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STUDY OF BIOACTIVE COMPOUNDS AND PHYTOCHEMISTRY OF *Halimeda macroloba* FROM THE SUMBERKIMA COASTAL AREA, BALI AS A BASIS FOR DOMESTICATION OF HIGH ECONOMIC VALUE AQUACULTURE COMMODITIES

Kajian Senyawa Bioaktif dan Fitokimia *Halimeda macroloba* dari Kawasan Pesisir Pantai Sumberkima, Bali Sebagai Dasar Domestikasi Komoditas Akuakultur Bernilai Ekonomi Tinggi

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ABSTRACT

Halimeda macroloba is a green macroalga with promising bioactive compounds that may serve as a basis for domesticating high-value aquaculture commodities, yet specific phytochemical and antioxidant activity data from its aqueous extract, particularly from the coastal area of Sumberkima Village, Bali, remain limited. This study aimed to determine the effectiveness of aqueous extraction and DPPH antioxidant activity, as well as to identify the phytochemical content of *Halimeda macroloba* from this area. The research used a descriptive method with an exploratory design. Samples were collected using purposive sampling at two stations representing shallow and moderate depth zones, then extracted by maceration with distilled water for 1×24 hours. The extract was quantitatively analyzed for flavonoid, tannin, and phenol content, tested for antioxidant activity using the DPPH method, and qualitatively screened for saponins, alkaloids, and terpenoids. The results showed the aqueous extract contained 345.31 mg/100 g phenol, 170.56 mg/100 g flavonoid, and 12.56 mg/100 g tannin, with an IC_{50} value of 6,949.4326 ppm classified as very weak antioxidant activity, while saponin, alkaloid, and terpenoid tests showed negative results. It is concluded that distilled water effectively extracts polar bioactive compounds but is ineffective in producing strong antioxidant activity, so this phenolic-dominated phytochemical profile can serve as baseline data for domesticating *Halimeda macroloba* as a high-value aquaculture commodity.

Keywords: Antioxidant, Aqueous Extract, Domestication, *Halimeda macroloba*, Phytochemicals

ABSTRAK

Halimeda macroloba merupakan makroalga hijau dengan potensi senyawa bioaktif yang dapat dikembangkan sebagai dasar domestikasi komoditas akuakultur bernilai ekonomi tinggi, namun data fitokimia dan aktivitas antioksidan dari ekstrak akuadesnya, khususnya dari kawasan pesisir Desa Sumberkima, Bali, masih sangat terbatas. Penelitian ini bertujuan mengetahui efektivitas ekstraksi akuades dan aktivitas antioksidan metode DPPH, serta mengidentifikasi kandungan fitokimia *Halimeda macroloba* dari kawasan tersebut. Penelitian bersifat deskriptif dengan rancangan eksploratif. Sampel diambil menggunakan teknik purposive sampling pada dua stasiun yang mewakili zona dangkal dan sedang, kemudian diekstraksi melalui maserasi dengan pelarut akuades selama 1×24 jam. Ekstrak yang diperoleh dianalisis kadar flavonoid, tanin, dan fenol secara kuantitatif, diuji aktivitas antioksidannya dengan metode DPPH, serta diskrining secara kualitatif untuk mengidentifikasi saponin, alkaloid, dan terpenoid. Hasil penelitian menunjukkan ekstrak akuades mengandung fenol sebesar 345,31 mg/100 g, flavonoid 170,56 mg/100 g, dan tanin 12,56 mg/100 g, dengan nilai IC_{50} sebesar 6.949,4326 ppm yang tergolong kategori antioksidan sangat lemah, sedangkan uji saponin, alkaloid, dan terpenoid menunjukkan hasil negatif. Disimpulkan bahwa akuades efektif mengekstraksi senyawa bioaktif polar namun tidak efektif menghasilkan aktivitas antioksidan tinggi, sehingga profil fitokimia yang didominasi senyawa fenolik ini dapat dijadikan data dasar bagi domestikasi *Halimeda macroloba* sebagai komoditas akuakultur bernilai ekonomi tinggi.

