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Antioxidant activity of *Eucheuma cottonii* Seaweed Extracts from Central Mawasangka Waters

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Abstract: *Eucheuma cottonii* is one type of seaweed that has the potential to be an antioxidant. This seaweed grows and is distributed in various waters of Indonesia, especially in Southeast Sulawesi. However, information regarding the antioxidant activity and the compound content of *E. cottonii* in this area is still limited. Therefore, this research aims to determine the compound and antioxidant activity profile in *E. cottonii*. The study used laboratory experimental methods. The research stages were preparation (seaweed sampling), extraction, qualitative phytochemical screening, and antioxidant assay. The initial identification of compounds using qualitative phytochemical screening methods with three extraction solvents: ethanol, ethyl acetate, and n-hexane. Meanwhile, the antioxidant test employed the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The results of qualitative phytochemical screening revealed that the seaweed extract of *E. cottonii* contains saponins, tannins, and terpenoids. The antioxidant assay using the DPPH method indicated that among the three extraction solvents used, ethanol extract exhibited a very strong antioxidant activity (with an IC_{50} of 30,036 ppm), compared to ethyl acetate extract (IC_{50} of 182,179 ppm) and n-hexane extract (IC_{50} of 641,454 ppm). This study concluded that *E. cottonii* seaweed has the potential to be developed as a source of natural antioxidants or raw material for the discovery of antioxidant medicines in the future.

Keywords: Antioxidant; *Eucheuma cottonii*; Phytochemistry; Seaweed.

Introduction

Seaweed has become one of the most promising resources today, evident in the increasing production and demand for seaweed worldwide. Seaweed production has significantly risen in the last 20 years, playing a crucial role in the fisheries industry of every country. According to data from the Food and Agriculture Organization (FAO, 2021), global seaweed production has nearly tripled from 118,000 tons in 2000 to 358,200 tons in 2019 [1]. In Indonesia, seaweed is a positively trending export commodity, experiencing a growth rate of 6.08% from 2017 to 2021, reaching USD 5.718 billion [2].

Seaweed is a macroalgae commonly found in intertidal, tidal, and subtidal zones. It is classified into three groups based on its pigment color: green seaweed, brown seaweed, and red seaweed. Green seaweeds include *Ulva*, *Enteromorpha*, *Codium*, *Chaetomorpha*, and *Caulerpa*. Brown seaweeds encompass *Sargassum*, *Turbinaria*, *Laminaria*, and *Dictyota*. Red seaweeds include *Gracilaria*, *Eucheuma*, *Gelidiella*, *Ceramium*, *and Acanthophora* [3]. Seaweeds are rich in various bioactive compounds such as proteins, minerals, vitamins, fibers, essential amino acids, pigments, and fatty acids. These compounds contribute to their remarkable properties, including antihypertensive, antidiabetic, antioxidant, anti-inflammatory, antitumoral, antiviral, and antimicrobial effects. Seaweed holds great potential in developing functional foods and nutraceuticals, contributing to global security in the future. Moreover, it could play a significant role in the pharmaceutical and biotechnological industries, as well as in drug development and other applications [4].

Antioxidants are compounds whose molecular structure can donate electrons freely to free radical molecules, thus breaking the chain reaction of these radical reactions. Antioxidants are inhibitory compounds that counteract reactions between free radicals and target molecules [5]. Antioxidants are compounds that neutralize the increase in free radicals, protect cells from the toxic effects they produce, and contribute to the prevention of diseases such as cancer, heart disease, cataracts, premature aging, and other degenerative diseases [6]. One of the seaweeds that has antioxidant activity is *Eucheuma cattoni* [7,8].

