ANTIGENOTOXIC ACTIVITY OF *GRACILARIA* SP. ON ERYTHROCYTES OF NILE TILAPIA EXPOSED BY METHOMYL-BASED PESTICIDE

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ABSTRACT

This research investigates the biochemical profile, antioxidant properties, and antigenotoxic activity of red seaweed (*Gracilaria* sp.) extract on micronuclei formation in peripheral blood erythrocytes of tilapia (*Oreochromis niloticus*) exposed to a methomyl-based pesticide. Results revealed a diverse phytochemical profile in the red seaweed extract, with the presence of alkaloids, triterpenoids, steroids, tannins, coumarins, terpenoids, quinine, phytosteroids, flavonoids, and phlobtannins. GC-MS analysis identified compounds such as Heptadecane, Neophytadine, Hexadecanoic acid, Oleic acid, and Adipic acid in the sample. The antioxidant activity exhibited a concentration-dependent response, demonstrating increased strength with higher extract concentrations. Antigenotoxic analysis indicated a significant reduction in micronuclei frequency in peripheral erythrocytes of tilapia. In conclusion, the research underscores the potential of red seaweed extract as a valuable natural resource with antioxidant properties and a mitigating effect on genotoxicity induced by pesticide exposure in aquatic organisms.

Key words: antigenotoxicity, biochemical, gc-ms analysis, micronucleus, gracilaria.

النشاط المضاد للسموم الجينية لـ *GRACILARIA* SP. من منطقة تربية الأحياء المائية الفرعية بمنطقة محمية باندا البحرية، على كريات الدم الحمراء لسمك البلطي النيلي المعرضة للمبيدات الحشرية التي تحتوي على الميثوميل

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المستخلص

يهدف البحث دراسة الصفات الكيميائية الحيوية ، وخصائص مضادات الأكسدة، والنشاط السمي المضاد للجينات لمستخلص الأعشاب البحرية الحمراء (*Gracilaria* sp.) على تكوين النوى الصغيرة في كريات الدم الحمراء الطرفية في أسماك البلطي (*Oreochromis niloticus*)المعرضة لمبيد آفات يحتوي على الميثوميل .كشفت النتائج عن وجود تنوع كيميائي نباتي في مستخلص الأعشاب البحرية الحمراء، مع وجود قلويدات، ترايتيربينويدات، ستيرويدات، عفص، كومارين، تربينويدات، كينين، فيتوستيرويدات، فلافونيدات، وفلوبتانينات .حدد تحليل GC–MS مركبات مثل هيبتاديكان، نيوفيتادين، حمض الهيكساديكانويك، حمض الأوليك، وحمض الأديبيك في العينة .أظهر نشاط مضادات الأكسدة استجابة تعتمد على التركيز، مما يدل على زيادة القوة مع تركيزات أعلى من المستخلص .أشار تحليل السمية المضادة للجينات إلى انخفاض كبير في تردد النوى الصغيرة في كريات الدم الحمراء المحيطية لسمك البلطي .في الختام، يؤكد البحث على إمكانات مستخلص الأعشاب البحرية الحمراء كمورد طبيعي قيم مع خصائص مضادة للأكسدة وتأثير مخفف على السمية الجينية الناجمة عن التعرض للمبيدات الحشرية في الكائنات المائية.

الكلمات المفتاحية: السمية المضادة للجينات، الكيمياء الحيوية، تحليلGC–MS ، النواة الصغيرة، الجراسيلاريا