Kata Kunci: Akuades, Antioksidan, Domestikasi, Fitokimia, *Halimeda macroloba*

INTRODUCTION

Indonesia is an archipelagic nation with exceptionally high marine biodiversity, making it one of the world's largest seaweed producers, offering tremendous potential for development through the aquaculture sector. Aquaculture itself is defined as the cultivation of aquatic commodities including fish, crustaceans, and aquatic plants, including various types of macroalgae with high economic and ecological value (Bina et al., 2019). Seaweed has long been recognized as a source of foreign exchange and a major source of income for coastal communities, due to its extensive uses, spanning food, pharmaceuticals, cosmetics, and animal feed (Soamole et al., 2018). However, Indonesia's aquaculture sector is still dominated by monotonous conventional commodities, increasing the risk of production failure due to disease outbreaks and market uncertainty. Therefore, commodity diversification through the exploration of potential local macroalgae is an urgent strategic step to increase national fisheries resilience (Subyakto et al., 2016).

Seaweed has been identified as containing various bioactive compounds beneficial to human health, such as antioxidants, anti-cancer, and anti-aging properties, as well as compounds that can maintain healthy skin and boost immunity. Secondary metabolites in seaweed include alkaloids, flavonoids, triterpenoids, steroids, tannins, and saponins, which provide strong evidence for its potential as a functional food and raw material for the pharmaceutical industry (Lantah et al., 2017). Research by Harahap et al. (2025) revealed that bioactive compounds from seaweed, particularly sulfated polysaccharides and polyphenols, exhibit significant antioxidant potential, making seaweed a promising resource for nutraceutical and pharmaceutical development. Furthermore, several types of algae have also been shown to contain compounds that function as synthons and biocatalysts in sustainable chemistry studies, opening up significant opportunities for the development of high-value-added products (Manuhara et al., 2016).

Among the various green seaweed species, the genus Halimeda shows particularly promising potential as a source of natural antioxidants worthy of further exploration. Gazali et al. (2019) screened *Halimeda opuntia* from the coast of West Aceh and found significant antioxidant activity, confirming that this genus has great potential as a bioactive raw material. Morphologically, *Halimeda macroloba* Decaisne is a green macroalga with a segmented, calcified thallus, approximately 5–10 cm tall, with fan-shaped segments connected by a flexible network that provides physical resistance even when exposed to water currents (Guiry & Guiry, 2024). This species also features a bulbous holdfast that functions to grip soft substrates such as sand, enabling it to achieve a survival rate of 85%–90% in shallow water sedimentary environments, making it superior to other non-calcified macroalgae species (Agustina et al., 2021).

Several previous researchers have conducted research on the antioxidant activity of *Halimeda macroloba*, with varying results depending on the sample location and extraction method used. Muzaki et al. (2018) reported that *Halimeda macroloba* from Teluk Awur Beach, Jepara, has very strong antioxidant activity in methanol extract with an IC50 value of 38.57 ppm, which is classified as a very strong antioxidant category. In line with this, Leibo et al. (2016) through testing the total extract of H. macroloba from Totok Bay waters also found a decrease in the intensity of the purple color in DPPH which indicates strong antioxidant activity, especially at a concentration of 75 ppm. Meanwhile, Putri et al. (2024) who studied *Halimeda* sp. obtained an IC50 value of 221.62 ppm which is classified as very weak, indicating that geographical variations and extraction methods have a major influence on the resulting antioxidant potential.

The phytochemical content of *Halimeda macroloba* has also been scientifically proven through various previous studies, confirming the presence of several important secondary metabolites. Soamole et al. (2018), in their study of three types of seaweed using ethanol extract, found that *Halimeda macroloba* contained alkaloids, flavonoids, terpenoids/steroids, saponins, and tannins, the main bioactive compounds found in macroalgae. Flavonoids play a crucial role as antioxidants due to their ability to donate hydrogen atoms to free radicals, thereby stabilizing radical molecules and preventing oxidative damage at the cellular level. Their production in algal tissue is influenced by the environmental conditions of the waters where they grow (Nurjanah et al., 2021). The phenol content in macroalgae also functions as a primary chemical defense against herbivores and pathogens, while also playing a role in adaptation to abiotic stressors such as fluctuations in salinity and sunlight intensity. Tropical macroalgae tend to have higher phenol levels as a form of protection against extreme UV radiation exposure (Nurjanah et al., 2021).