Mawasangka Tengah is one of the districts in Central Buton Regency, Southeast Sulawesi, Indonesia, with significant potential for developing marine resources. This is evident from the dominant livelihood of the local population, primarily fishermen and marine product cultivators [9]. *Eucheuma cattoni* seaweed cultivation in Mawasangka Tengah District has started to grow and become one of the livelihoods in the area. The local community competes to cultivate this seaweed because it has a high market value, sometimes reaching 40 thousand rupiahs per kg. However, the chemical content and biological activities, especially the antioxidant activities of seaweed from the waters of Mawasangka Tengah, have not been reported until now. Therefore, based on this information, researchers are very interested in identifying the chemical components (secondary metabolites) in

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seaweed extracts *Eucheuma cattoni* originating from the waters of Central Mawasangka using three types of solvents: ethanol, ethyl acetate, and n-hexane, as well as studying their antioxidant activities

This research is very important to carry out because seaweed has the potential to be developed as a functional food ingredient and Indonesian nutraceuticals [2], especially the use of local Indonesian marine resources originating from the waters of Central Mawasangka, Southeast Sulawesi province in the pharmaceutical industry, especially as raw materials for antioxidant.

Research Methods

This research is an experimental study to determine the type of secondary metabolite qualitatively through a phytochemical screening test and determine its antioxidant effect in several different solvents, namely n-hexane, ethyl acetate, and ethanol. The first stage was to take samples of *E. cottoni* seaweed in Langkomu village, Central Mawasangka sub-district, Central Buton Regency. The second stage is sample extraction. At this stage, the seaweed samples are dried before extraction using three solvents. The second stage is a phytochemical screening test, and the final stage is an antioxidant test using the DPPH method. Tools, materials, and research stages are described as follows:

Chemicals and Instruments

The research materials used include dried seaweed sourced from Langkomu Village, Mawasangka Tengah District, methanol (CH_3OH), ethanol (C_2H_5OH), ethyl acetate ($C_4H_8O_2$), n-hexane (C_6H_{14}), magnesium (Mg), chloroform ($CHCl_3$), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), gallic acid, iron (III) chloride ($FeCl_3$), distilled water (H_2O), Dregendroff reagent, and DPPH (1,1-diphenyl-2-picrylhydrazyl). The equipment used includes a rotary evaporator, digital scale, oven, blender, funnel, pipette, spatula, reaction tube, Erlenmeyer flask, filter paper, vial, and UV spectrophotometer (Thermo Scientific Genesys 10 UV Vis).

Extraction

Freshly sample harvested seaweed, approximately 2 kg, was cleaned and washed with water, then dried in an open space for 3 to 5 days. Once dried, it was cut into small pieces with a size of approximately ± 1 cm. The sample was further dried in an oven at a temperature of 40°C until completely dry. Subsequently, the dried sample was finely ground using a blender. The obtained sample was then weighed. Seaweed extraction was performed through maceration using three organic solvents: ethanol, ethyl acetate, and n-hexane. One hundred grams of the sample was placed into containers for each solvent (Ethanol, ethyl acetate, and n-hexane) and macerated with 200 ml of the respective solvent. The maceration process was carried out for 3x24 hours. The resulting macerate was filtered using filter paper. The filtrate of the seaweed extract obtained was then evaporated using a rotary evaporator. The percentage yield of the extract was calculated by comparing the weight of the extract with the initial weight of the sample. The yield (%) was calculated based on the following equation [10]

$$\text{Yield :(\%)} = \frac{\text{mass of crude extract (g)}}{\text{mass of sample (g)}} \times 100\%$$

Phytochemical screening

Phytochemical screening was carried out according to [11] (Table 1).

Table 1. A qualitative phytochemical screening of the *Eucheuma cottonii* extract.