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INTRODUCTION

Pesticide widely used for pest control and boost agricultural production (15). Methomyl is a broad-spectrum pesticide that is widely used on agriculture, plantations and ornamental plant cultivation. This pesticide is substitute for organophosphorus pesticides and effective to counter various insect pests. The half-life of methomyl base pesticide in aquatic environments is reported to be 6–262 days (2,3). Methomyl has caused serious pollution problems due to its abuse and also causes pest resistance. Several insects such as Bemisia tabaci, cotton bollworm, and Helicoverpa armigera have been reported to develop resistance to methomyl (2). Additionally, there have been numerous reports on the pesticide's harmful effects on various non-target pests. Methomyl, recognized as a potent genotoxic substance, is capable of causing DNA damage and apoptosis in vitro at concentrations below lethal levels, specifically in the range of 30–34 mg/kg (4). Methomyl based pesticide significantly increases the concentration of that damage DNA and lead to genetic mutations (5). Toxicity assessment of fish showed serious issues (in fact; methomyl caused significant genetic damage to the tissues (6,7). Methomyl can cause genetic demage on tilapia and at higher concentrations can destroy the enzyme activity of tilapia liver (8). This pesticide also cause mass death of Damphnia magna (9). Published research reported that short-term exposure (96 h) of methomyl caused no death in adult male Gammarus fossarum but resulted in 66% inhibition of acetylcholinesterase (AChE) activity (10). However, long-term exposure to methomyl can cause serious adverse effects to the nontarget organisms (11). Marine macroalgae are important ecologically and commercially to many regions of the world, especially in Asian countries such as China, Japan and Korea (12). Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites (13). The marine macroalga synthetize a variety of compounds such as carotenoids, terpenoids, xanthophylls, chlorophyll, vitamins, saturated and polyunsaturated fatty acids, amino acids, acetogenins, antioxidants such as polyphenols, alkaloids, halogenated compounds and polysaccharides such as agar, carrageenan, proteoglycans, alginate, laminaran, rhamnan sulfate, galactosyl glycerol and fucoidan. These compounds probably have diverse simultaneous functions for the seaweeds and can act as allelopathic, antimicrobial, antifouling, and herbivore deterrents, or as ultraviolet-screening agents (14,15). Nowadays seaweed represents several biomedical compounds. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activity have been detected in green, brown and red algae. (16,17, 25). Published research showed that the genus Gracilaria was the most attractive candidate because of its ability to achieve high yields and while producing commercially valuable extracts (18). Red seaweed (Gracilariales, Rhodophyta) is a group of macroalgae with more than 300 species and about 160 have been accepted taxonomically. The red seaweed species are of great importance for the biotechnological and industrial uses due to they phycocolloids, the main source of agar α-(1,4)-3,6-anhydro-l-galactose and β-(1,3)-d-galactose with little esterification in cell wall (19). The research aims to investigate the biochemical profile, antioxidant properties, and antigenotoxic activity of red seaweed (*Gracillaria* sp.) extract on the micronuclei formation in peripheral blood erythrocytes of tilapia (*Oreochromis niloticus*) exposed to a methomyl-based pesticide. Essentially, the study seeks to understand how the seaweed extract may impact the fish's blood cells in the presence of the pesticide, focusing on potential protective effects, such as antioxidative properties and mitigation of genotoxic damage. This research contributes to our knowledge of natural compounds, like red seaweed extract, that may offer protective benefits in the context of environmental stressors like pesticides.

MATERIAIS AND METHODS

Seaweed extract preparation: The red seaweed (*Gracilaria* sp.) (Figure1) was collected from Integrated Multi-Trophic Aquaculture (IMTA) System at Aquaculture Sub-Zone of the Banda Marine Conservation Area. The samples were thoroughly washed

with fresh water to eliminate salt and another substance. Subsequently, they were soaked in room temperature freshwater overnight and then air-dried for three days in greenhouse. The methanolic extract was prepared following established methods (14,15,20,21). Initially, the dried seaweed underwent grinding into a powder using a blender, followed by freeze-drying and storage in polythene bags before the extraction process. Various solven has been studied for several chemical analysis (22,23). For this extraction, 30 grams of seaweed powder were extracted with 500 ml of 100% ethanol using a Soxhlet apparatus for 24 hours, and the resulting solution was evaporated in a rotary evaporator at 60 °C until dry. The obtained extracts were stored at -20 °C until needed, with an observed yield of 3.5%. A stock solution of the extract (100 mg/ml) was prepared in sterile Milli-Q water. The furanone (C-30) compound, specifically [(5Z)-4-bromo-5-(bromomethylene)-2(5H)-furanone], was procured from Sigma-Aldrich in Buchs, Switzerland. This furanone was dissolved in absolute ethanol to achieve a concentration of 50 mg/ml and was stored at -20°C. The stock solution was further diluted in media to create a working solution.