Most seaweed antioxidant research to date has focused on the use of organic solvents such as methanol and ethanol, while the use of aquadest (water) as a solvent is still rare despite its advantages. The use of aquadest extracts is environmentally friendly and capable of extracting water-soluble polar compounds, such as certain polysaccharides and phenolic compounds, which may play a significant role in antioxidant activity. Furthermore, aqueous extracts are more relevant for direct application in functional food or beverage products due to their safe consumption and the absence of complex solvent separation processes. The choice of maceration with aquadest as a solvent in this study aligns with the findings of Martiningsih et al. (2016), who stated that maceration was chosen because it is easier to perform, requires simple equipment, and is suitable for extracting heat-labile compounds.

The waters of Sumberkima Village, Bali, are a natural habitat for *Halimeda macroloba*, which thrives in a well-maintained ecosystem. This algae grows abundantly on sand flats and coral reefs, supported by clear waters, optimal sunlight penetration, and stable temperature and salinity parameters, forming a rigid green carpet that serves as an indicator of good aquatic environmental quality. Ecophysiologically, H. macroloba demonstrates resilience to global warming by maintaining the stability of its chlorophyll pigments at temperatures up to 31°C, resulting in a stable growth success rate of around 82%, making it a resilient commodity in tropical waters (Sinutok et al., 2022). However, phytochemical data and specific antioxidant activity for *Halimeda macroloba* samples from the Sumberkima Village cultivation area are still very limited, considering that the bioactive compound content in macroalgae can vary significantly depending on geographic location, cultivation conditions, and extraction method used.

Based on this description, this study aims to determine the effectiveness of extracting the marine macroalga *Halimeda macroloba* using distilled water as a solvent and to test its antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. It also aims to identify the phytochemical compounds contained in the extract. The DPPH method was chosen because it is one of the most common and standardized methods for measuring antioxidant activity, where the decrease in the intensity of the DPPH purple color measured at a wavelength of 515–517 nm reflects the ability of antioxidant compounds to donate electrons or hydrogen atoms to stabilize free radicals (Adawiah et al., 2015). The initial assumption of this study was that the aqueous extract of *Halimeda macroloba* from the waters of Sumberkima Village contains active phytochemical compounds such as flavonoids, tannins, saponins, alkaloids, and triterpenoids that contribute to antioxidant activity that can be measured through the IC50 value, considering that tropical water conditions rich in UV light encourage the synthesis of chemical defense compounds in macroalgae (Nurjanah et al., 2021). This research is positioned as a preliminary study (baseline data) for the domestication process of *Halimeda macroloba* which is expected to provide scientific contributions to the development of potential local macroalgae-based aquaculture while supporting sustainable national fishery commodity diversification policies.

RESEARCH METHODS

This research was conducted from May to September 2025 by sampling the macroalgae *Halimeda macroloba* in the coastal area of Sumberkima Village, Gerokgak District, Buleleng Regency, Bali Province, in two depth zones, namely the shallow zone (1–3 meters) representing high light exposure conditions and the medium zone (4–6 meters) representing moderate conditions, with a purposive sampling technique to select healthy, intact thallus that has reached adult size. The sample preparation process, soaking using aquadest solvent, and heating were carried out at the Research Laboratory of the Faculty of Mathematics and Natural Sciences, Ganesha University of Education, while the extract concentration using a rotary evaporator was carried out at the Forensic Laboratory of Udayana University, and phytochemical screening and antioxidant activity testing were carried out at the FTP Laboratory of Udayana University. The type of research used was descriptive exploratory which aims to qualitatively describe the content of phytochemical compounds while quantitatively measuring the antioxidant activity through the IC_{50} value of the macroalgae extract *Halimeda macroloba* from Sumberkima waters. As supporting data for the suitability of the cultivation site, secondary water quality data was used, referring to Soumokil (2024), which recorded temperature parameters of 31.1–32.5°C, salinity of 31.1–32.5 ppt, pH of 8.2–8.9, phosphate of 0.02–0.06 mg/L, and nitrate of around 0.029 mg/L, all indicating water conditions conducive to the growth of *Halimeda macroloba*.