Test	Procedure	Positives indication
Flavonoids	Extract (20 mg) + 10 mL MeOH (dissolved, filtered). Filtrate + 1 mL concentrated HCl + Mg strip (0.5 cm)	Change in the color of the solution to reddish
Tannins	Extract (20 mg) + 5 mL H_2O (dissolved, filtered). Filtrate + 5 drops of 0.1% FeCl3	Change the color of the solution to green-brown/blue-darkening.
Terpenoids	Extract (20 mg) + 2 mL CH_3Cl (dissolved) + 3 mL concentrated H_2SO_4 (added slowly until a single layer is formed)	The reddish-brown color changes between layers.
Saponins	Extract (20 mg) + 5 mL H_2O (heated to boiling, filtered). Filtrate (vigorously shaken)	Formation of foam on the top of the solution
Alkaloids	Extract (20 mg) + 2.5 mL 1% HCl (shaken for 5 minutes, filtered). Filtrate + 5 drops of Dragendorff reagent	Formation of a reddish-orange color in the solution

Antioxidant Assay

The antioxidant assay used the DPPH (2,2-diphenyl-1-picrylhydrazyl) method according to [12] with some modifications. Test solutions of ethanol extract (62.5 ppm, 31.25 ppm, 15.63 ppm, 7.81 ppm, 3.91 ppm, 1.95 ppm, and 0.98 ppm), ethyl acetate extract (250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.63 ppm, 7.81 ppm, 3.91 ppm, and 1.95 ppm), n-hexane extract (1000 ppm, 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.63 ppm, 7.81 ppm, 3.91 ppm, and 1.95 ppm), and positive control Gallic Acid (standard)(0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm, and one ppm) were prepared using methanol p.a. as the solvent. Next, 0.5 mL of each extract solution or standard chemical was mixed with 0.5 mL of DPPH 0.3 mM in a flask. The mixture was vortexed and left in a dark room for 30 minutes at room temperature. Each test sample was then measured for its absorbance using a UV-Vis spectrophotometer (Thermo Scientific Genesys 10 UV Vis) at a wavelength of 517 nm. This measurement was performed in triplicate. The absorbance data obtained were used to calculate the % inhibition using the following equation:

$$\% \text{ Inhibition } = 100 - \frac{(\text{Abs sample} - \text{Abs Blank})}{\text{Abs Control}} \times 100\%$$

Note:
Abs sample: absorbance of sample/standard (Extract/(standar+Methanol+DPPH))
Abs blank: absorbance of the blank without additions of DPPH control (Extract+Methanol)
Abs control: absorbance of DPPH solution in methanol (DPPH+Methanol)

Statistics analysis

Data analysis was used to find the linear regression equation in this research, and Microsoft Excel was utilized [13]. The linear regression equation y = ax + b was obtained by plotting the concentration values of the test solution (X-axis) against the % inhibition (Y-axis). From this linear regression equation, the y value is 50 (the determined value of IC_{50}), while the x value is the antioxidant activity value of the test sample to be determined (IC_{50}). The determination of the IC_{50} value uses the formula in Equation 2:

$$IC_{50} = 50 - ab \quad \text{(Equation 2)}$$

Note:
a: the intercept value from the linear regression equation
b: the slope value from the linear regression equation

Results and Discussion

The plant tissue used is the entire part of seaweed (Figure 1). Extraction is carried out using three types of solvents: ethanol, ethyl acetate, and n-hexane. The highest extraction yield percentage was obtained with ethanol as the solvent, namely 2.43%, followed by ethyl acetate with 1.78% and n-hexane with 0.54% (Table 2). The yield percentage in extraction is determined by the type of plant components, solvent, and harvest age [14].

Figure 1. *Eucheuma cattoni* Seaweed

Phytochemical screening serves as a straightforward analytical approach to classify the diversity of secondary metabolites found in plants and natural products, with the potential to unveil compounds valuable for drug discovery. This method offers preliminary information into the biologically active compounds within the tested sample. In this study, the results indicated the presence of metabolic groups consisting of terpenoids, saponins, and tannins (Table 3). The phytochemical screening results of the *Eucheuma cottonii* extract suggest that the secondary metabolite in ethanol extract contains saponin, tannins, and terpenoids. Ethyl acetate extract contains saponin and terpenoid, while n-hexane extract contains terpenoid.