Figure 1. Red seaweed (*Gracilaria* sp.) from Integrated Multi-Trophic Aquaculture (IMTA) System at Aquaculture Sub-Zone of the Banda Marine Conservation Area

Yield and phytochemical Analysis: The measurement of the extracted soluble mass was conducted in relation to the initial mass of powdered red seaweed, as outlined in a previously published methods (24). Phytochemical tests of red seaweed (*Gracilaria* sp.) extracts include Alkaloids, Triterpenoids, Steroids, Tannin, Saponin, Coumarins, Terpenoidas, Quinine, Phytosteroids, Flavonoids, Phlobtannins (20,25), which are carried out using published methods.

Biochemical compound analysis

The crude extract of red seaweed (*Gracillaria* sp.) was partially purified via thin layer chromatography (TLC) plates (Merck, Germany) using the solvent system chloroform: ethyl acetate: methanol (6:3:1). Next, the partially purified fraction was loaded onto a silica gel packed column (Hi-media, India) (20 cm long and 2 cm diameter) and eluted with n-hexane: ethyl acetate (50:50 v/v). Fractions were characterized by gas chromatography (GC-2010) connected to a quadrupole mass spectrometer analyzer (QP-2010) (Shimadzu, Japan) to determine their chemical content using an Rtx-PCB capillary column (identity 60 m × 0.25 mm, film thickness 0 .25 mm, RESTEK, Bellefonte, PA). GCMS is widely used for biochemical determinations. Helium with a purity of 99.99% was used as the carrier gas at a flow rate of 1 ml/min. One ml of extract was injected in a split mode using an autosampler. The injector port, interface and ion source temperatures were set at 250, 270 and 230 °C, respectively. GC temperature was programmed as follows: 50 °C (1 min), 10 °C (1 min) ramp to 320 °C (10 min hold). The mass spectrometer was operated in electron ionization (EI) mode at 70 eV and at an emission current of 60 mA. Full scan data were obtained in a mass range of m/z 50–500. Interpretation of mass spectrum analysis was done by using database of PubChem. The spectrums of the unknown components were compared with the spectrum of known components stored in the library (26).

Antioxidant activity using DPPH assay

The antioxidant activity of red seaweed (*Gracillaria* sp.) extracts was measured using the stable radical, DPPH as a standard reagent. This was determined as described by published methods (27) with minor modifications. Briefly, stock solutions of the red seaweed (*Gracillaria* sp.) extracts were prepared in methanol. Dilutions were made to obtain concentrations from 0.1 to 1.0 mg/ml. Diluted solutions (2.0 ml) were mixed with 2.0 ml of

0.16 mM DPPH in methanol. The mixtures were shaken vigorously and maintained for 30 min at room temperature in the dark. The absorbance (OD) of mixtures was measured at 517 nm against a reagent blank by using a UV–Vis spectrophotometer. The tests were performed in triplicate. The plot of scavenging activity of DPPH was recorded and the IC50 value (concentration of the sample to scavenging 50% of the DPPH radicals; mg/ml) was then calculated.

Animal preparation

A Methomyl base pesticide was procured from the agricultural market in Batu, East Java, Indonesia. Tilapia specimens, measuring 9 to 12 cm, were obtained from the Freshwater Laboratory of Sumberpasir, Malang, East Java. Genotoxicity tests and micronucleus assays were conducted at the Fish Cultivation Laboratory within the Division of Parasites and Fish Health, Faculty of Fisheries and Marine Sciences, Brawijaya University. The acclimatization of the fish involved placing them in a tank and providing commercial feed once a day. After a holding period of 14 days, the fish were divided into six groups of 10 individuals each. They were then transferred and acclimatized in an aquarium equipped with an aeration system (dimensions: 60x30x25cm) for a duration of 2 days. If the mortality rate in the fish population remained below 3% over the next 48 hours, the Carp population treatment would be deemed suitable for testing. However, if the mortality exceeded 3%, the affected fish would be replaced with new specimens from the holding tank and reacclimatized for an additional 2 days.