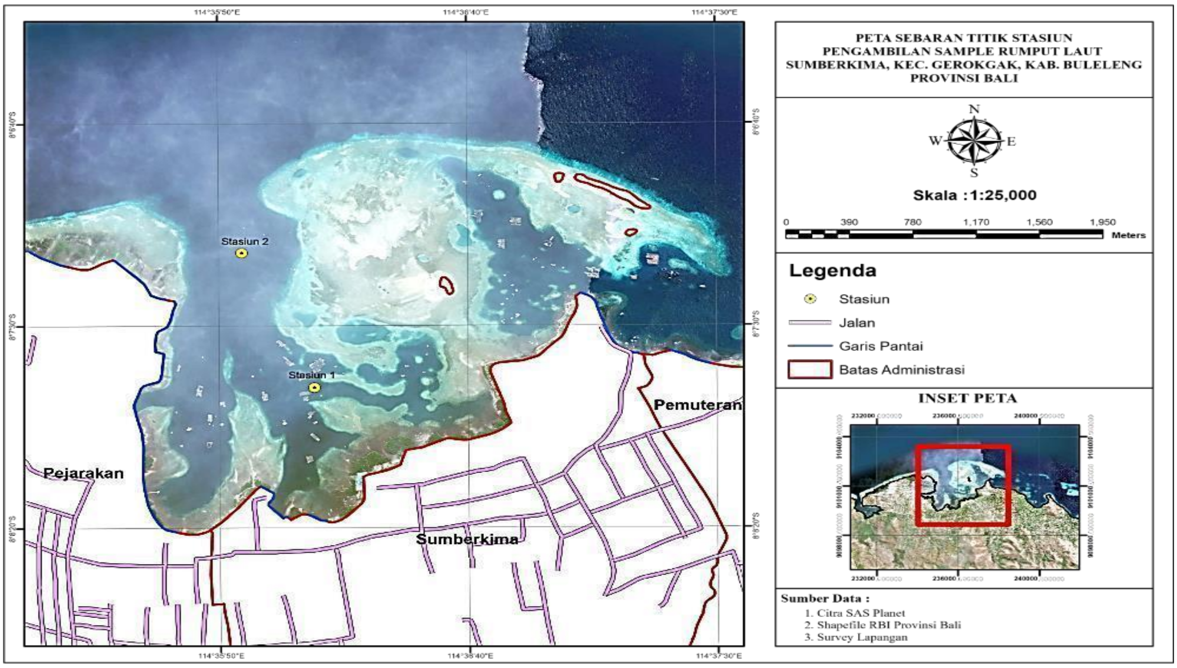

Figure 1. Research Location

The research procedure began with sample preparation. The collected samples were thoroughly washed with seawater to remove dirt and epiphytes, rinsed with distilled water, dried by airing in the shade or using an oven at 40–50°C, then ground into a powder using a blender and stored in an airtight container until ready for extraction. The extraction process was carried out using the maceration method, namely soaking 100 grams of *Halimeda macroloba* simple powder in 1 liter of distilled water (ratio 1:10 w/v) for 1×24 hours at room temperature with occasional stirring, then the filtrate was filtered using filter paper and concentrated using a rotary evaporator at a temperature of ±60°C until a thick extract was obtained (Prasetiyo et al., 2023). Phytochemical screening was carried out qualitatively on the thick extract to identify the flavonoid, tannin, total phenol, saponin, alkaloid, triterpenoid, and steroid compounds, where the saponin test was characterized by the formation of stable foam after the addition of distilled water, the alkaloid test was characterized by the formation of orange to brick red precipitate after the addition of Dragendorff or Mayer reagent, and the triterpenoid and steroid tests were distinguished based on the reddish color change (triterpenoid) and the formation of a red ring at the solution interface (steroid) after the addition of concentrated H_2SO_4 (Prasetiyo et al., 2023). The antioxidant activity test was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method using a spectrophotometer at a wavelength of 515–517 nm, where the decrease in the intensity of the purple color of the DPPH solution after reacting with the extract reflects the antioxidant's ability to ward off free radicals (Adawiah et al., 2015).

The data analysis techniques in this study are divided into two complementary approaches, namely qualitative descriptive analysis for phytochemical screening data and quantitative analysis for antioxidant test data. Phytochemical screening data are presented in tabular form with positive (+) notation if the compound is detected and negative (-) if not detected, so that the secondary metabolite content profile of *Halimeda macroloba* extract can be described systematically and easily understood. Quantitative data from the antioxidant test in the form of percent inhibition at various extract concentrations are then analyzed using linear regression analysis to obtain the equation of the line $y = ax + b$, where the IC_{50} value is obtained by entering the value of $y = 50$ into the equation to obtain the extract concentration that is able to inhibit 50% of DPPH free radical activity. The IC_{50} values obtained were then categorized based on the antioxidant strength classification, namely very strong (IC_{50} < 50 ppm), strong (50–100 ppm), moderate (100–150 ppm), weak (150–200 ppm), and very weak (> 200 ppm) according to the FTP Laboratory standards of Udayana University, so that the antioxidant potential of *Halimeda macroloba* aqueous extract from the waters of Sumberkima Village can be evaluated objectively and standardized.