A positive indication of the presence of saponin in the sample is the formation of stable foam after adding water or alcohol and stirring for some time. This happens because the compound structure of saponins contains aglycone groups (triterpenoids or steroids), which are lipophilic, and glycones (sugars), which are hydrophilic and give them soap-like properties. So, when stirred with water or alcohol, it will produce abundant foam because the surface tension has been reduced [15]. A positive indication in the tannin test is marked by a change in the color of the sample to brownish-green or blackish-blue. This color change is due to forming a complex (coordinating covalent bond) of Fe^{3+} with the tannin compound in the sample [11]. A positive test for terpenoids is indicated by a reddish-brown color between the layers of the sample solution when reacted with sulfuric acid. The reaction is a condensation reaction followed by the release of the H_2O and the formation of a carboaction. The reddish brown color is formed due to an electrophilic addition reaction [16].

The results of the bioactive compounds in this study were compared with the results of [17], which stated that the bioactive compounds *E. cottonii* contained were flavonoids, triterpenoids, and phenol hydroquinone; [16] states that *E. cottonii* contains alkaloids, flavonoids, phenol hydroquinone, and tannin. Meanwhile, [8] stated that *E. cottonii* contains alkaloids, flavonoids, saponins, and tannins. Differences in secondary metabolite content in the seaweed *E. cottonii* are influenced by the biosynthesis process of each plant. Plant biosynthesis is influenced by influenced by various factors, namely genetic characteristics, environment (salinity, metals, temperature, exposure to sunlight (UV), pathogenic organisms that cause disease), and nutritional sources [18].

Antioxidant analysis of *Eucheuma cottonii* extract was carried out using the DPPH method, which is the most common method used to test antioxidant activity because this method is relatively fast and simple, and only a few ingredients are used. This analysis was carried out by measuring the inhibitory activity value against DPPH free radicals using UV-Vis spectrophotometry. **Figures 2, 3, 4, and 5** explain the linear regression of gallic acid and *Eucheuma cottonii* extract using three different solvents: n-hexane, ethanol, and ethyl acetate. The IC_{50} values are determined using the linear regression equation that has been obtained. The smaller the IC_{50} value, the greater the antioxidant activity.

Table 2. Yield (%) of *E.cottonii* extract from various solvents

Solvent	Color	Sample Mass (g)	Extract Mass (g)	yield % (b/b)
Ethanol	brownish	100 g	2.43	2.43%
Ethyl acetate	brownish	100 g	1.78	1.78%
n-Hexsana	yellowish-brown	100 g	0.54	0.54%

Table 3. Qualitative analysis of phytochemical screening of *Eucheuma cattonii* extracts

Extract	Saponins	Alkaloids	Tannins	Flavonoids	Terpenoids
Ethanol	+	-	+	-	+
Ethyl acetate	+	-	-	-	+
n-Hexsana	-	-	-	-	+

Key: present (+) dan absent (-)