Observation of antigenotoxicity: In Phase 1 of the research, the aquariums (60x30x30 cm) were prepared and labeled for control (without treatment) and test (three replications each). Fresh water was refilled into each aquarium. Tilapia fish were acclimated and then transferred into the designated aquariums, with ten fishes in each. Methomyl-based pesticide, previously measured, was dissolved in each aquarium. The fish were allowed up to 2 x 24 hours before sampling their blood in each aquarium. In Phase 2 of the research, the aquariums (60x30x30 cm) were prepared and labeled for test and control (three replications each). Fresh water was refilled into these aquariums. The remaining fish from Phase 1 were gently transferred into the designated aquariums. Extract of red seaweed (*Gracillaria* sp) was dissolved in each aquarium, with concentrations including Negative Control, 25 ppm, 50 ppm, 100 ppm, 200 ppm, and positive control, modified from published concentrations (8). The fish were allowed up to 96 hours before sampling their blood in each aquarium. After treatment, peripheral erythrocyte blood from each fish group was sampled and smeared on clean microscope slides. After fixation in absolute methanol for about 20 min, the slides were air-dried and stained with 10% of giemsa for about 25 minutes then observed. Coding using a microscope (Olympus CX21) with 400x magnification to determine the frequency of micronucleus cell and another different pattern of morphologically altered erythrocyte and then counted as cell per 1000 (‰). The micronucleus frequency is then counted base on the published formulation (28)

Data analysis: The data that has been obtained were analyzed by using the ANOVA test. This data is used to determine how exposure to batik waste on levels of SOD (Superoxide Dismutase) in Tilapia. This ANOVA analysis used a confidence level of 95% and an error rate of 5%. The follow-up test used was the Duncan test

RESUITS AND DISCUSSION

Mass yield and phytochemical analysis: Fresh red seaweed (*Gracilaria* sp.) obtained from farmers as much as 1000 grams each (net weight) then carried out the drying process with the dry shade method. After 2x24 hours obtained dry weight as follows table 1.

Table 1. Dry weight and ethanol extract weight of red seaweed (*Gracilaria* sp.)

Repetitions	Dry weight (Gram)	Dry weight (%)	Ethanolic Extract (gram)	Ethanolic Extract (%)
1	810	81%	9.83	9.80%
2	740	74%	7.22	7.22%
3	790	79%	8.09	8.09%
Average	780	78%	8.38	8.38%

Based on table 1, the dry weight (net weight) in replicate 1 was 810 grams (81%), in replicate 2 was 740 grams (74%) and in replicate 3 was 790 grams (79%). The average dry weight (net weight) obtained from 1000 grams of sargassum is 780 grams (78%). Dried seaweed is then milled and the extraction process. The extraction process is done by maceration method with soaking period for 24 hours. While the solvent used is methanol (polar) with the ratio of solvent and dry *Gracilaria* material is 100gr:500ml (1:5 b/v). Simplisia obtained then evaporated using RV 10 digital rotary evaporator (temperature 50^{o}C and speed 80 rpm) until the resulting crude extract in the form of paste. Methanol liquid extract from red seaweed (*Gracilaria* sp.) after evaporation process produces concentrated methanol extract. Weight yield obtained in replicate 1 amounted to 9.83 grams (9.8%), in replicate 2 amounted to 7.22 grams (7.22%), in replicate 3 amounted to 8.09 grams (8.09%). The average weight of extract obtained from 100 grams of red seaweed powder (*Gracilaria* sp.) is 8.38 grams (8.38%). The phytochemical assay of the ethanolic extract of Red Seaweed (*Gracillaria* sp.) provides valuable insights into the diverse classes of secondary metabolites present in the seaweed. The results indicate the presence or absence of specific phytochemical compounds, contributing to the overall understanding of the potential bioactive constituents within the extract (table 2).

Phytochemical test of neem leaves: The results of the fitokima analysis qualitatively showed that the positive neem leaf extract contained several compounds namely flavonoids, tannins, saponins, and triterpenoids. More can be seen in table 3

Table 2. The results of a qualitative phytochemical analysis

Compounds	Confirmation
Alkaloids	+
Triterpenoids	+
Steroids	+
Tannin	+
Saponin	−
Coumarins	+
Terpenoidas	+
Quinine	+
Phytosteroids	+
Flavonoids	+
Phlobtannins	+

Note: + = present; − = absent

Alkaloids, triterpenoids, steroids, tannins, coumarins, terpenoids, quinine, phytosteroids, flavonoids, and phlobtannins were tested in this study. The confirmation of the presence of alkaloids, triterpenoids, steroids, tannins, coumarins, terpenoids, quinine, phytosteroids, flavonoids, and phlobtannins suggests a rich and diverse phytochemical profile in the ethanolic extract of Red Seaweed (*Gracillaria* sp.). These compounds are known for their potential bioactive properties and have been associated with various biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. The absence of saponins in the extract is noteworthy. Saponins are glycosides with known surfactant properties and have been linked to various pharmacological activities, such as anti-inflammatory and antifungal effects. The absence of saponins in the ethanolic extract may indicate that these specific properties are not prominent in this particular seaweed species or may be present in minimal quantities. The confirmation of the presence of quinine is interesting, as it is a compound known for its antimalarial properties. This finding adds potential therapeutic value to the red seaweed extract, suggesting it could be explored further for its antimalarial potential.