RESULT

Sample Description and Extraction Process

Samples of the macroalgae *Halimeda macroloba* were obtained from the coastal area of Sumberkima Village, Gerokgak District, Buleleng Regency, Bali, on muddy sand substrate in shallow to medium water zones. The samples exhibited green, segmented, and calcareous thallus, typical of the genus Halimeda. The samples were washed with seawater to remove dirt and epiphytes, rinsed with distilled water, dried, and ground into a simple powder before extraction.

Extraction was carried out using a maceration method using distilled water (1:10 w/v) for 24 hours at room temperature. The macerated filtrate was filtered and then concentrated using a rotary evaporator at ±60°C to obtain a thick extract, which was used for all phytochemical and antioxidant tests in subsequent stages.

Bioactive Compound Analysis Results

Bioactive compound analysis was conducted quantitatively using UV-Vis spectrophotometry (flavonoids at λ ±415 nm, tannins at λ ±760 nm, and phenols at λ ±765 nm) on *Halimeda macroloba* aqueous extract. The analysis results are presented in Table 1.

Table 1. Phytochemical Screening Results of *Halimeda macroloba* Aqueous Extract

No	Test Parameters	Analysis Results
1.	Flavonoid Content	170.56 mg/100 g
2.	Tannin Content	12.56 mg/100 g
3.	Phenol Content	345.31 mg/100 g

Source: Laboratory Analysis Results, Faculty of Agricultural Technology, Udayana University (2025)

According to Table 1, phenol content was the parameter with the highest value (345.31 mg/100 g), followed by flavonoid content (170.56 mg/100 g), while tannin content showed the lowest value among the three parameters (12.56 mg/100 g).

Antioxidant Activity Test Results

The antioxidant activity of *Halimeda macroloba* aqueous extract was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method at a wavelength of 517 nm, with IC_{50} values obtained through a linear regression equation ($y = ax + b$) from the percent inhibition data at six concentrations (0, 2, 4, 6, 8, and 10 mg/mL). The measurement results are presented in Table 2.

Table 2. Table of IC50 Values of *Halimeda macroloba* Seaweed Extract

No	Sample Code	IC50 Analysis Results (ppm)	Antioxidant Category
1.	*Halimeda macroloba*	6,949.4326	Very weak

Source: Laboratory Analysis Results, FTP, Udayana University (2025)

The determination of antioxidant strength categories in Table 4.2 refers to the following IC_{50} value criteria: very strong (<50 ppm), strong (50–100 ppm), moderate (100–150 ppm), weak (150–200 ppm), and very weak (>200 ppm). Based on these criteria, the IC_{50} value of

Halimeda macroloba aqueous extract of 6,949.4326 ppm is classified as very weak, as it far exceeds the threshold of 200 ppm.

Qualitative Phytochemical Screening Results

Qualitative phytochemical screening was conducted to identify the presence of saponins, alkaloids, and terpenoids in *Halimeda macroloba* aqueous extract, using a foam test (saponins), a color test with Mayer/Wagner reagent (alkaloids), and a color test with concentrated H_2SO_4 (terpenoids), respectively. The results are presented in Table 3.

Table 3. Phytochemical Screening Results of *Halimeda macroloba* Aqueous Extract

No	Test Parameters	Analysis Results	Keterangan
1.	Saponins	Negative (-)	No stable foam formation
2.	Alkaloids	Negative (-)	No specific sediment/color change
3.	Terpenoids	Negative (-)	No brownish/reddish ring formation

Source: Laboratory Analysis Results of FTP, Udayana University (2025)

Based on Table 3, the *Halimeda macroloba* aqueous extract showed negative results for all three qualitative test parameters analyzed: saponins, alkaloids, and terpenoids.