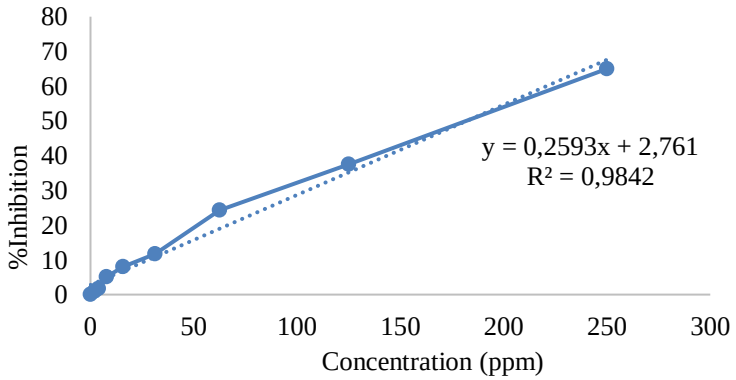

Figure 2. DPPH Scavenging activity of *E. cattonii* Ethyl acetate extract

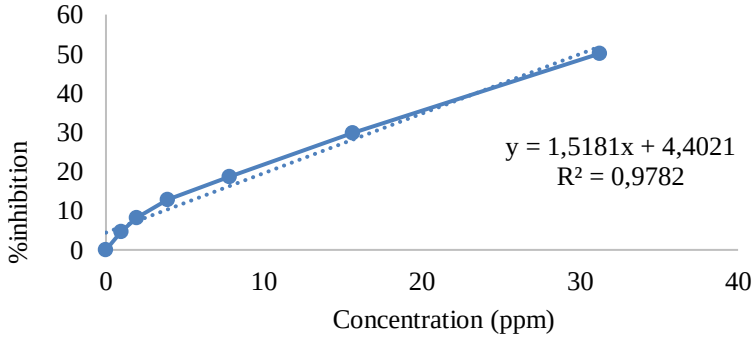

Figure 3. DPPH Scavenging activity of *E. cattonii* Ethanol extract

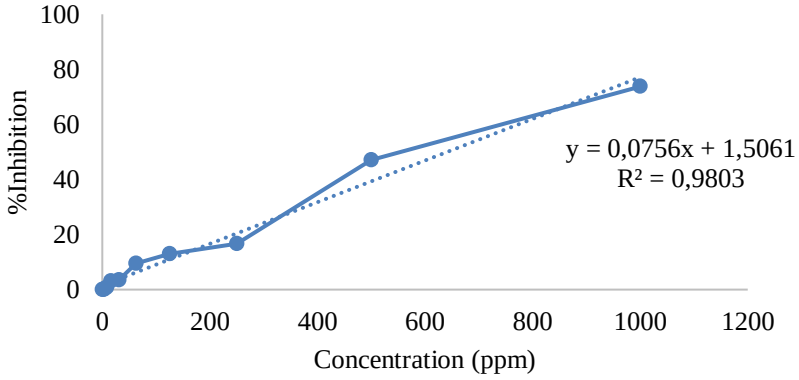

Figure 4. DPPH Scavenging activity of *E. cattonii* n-hexane extract

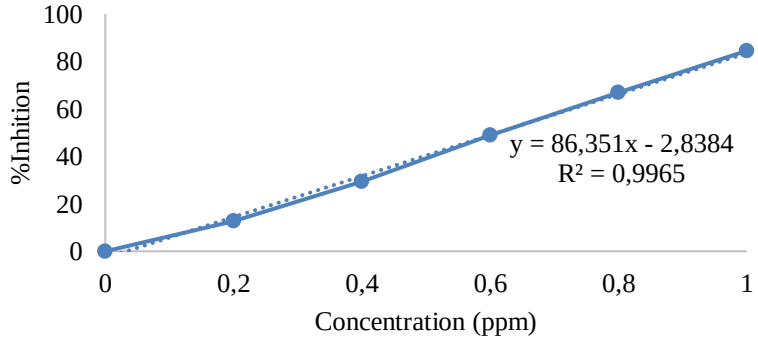

Figure 5. DPPH Scavenging activity Of Gallic acid

Table 4 shows the results of antioxidant activity tests on *E. cattonii* seaweed. In **Table 4**, it is indicated that the ethanol extract has an IC_{50} value of 30,036 ppm, the ethyl acetate extract has 182,179 ppm, and the n-hexane extract has 641,454 ppm. Based on these IC_{50} values, the ethanol extract exhibits very strong antioxidant activity compared to the ethyl acetate and n-hexane extracts. As a comparison, the synthetic antioxidant Gallic Acid (positive control) with a very strong antioxidant activity has an IC_{50} value of 0.6107 ppm.