Biochemical compound analysis

The purification and characterization of active compounds play a pivotal role in understanding and harnessing the potential benefits of natural substances. In this study, we focus on the application of gas chromatography-mass spectrometry (GC-MS) as a powerful analytical technique for the identification and analysis of bioactive compounds. The integration of GC-MS enables the separation of complex mixtures and the precise determination of the molecular structure of individual compounds based on their mass spectra. As a preliminary step, the purification process aims to isolate the active compound of interest from the complex mixture. This step is crucial to enhance the specificity of the analysis and eliminate interfering substances. The subsequent characterization of the purified compound involves the use of GC-MS, a technique known for its high sensitivity and ability to

provide detailed information about the chemical composition of a sample. The combination of gas chromatography, which separates individual compounds, and mass spectrometry, which identifies and quantifies these compounds, allows for a comprehensive understanding of the active ingredient's structure and properties.

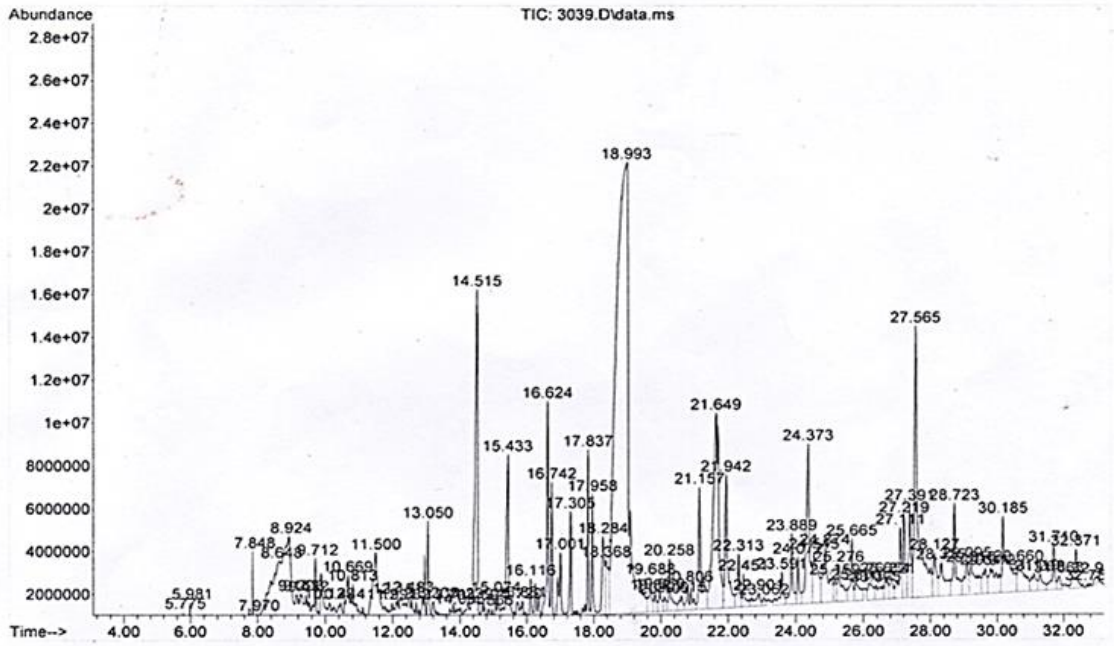

Figure 1. Purification and characterization of active compound of red seaweed (*Gracillaria* sp.) by gas chromatography-mass spectrometry (GC-MS)

Table 3. Biochemical compound of Red Seaweed (*Gracilaria* sp.)