DISCUSSION

Bioactive Compound Content of *Halimeda macroloba* Aquated Extract

Quantitative phytochemical analysis results showed that *Halimeda macroloba* aqueous extract contained 345.31 mg/100 g of phenolic compounds, 170.56 mg/100 g of flavonoids, and 12.56 mg/100 g of tannins. The high phenolic content compared to other compounds indicates that phenolic compounds were the dominant secondary metabolites successfully extracted using aqueous solvent. The presence of phenolic compounds in *Halimeda macroloba* extract indicates that this macroalgae possesses a chemical defense mechanism against aquatic environmental conditions. According to Nurjanah et al. (2021), phenolic compounds in macroalgae function as protection against oxidative stress, ultraviolet light exposure, and pathogenic microorganisms. The coastal area of Sumberkima Village, with its high light intensity and tropical water conditions, is suspected to be a factor triggering increased phenolic compound biosynthesis in *Halimeda macroloba*, in line with the theory that tropical macroalgae tend to produce higher phenol content as an adaptation to environmental stress (Nurjanah et al., 2021). This finding strengthens the argument that the habitat conditions in Sumberkima ecologically support the synthesis of polar secondary metabolites that can be extracted using water solvents.

The flavonoid content of 170.56 mg/100 g indicates that the *Halimeda macroloba* aqueous extract also contains quite potent natural antioxidant compounds. Flavonoids, as a group of polyphenolic compounds, are able to donate hydrogen atoms to stabilize free radicals, thus inhibiting oxidative damage at the cellular level. The flavonoid content in this study is suspected to be influenced by *Halimeda macroloba* growth habitat, which is in shallow to moderate water zones with relatively high light penetration. According to Nurjanah et al. (2021), algae living in shallow waters tend to produce higher levels of flavonoids as a form of protection against solar radiation. Meanwhile, the tannin content obtained was 12.56 mg/100 g, indicating that the *Halimeda macroloba* extract also contained tannins, albeit in lower amounts. This is thought to be due to the limited ability of aqueous solvents to optimally extract all secondary metabolites (Harborne, 1987).

The results of this study indicate that the use of aqueous solvents is capable of extracting polar secondary metabolites such as phenols, flavonoids, and tannins, in accordance with the principle of "like dissolves like," where polar solvents are more effective at dissolving polar compounds than semi-polar or non-polar compounds. Nevertheless, the limitations of aqueous solvents in extracting semi-polar and non-polar compounds are factors that need to be considered in further research to obtain a more complete and comprehensive phytochemical profile of *Halimeda macroloba*. The finding of significant phenol and flavonoid contents in this aqueous extract provides a strong scientific basis that *Halimeda macroloba* from the Sumberkima area has the potential as a source of natural secondary metabolites worthy of further development, both for pharmaceutical purposes, functional foods, and sustainable aquaculture.

Antioxidant Activity of *Halimeda macroloba* Aquated Extract

The results of the antioxidant activity analysis using the DPPH method showed that the *Halimeda macroloba* aqueous extract had an IC_{50} value of 6,949.4326 ppm, which is categorized as a very weak antioxidant. An IC_{50} value above 200 ppm is categorized as a very weak antioxidant according to the standards of the Udayana University FTP Laboratory. The low antioxidant activity in this study is thought to be influenced by the type of solvent used during the extraction process, as aquadest, as a polar solvent, has limitations in extracting semi-polar and non-polar bioactive compounds such as terpenoids, steroids, carotenoid pigments, and several chlorophyll derivatives, which are generally more soluble in organic solvents such as methanol, ethanol, and ethyl acetate (Harborne, 1987). These results contrast with the research of Muzaki et al. (2018) reported that the methanol extract of *Halimeda macroloba* had very strong antioxidant activity with an IC_{50} value of 38.57 ppm. This highly significant difference clearly indicates that the type of solvent significantly influences the extraction capacity of antioxidant compounds in macroalgae.

Although the phenol content obtained was quite high, at 345.31 mg/100 g, high phenol content does not directly result in high antioxidant activity because the antioxidant activity of an extract is not only determined by the total amount of phenolic compounds, but is also influenced by the chemical structure, position of the hydroxyl group, degree of polymerization, and stability of the phenolic compounds (Badarinath et al., 2010). Pratama et al. (2022) also explained that macroalgae extracts using water solvents generally show lower antioxidant activity because the use of distilled water allows the activity of the polyphenol oxidase enzyme which is still active in fresh macroalgae tissue, so that the enzyme can oxidize phenolic compounds into quinone forms which are less active as antioxidants and cause some phenolic compounds to lose their ability to donate hydrogen atoms to DPPH free radicals. In addition to solvent and compound structure factors, low antioxidant activity can also be influenced by the sample preparation process, temperature during evaporation, physiological age of the macroalgae, sampling season, and environmental conditions where they grow, considering that phenolic compounds in macroalgae are sensitive to heat, light, and oxidation so they are easily degraded during the processing process (Sari et al., 2023).