In this research, antioxidant tests were carried out using DPPH reagent and gallic acid (synthetic antioxidant) as a comparison. DPPH reagent (2,2-diphenyl-1-picryhydrazyl) is a free radical that accepts an electron or hydrogen to be converted into a diamagnetic molecule. There are antioxidant compounds in E. cottoni seaweed extract (saponins, tannins, and terpenoids) that capture free radicals from DPPH by donating their radical hydrogen atoms to DPPH radicals so that they are reduced to DPPH-H (2,2-dephenyl-1-picrylhydrazine). The characteristic of the reaction is a color change from purple (DPPH• or DPPH-R) to yellow (DPPH-H) [20].

Research on the antioxidant activity of the seaweed *Eucheuma cottonii* in various waters in Indonesia has been conducted previously. The antioxidant activity of *E.cottonii* seaweed in the waters of Sumenep, East Java, is considered weak, with an IC_{50} value of 757.05 ppm in methanol solvent, 770.81 ppm in n-hexane solvent, and 1,751.97 ppm in aquades solvent [8]. Meanwhile, from the waters of Dahi'ae, East Nusa Tenggara, the seaweed extract is also classified as weak with an IC_{50} value of 169.06 ppm in methanol solvent [7] When compared to seaweed from Mawasangka Tengah district (in this study), the antioxidant activity of *E. cottonii* seaweed is considered very strong with an IC_{50} value of 30.036 ppm in ethanol solvent. However, ethyl acetate solvent is classified as weak with an IC50 value of 182.179 ppm, and n-hexane solvent is considered very weak with an IC_{50} value of 641.454 ppm.

The antioxidant activity of *E. cottoni* seaweed from the waters of Central Mawasangka district is classified as very strong (IC_{50} 30.036 ppm) in ethanol solvent. The compound content influences this in the seaweed ethanol extract, which, based on the results of phytochemical screening, contains saponins, tannins, and terpenoids. Saponins, tannins, and terpenoids are secondary metabolites with antioxidant activity [15, 21-23]. The antioxidant activity of a compound is measured by its ability to delay, slow down, or prevent the oxidation process. Antioxidants are bioactive compounds that can delay or prevent the formation of free radical reactions [24]. Based on the source, antioxidants are divided into synthetic and natural. Synthetic antioxidants are obtained from synthetic results in the laboratory and have been clinically tested for their antioxidant effects. Meanwhile, natural antioxidants come from animals and plants. According to [25], phenolic hydroxyl groups play a key role in the free radical scavenging capacity, and the number and position of hydroxyl groups and other groups are also the main influencing factors. Therefore, the saponin, tannin, and terpenoid compounds in seaweed extract *E. cattonii* from the waters of the Central Mawasangka district are thought to have hydroxyl groups that can inhibit or prevent free radicals naturally.

Based on these data, it is suggested that *E. cottonii* seaweed has the potential for further development, including a more specific analysis of antioxidant activities, to support the development of information and technology related to the utilization of *E. cottonii* seaweed from Indonesia.

Table 4. Results of measurement for antioxidant activity *E. cattonii* extract and gallic acid

Test Solution	Regression Equation (in 517 nm)	IC_{50} (ppm)	Category[19]
DPPH Ethanol Sample	y = 1.5181x + 4.4021	30.036	Very strong, IC_{50}= <50 ppm
DPPH Ethyl Acetate Sample	y = 0.2593x + 2.761	182.179	Weak, IC_{50}= 150-200 ppm
DPPH n-Hexane Sample	y = 0.0756x + 1.5061	641.454	Very weak, IC_{50}= >200 ppm
Gallic Acid	y = 86.351x - 2.8384	0.6107	Very strong, IC_{50}= <50 ppm

Conclusion

Based on the research results, the compound profiles in three extracts of *Eucheuma Cottoni* seaweed from Central Mawasangka waters are in ethanol extract containing saponins, tannins, and terpenoids. Ethyl acetate extract contains saponin and terpenoid, while n-hexane extract contains terpenoid. Meanwhile, the results of antioxidant tests using the DPPH method show that the ethanol extract has very strong activity with an IC_{50} value of 30.036 ppm, the ethyl acetate extract has weak antioxidant activity with an IC_{50} value of 182.179 ppm, and the n-hexane extract has very weak antioxidant activity with an IC_{50} value of 641.454 ppm.