Retention time (minutes)	% Area	Compound name	Chemical formula	Function
14.514	**3.69**	**Heptadecane**	**$C_{17}H_{36}$**	**Antioxidant (26)**
16. 625	**1.44**	**Neophytadine**	**$C_{20}H_{38}$**	**Antimicrobial and anti- inflammatory (26)**
18.994	**24.6**	**Hexadecanoic acid**	**$C_{16}H_{32}O_2$**	**Antioxidant (26)**
21.649	**4.82**	**Oleic acid**	**$C_{18}H_{34}O_2$**	**Antioxidant (26)**
24,373	**3. 12**	**Adipic acid**	**$C_6H_{10}O_4$**	**antioxidant, anti-inflammatory (26)**
27.626	**1.22**	**Hexadecanoic acid**	**$C_{16}H_{32}O_2$**	**Antioxidant (29,30)**

The gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of various compounds in the sample (Figure 1 and table 3), each identified based on their retention time, percentage area, compound name, chemical formula, and similarity to the instrument library. Notably, heptadecane ($C_{17}H_{36}$) was detected at a retention time of 14.514 minutes, constituting 3.69% of the total area. Heptadecane is recognized for its antioxidant properties, as corroborated by studies conducted by published methods (24). Neophytadine ($C_{20}H_{38}$) eluted at 16.625 minutes, contributing 1.44% to the overall composition. This compound has been associated with antimicrobial and anti-inflammatory effects, in line with findings by published research (31). Hexadecanoic acid ($C_{16}H_{32}O_2$) appeared at two distinct retention times: 18.994 minutes and 27.626 minutes, with percentage areas of 24.6% and 1.22%, respectively. Its prevalence aligns with its antioxidant properties, as evidenced by studies conducted by published article (29). Additionally, oleic acid ($C_{18}H_{34}O_2$) exhibited a retention time of 21.649 minutes, representing 4.82% of the total area. This compound is also recognized for its antioxidant effects, as reported by published research. Adipic acid ($C_6H_{10}O_4$) was identified at 24.373 minutes, constituting 3.12% of the area. The presence of these compounds in the sample underscores the potential health benefits associated with the analyzed substance, ranging from antioxidant and anti-inflammatory properties to antimicrobial effects. The reliability of the identifications is bolstered by high similarity percentages to the PubChem database (26), suggesting a robust analytical methodology. The findings contribute valuable insights into

the chemical composition of the sample and its potential applications in various fields, including medicine and food science

The antioxidant activity: This inhibition percentage is calculated based on the variance in absorbance between the DPPH absorbance and the sample absorbance, measured using a UV-Vis spectrophotometer. The level of antioxidant activity is denoted by the IC_{50} value, representing the concentration of the sample solution needed to inhibit 50% of the DPPH free radicals. The antioxidant activity of flavonoids from red seaweed (*Gracilaria* sp.) can be observed in the figure 2 below. Based on Figure 2 above, it can be observed that the higher the concentration of extract provided, the stronger the antioxidant activity. Based on the linear equation obtained from the graph of antioxidant activity testing of red seaweed (*Gracilaria* sp.), the IC_{50} value is determined to be 8.67 (µg/ml).

