contains flavonoid components. The extract also contains other beneficial chemicals such as DHA, EPA,²⁰ and carotenoid in addition to the flavonoid.²¹ Several investigations have demonstrated that there is a link between the chemicals' ability to stabilize membranes and their chemical composition.²² Flavonoids are compounds that have anti-inflammatory activity by protecting the erythrocyte membrane against membrane damage,

causing hemolysis because flavonoids can inhibit inflammatory mediators and free radicals.²³ Flavonoid compounds have the ability to inhibit the work of the enzymes cyclooxygenase and lipoxygenase in converting arachidonic acid into prostaglandins, which are inflammatory mediators.²⁴ Also assumed to be responsible for the stability of the red blood cell membrane in the presence of hemolysis-induced hypotonic solutions include flavonoids, EPA, DHA, and carotenoid chemicals, among other molecules.

Table-4: Summary of The Identification of Compounds in Chloroform Extract

Compound	Labeled
Alkaloid	+
Phenolic	+
Flavonoid	+
Steroid	+
Terpenoid	+

Note: + was identified

In conclusion, the marine microalgae extract *Navicula salinicola* is known to have anti-inflammatory activity. The IC₅₀ value of diclofenac sodium was $54.128 \pm 0.033\%$, while *n*-hexane extract, chloroform extract and ethanol extract were 154.905 ± 0.056 , 80.395 ± 0.372 , and $106.915 \pm 0.153\%$, respectively. Chloroform extract has the most active anti-inflammatory activity because the IC₅₀ value closest to the diclofenac sodium.

CONCLUSION

We have given our findings on the cultivation, extraction, and anti-inflammatory evaluation of *Navicula salinicola*, which were conducted using HRBC. After seven days of cultivation, the *Navicula salinicola* was found to be in optimal condition. The chloroform extract was shown to be the most effective at inhibiting hemolysis and contained alkaloid, phenolic, flavonoid, steroid, and terpenoid compounds.

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REFERENCES

1. I. Setyaningsih, L. Hardjito, D.R. Monintja, M.F.A. Sondita, M. Bintang, N. Lailati, and L. Panggabean, *Jurnal Biologi Indonesia*, **5(1)**, (2017), <https://doi.org/10.14203/jbi.v5i1.3203>
2. E. NT, *Journal of Nutrition & Food Sciences*, **06**, 507(2016), <https://doi.org/10.4172/2155-9600.1000507>
3. M. Mourelle, C. Gómez, and J. Legido, *Cosmetics*, **4**, 46(2017), <https://doi.org/10.3390/cosmetics4040046>
4. W.-T. Choo, M.-L. Teoh, S.-M. Phang, P. Convey, W.-H. Yap, B.-H. Goh, and J. Beardall, *Frontiers in Pharmacology*, **11**, 1086(2020), <https://doi.org/10.3389/fphar.2020.01086>
5. R.A. Akmalia, H. Hajrah, and L. Rijai, AKTIVITAS ANTIINFLAMASI EKSTRAK RIMPANG TEMU KUNCI (*Boesenbergia pandurata*) SECARA IN-VITRO, in: Proceeding 4th Mulawarman Pharm. Conf., Fakultas Farmasi, Universitas Mulawarman, Samarinda, 2016, <https://doi.org/10.25026/mpc.v4i1.195>
6. M. Ghasemian, S. Owlia, and M.B. Owlia, *Advances in Pharmacological Sciences*, **2016**, 1(2016), <https://doi.org/10.1155/2016/9130979>
7. R.R. Ambati, D. Gogisetty, R.G. Aswathanarayana, S. Ravi, P.N. Bikkina, L. Bo, and S. Yuepeng, *Critical Reviews in Food Science and Nutrition*, **59**, 1880(2019), <https://doi.org/10.1080/10408398.2018.1432561>
8. I. Telussa, Rusnadi, and Zeily Nurachman, *Algal Research*, **41**, 101530(2019), <https://doi.org/10.1016/j.algal.2019.101530>
9. H. Abrahamse, and M.R. Hamblin, *Biochemical Journal*, **473**, 347(2016), <https://doi.org/10.1042/BJ20150942>
10. C. Galasso, C. Corinaldesi, and C. Sansone, *Antioxidants*, **6**, 96(2017),

- <https://doi.org/10.3390/antiox6040096>
11. P. Lavens, and P. Sorgeloos, Manual on the Production and Use of Live Food for Aquaculture, Food and Agriculture Organization (FAO)., 1996.
 12. S. Yesmin, A. Paul, T. Naz, A.B.M.A. Rahman, S.F. Akhter, M.I.I. Wahed, T. Bin Emran, and S.A. Siddiqui, *Clinical Phytoscience*, **6**, 59(2020), <https://doi.org/10.1186/s40816-020-00207-7>
 13. D. Kurnia, *Jurnal Kartika Kimia*, **3**, 53(2020), <https://doi.org/10.26874/jkk.v3i2.65>
 14. J. D. Wwhr, *Journal of Phycology*, **43**, 412(2007), <https://doi.org/10.1111/j.1529-8817.2007.00335.x>
 15. M. Kurokawa, P.M. King, X. Wu, E.M. Joyce, T.J. Mason, and K. Yamamoto, *Ultrasonics Sonochemistry*, **31**, 157(2016), <https://doi.org/10.1016/j.ultsonch.2015.12.011>
 16. V.J. Skinner, D. Lekkas, T.A. Winning, and G.C. Townsend, *Creative Education*, **03**, 890(2012), <https://doi.org/10.4236/ce.2012.326134>
 17. M. Gebrelibanos, *Momona Ethiopian Journal of Science*, **4**, 16(2012), <https://doi.org/10.4314/mejs.v4i2.80113>
 18. H. Chairul, and E. Putri, uji aktivitas antiinflamasi ekstrak daun kelor (*moringa oleifera* lam.) dengan metode Stabilisasi membran sel darah merah secara *In vitro* , Uin Syarif Hidayatullah Jakarta, 2013.
 19. C.A. Burtis, E.R. Ashwood, and D.E. Bruns, Tietz Textbook of Clinical Chemistry and Molecular Diagnostics-e-book, Elsevier Health Sciences, 2012.
 20. Z. Montero-Lobato, M. Vázquez, F. Navarro, J. Fuentes, E. Bermejo, I. Garbayo, C. Vilchez, and M. Cuaresma, *Marine Drugs*, **16**, 478(2018), <https://doi.org/10.3390/md16120478>
 21. M. Raposo, A. de Moraes, and R. de Moraes, *Marine Drugs*, **13**, 5128(2015), <https://doi.org/10.3390/md13085128>
 22. T.A. Conde, I. Zabetakis, A. Tsoupras, I. Medina, M. Costa, J. Silva, B. Neves, P. Domingues, and M.R. Domingues, *International Journal of Molecular Sciences*, **22**, 9825(2021), <https://doi.org/10.3390/ijms22189825>
 23. S.J. Maleki, J.F. Crespo, and B. Cabanillas, *Food Chemistry*, **299**, 125124(2019), <https://doi.org/10.1016/j.foodchem.2019.125124>
 24. A.N. Panche, A.D. Diwan, and S.R. Chandra, *Journal of Nutritional Science*, **5**, e47(2016), <https://doi.org/10.1017/jns.2016.41>

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