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References

[1] Zhang, L., Liao, W., Huang, Y., Wen, Y., Chu, Y., & Zhao, C. (2022). Global seaweed farming and processing in the past 20 years. In *Food Production, Processing and Nutrition* (Vol. 4, Issue 1). BioMed Central Ltd.

[2] Anonymous (2023). *Laporan Capaian Kinerja 2022 Deputi Bidang Koordinasi Sumber Daya Maritim*. The Coordinating Ministry for Maritime and Investment.

[3] Munaeni, W., Lesmana, D., Irawan, H., Hamka, M. S., & Nafsiyah, I. (2023). *Potensi Budidaya dan Olahan Rumput Laut di Indonesia*. TOHAR MEDIA.

[4] Salido, M., Soto, M., & Seoane, S. (2024). Seaweed: Nutritional and gastronomic perspective. A review. In *Algal Research* (Vol. 77). Elsevier B.V.

[5] Nisa, H.(2023). *Rumput Laut Eucheuma cottonii Sebagai Antioksidan Alami*. Fakultas Perikanan Dan Kelautan Universitas Airlangga.

[6] Nurkhasanah, Bachri, M., S., & Yuliani., S. (2023). E-book : *Antioksidan dan Stress Oksidatif*. UAD Press.

[7] Kore, M. Melkianus Mola Kore, Sonya Titin Nge, M. N. (n.d.). Uji Aktivitas Antioksidan Pada Ganggang Cokelat (*Sargassum polycystum)* Dan Ganggang Hijau (*Euchema cottonii*) Pada Perairan Dahi' ae. In *Indigenous Biologi Jurnal pendidikan dan Sains Biologi* (Vol. 1, Issue 3).

[8] Syafitri, T., Hafiludin, H., & Chandra, A. B. (2022). Pemanfaatan Ekstrak Rumput Laut (*Eucheuma cottonii*) Dari Perairan Sumenep Sebagai Antioksidan. *Jurnal Kelautan: Indonesian Journal of Marine Science and Technology*, *15*(2), 160–168.

[9] Anonymous. (2022). Dinas Perikanan (DP) Mawasangka Tengah.

[10] Ngamkhae,N., Monthakantirat, O., Chulikhit, Y., Boonyarat, C., Maneenet, J., Khamphukdee, C. Kwankhao, P., Pitiporn, S., Daodee, S. (2022). Optimization of extraction method for Kleeb Bua Daeng formula and comparison between ultrasound-assisted and microwave-assisted extraction. *Journal of Applied Research on Medicinal and Aromatic Plants*, 28, 100369

[11] Hendrisno, H., Megawati, M., Agusriyadin, A., & Carla Wulandari , S. (2023). A Phytochemical Profile and Acute Toxicity of *Meistera aculeata* (Roxb). Skornick. & M.F. Newman Fruits (Zingiberaceae). *Jurnal Akta Kimia Indonesia (Indonesia Chimica Acta)*, *16*(1), 17-22.

[12] Deuschle, V. C. K. N., Norbert Deuschle, R. A., Bortoluzzi, M. R., & Athayde, M. L. (2015). Physical chemistry evaluation of stability, spreadability, in vitro antioxidant, and photo-protective capacities of topical formulations containing *Calendula officinalis* L. Leaf extract. *Brazilian Journal of Pharmaceutical Sciences*, *51*(1), 63–75.

[13] Ulfa, A. M., Lukman, I. R., Widiyanti, B. L., Hartini, H., Hilmi, A., & Darmawan, M. I. (2021). Antioxidant activity of seaweed (*Eucheuma cottonii*) and coconut (Cocos nucifera) masks. *IOP Conference Series: Earth and Environmental Science*, *712*(1).