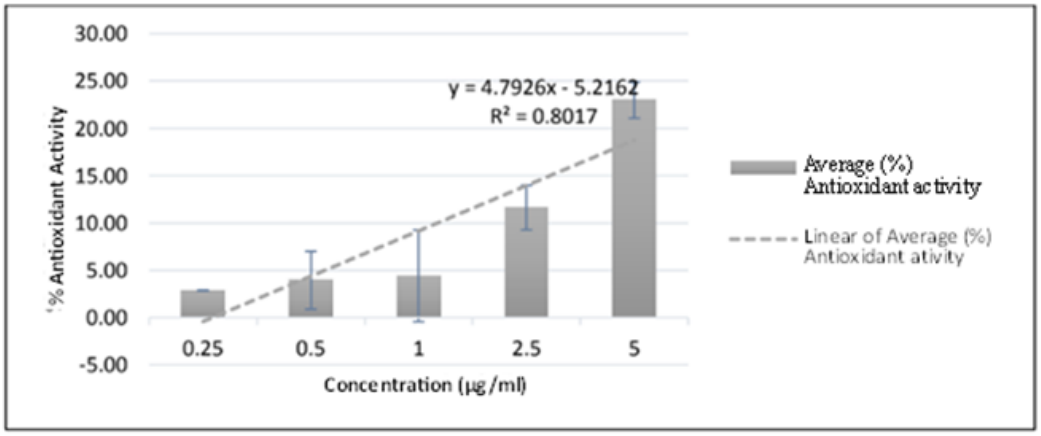

Figure 2. The antioxidant activity of red seaweed (*Gracilaria* sp.) extract

The antioxidant activity of marine algae is attributed to a diverse array of compounds, including pigments such as chlorophylls and carotenoids, vitamins and their precursors (cophenol, carotene, niacin, thiamine, and ascorbic acid), and phenolic compounds like polyphenols, hydroquinones, and flavonoids. Additionally, substances like phospholipids (especially phosphatidylcholine), terpenoids, peptides, and other antioxidative compounds contribute directly or indirectly to the inhibition or suppression of oxidation processes. To evaluate antioxidant activity, various standard methods are employed, and the strength of antioxidant activity is often categorized as strong, moderate, or weak based on the obtained results. These categorizations are generally determined by comparing the activity of the sample with standard antioxidants like ascorbic acid or Trolox. In the context of *Gracilaria*, a red seaweed, the antioxidant activity has been explored in several published research studies. The assessment of antioxidant activity often involves methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, FRAP (Ferric Reducing Antioxidant Power) assay, or total phenolic content determination. Strong antioxidant activity in *Gracilaria* is often associated with high concentrations of specific bioactive compounds. These compounds, which may include polyphenols, flavonoids, and carotenoids, contribute to the scavenging of free radicals and inhibition of oxidative processes. Moderate antioxidant activity may be observed when the concentration of these compounds is lower but still substantial enough to confer protective effects against oxidative stress. Weak antioxidant activity suggests a lower concentration of these bioactive compounds, and the seaweed may have a more limited capacity to neutralize free radicals. It's crucial to note that the specific antioxidant activity of *Gracilaria* can vary based on factors such as species, geographical location, harvesting conditions, and processing methods. Therefore, a comprehensive understanding of the antioxidant potential requires a detailed examination of individual studies and their methodologies, as well as the specific bioactive compounds identified in the seaweed.

The antigenotoxic activity: The pesticide exposure to fish is one of the reasons for the increased frequency of micronuclei in fish

erythrocytes. According to a study conducted by published methods (8) on tilapia (*Oreochromis mossambicus*), the concentration and length of time of pesticide exposure to fish can bind the induction of micronuclei in fish peripheral erythrocytes. The picture of micronuclei can be seen in the figure 3.

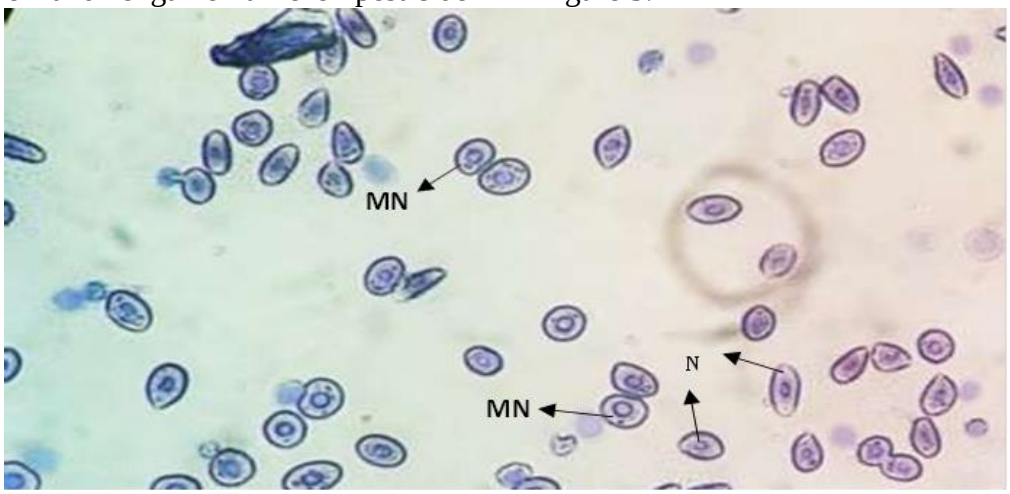

Figure 3. Peripheral blood erythrocyte cells of Tilapia (Oreochromis niloticus) during the research. (MN= Micronuclei; N= Normal)

Carcinogenic compounds such as pesticides made from active methomyl can interfere with the process of cell division by inhibiting the formation of spindle threads resulting in incomplete chromosome segregation. If this cell division process is disrupted because the chromosomes are damaged by chemicals or radiation, then the distribution of genetic material in the new nucleus during cell division will be affected and some or perhaps all of the chromosomes will fail to be included in one of the two new nuclei. When this happens, the genetic material that is not incorporated into the nucleus will form its own nucleus that is smaller than the main nucleus that is clearly visible under a microscope. Chromosomal aberrations induced by chemicals can trigger the formation of micronuclei.

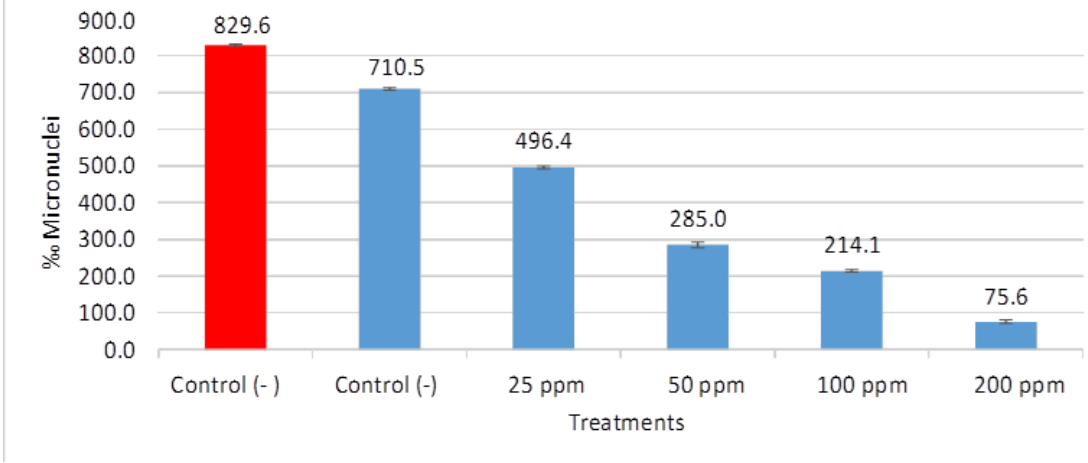

Figure 4. Peripheral blood erythrocyte cells of Tilapia (Oreochromis niloticus) during the research. (MN= Micronuclei; N= Normal)

Fish that had been exposed to the LC_{50} dose of methomyl active pesticide for 96 hours were then transferred to an aquarium containing fresh water without pesticide application. Fish were then injected with flavonoids. After a 96-hour rearing period, the frequency of micronuclei in the control dose treatment (-) was 710.5 ± 14.2 ‰, in dose treatment a was 496.4 ± 18.4 ‰, in dose treatment b was 285.0 ± 30.7 ‰, and in dose treatment c was 214.1 ± 10.45 ‰. The further effect of giving red seaweed extract (*Gracillaria* sp.) at higher doses can significantly reduce the frequency of micronuclei in Tilapia erythrocytes ($p<0.05$). This happens because flavonoids are compounds that have the potential as co-

chemotherapeutic agents and are able to reduce cells that are suspected of cancer by modulating the cell cycle and inducing cell apoptotic activity so that they can kill potentially cancerous cells such as micronuclei cells (1, 12, 31) . Flavonoids are also known as compounds that can act as immunostimulants, antitumors, anti-HIV and antioxidants. These compounds are able to protect healthy fish body cells from exposure to methomyl pesticides and help the regeneration process of red blood cells. The role of flavonoid compounds on blood, where flavonoid compounds are able to protect cell membrane lipids, prevent blood cell damage and are able to increase erythropoiesis, which is the process of erythrocyte formation in the bone marrow. Therefore, the provision of flavonoids is able to destroy potentially cancerous cells and help form new blood cells so as to restore the condition of fish blood (2, 24). The red seaweed (*Gracillaria* sp.) confirms presence of several phytochemical compounds, has antioxidant and antigenotoxic activity through micronuclei assay in peripheral erythrocytes of tilapia. The authors would like to thank the Indonesia Endowment Fund for Education (LPDP) from the Ministry of Finance, Republic of Indonesia, for granting the scholarship and supporting this research. The authors also acknowledge the Integrated Research Laboratory (IRL) Brawijaya University, and the SATU JRS 647/UN3/3023 funding for all support and facilities.

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