[14] Purbosari, N., Warsiki, E., Syamsu, K., & Santoso, J. (2020). Effect of Harvest Age and Solvents on the Phenolic Content of *Eucheuma cottonii* Extract. *Makara Journal of Science*, *24*(3), 141–147.

[15] Timilsena, Y.P., Phosanam, A., and Stockmann, R. (2023). Perspectives on Saponins: Food Functionality and Applications. Int. J. Mol. Sci., 24, 13538.

[16] Melati dan Parbuntari, H. (2022). Screening Fitokimia Awal (Analisis Qualitative) Pada Daun Gambir (Uncaria Gambir Roxb) Asal Siguntur Muda. *Periodic Chemistry Journal of Universitas Negeri Padang , Vol 11(3):88-92*

[17] Maharany, F., Nurjanah, Suwandi, R., Anwar, E., & Hidayat, T. (2017). Kandungan Senyawa Bioaktif Rumput Laut *Padina australis* dan *Eucheuma cottonii* Sebagai Bahan Baku Krim Tabir Surya. *Jphpi*, *20*(1), 10–17.

[18] Safia, W., Budiyanti, & Misrif. (2020). Kandungan Nutrisi dan Senyawa Bioaktif Rumput Laut (*Euchema cottonii*) yang Dibudidayakan dengan Teknik Rakit Gantung pada Kedalaman Berbeda. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *23*(2), 261–271.

[19] Selwal, N., Rahayu, F., Herwati, A., Latifah, E., Supriyono, Suhara, C., Kade Suastika, I. B., Mahayu, W. M., & Wani, A. K. (2023). Enhancing secondary metabolite production in plants: Exploring traditional and modern strategies. *Journal of Agriculture and Food Research*, *14*.

[20] Zamzani, I., & Triadisti, N. (2021). Limpasu Pericarpium : an Alternative Source of Antioxidant From Borneo with Sequential Maceration Method. *Jurnal Profesi Medika : Jurnal Kedokteran Dan Kesehatan*, *15*(1).

[21] Flieger, J., & Flieger, M. (2020). The [DPPH●/DPPH-H]-HPLC-DAD Method on Tracking the Antioxidant Activity of Pure Antioxidants and Goutweed (*Aegopodium podagraria* L.) Hydroalcoholic Extracts. *Molecules (Basel, Switzerland)*, *25*(24), 6005.

[22] Cano, A., Contreras, C., Chiralt, A., & González-Martínez, C. (2021). Using tannins as active compounds to develop antioxidant and antimicrobial chitosan and cellulose based films. *Carbohydrate Polymer Technologies and Applications*, *2*, 100156.

[23] Konfo, T. R. C., Djouhou, F. M. C., Koudoro, Y. A., Dahouenon-Ahoussi, E., Avlessi, F., Sohounhloue, C. K. D., & Simal-Gandara, J. (2023). Essential oils as natural antioxidants for the control of food preservation. *Food Chemistry Advances*, *2*, 100312.

[24] Siddiqui, T., Sharma, V., Khan, M. U., & Gupta, K. (2024). Terpenoids in Essential Oils: chemistry, classification, and potential impact on human health and industry. *Phytomedicine plus*, 100549.

[25] Sulasiyah, Sarjono, P.R., Aminin, A. L. N.(2018). Antioxidant from Turmeric Fermentation Products (Curcuma longa) by Aspergillus Oryzae. Jurnal Kimia Sains dan Aplikasi 21 (1): 13 – 18

[26] Lang, y., Gao, N., Zang, Z., Meng, X., Lin, Y., Yang, S., Yang, Y., Jin, Z., Li, B. (2024). Classification and antioxidant assays of polyphenols: a review. Journal of Future Foods, Volume 4(3):193-204.