Although the antioxidant activity is classified as very weak, the presence of phenolic and flavonoid compounds in *Halimeda macroloba* extract still indicates the presence of bioactive potential that can be further developed, because phenolic compounds are known to be able to inhibit oxidation reactions through the mechanism of donating protons or hydrogen atoms to free radicals, thus forming more stable compounds (Pangestuti & Kim, 2011). Therefore, optimization of extraction methods using organic solvents or a combination of solvents is needed to increase the effectiveness of isolating antioxidant compounds from *Halimeda macroloba*, because the use of extraction methods with low temperatures and shorter extraction times can also be an alternative to maintain the stability of bioactive compounds so that the antioxidant activity obtained is more optimal. The difference in antioxidant activity between these studies confirms that the content of secondary metabolites in *Halimeda macroloba* is very dynamic and is influenced by extraction methods and habitat conditions, so further research with a multi-solvent approach is highly recommended to reveal the full potential of Halimeda macroloba as a source of natural antioxidants.

Qualitative Phytochemical Screening Results

Qualitative phytochemical screening results showed that the aqueous extract of *Halimeda macroloba* yielded negative results for saponins, alkaloids, and terpenoids. The saponin test showed no stable foam on the surface of the solution, the alkaloid test showed no precipitate formation or specific color change after the addition of the reagent, and the terpenoid test showed no brownish or reddish ring formation, which would indicate the presence of terpenoids. The failure to detect these three groups of compounds is thought to be influenced by the type of solvent used, as aqueous, as a polar solvent, is less effective in extracting semi-polar and non-polar compounds such as terpenoids, which are generally more soluble in organic solvents such as n-hexane, chloroform, ethyl acetate, and methanol (Harborne, 1987). This is consistent with the differences in results compared to the study by Soamole et al. (2018) using ethanol as a solvent found positive results for alkaloids, flavonoids, terpenoids/steroids, saponins, and tannins in *Halimeda macroloba*, indicating that the organic solvent extraction method provides a broader range of secondary metabolite polarities than distilled water (Saifudin, 2014).

Negative results in the alkaloid test can also be influenced by low alkaloid concentrations in the sample or compound degradation during the preparation and extraction process, as alkaloid content in marine organisms is strongly influenced by physiological conditions, tissue age, and environmental pressures in which the organism lives (Sitorus & Hutabarat, 2024). The low alkaloid content in *Halimeda macroloba* from the Sumberkima area is thought to be related to relatively stable habitat conditions, resulting in a low production of chemical defense compounds such as alkaloids. Meanwhile, the absence of stable foam in the saponin test indicates that saponin glycosides were not detected in significant amounts in the extract, considering that saponin content in algae is strongly influenced by species, growth phase, and habitat conditions (Gunawan, 2018). Ecological factors also play a role, as macroalgae living in environments with high ecological stress, such as exposure to ultraviolet radiation, salinity fluctuations, herbivory, and competition for space, generally produce more complex secondary metabolites. While relatively stable environmental conditions can lead to lower production of certain secondary metabolites (Pangestuti et al., 2017).

Although qualitative screening results were negative for saponins, alkaloids, and terpenoids, this study still demonstrated the bioactive potential of *Halimeda macroloba* through quantitatively detected phenol, flavonoid, and tannin content. This demonstrates that *Halimeda macroloba* aqueous extract still holds potential as a source of polar secondary metabolites associated with antioxidant activity and physiological protection. Geographical factors and the habitat conditions of the sampling location also influence the composition of secondary metabolites, as *Halimeda macroloba* growing in environments with different nutrient, salinity, and light intensity conditions can produce different secondary metabolite compositions, given the high metabolic adaptability of tropical macroalgae to environmental changes (Nurjanah et al., 2021). Therefore, further research using organic solvents with a wider polarity range is highly recommended to obtain a completer and more accurate phytochemical picture of *Halimeda macroloba* from the Sumberkima area, Bali.

Linking Research Results to Aquaculture Domestication Potential

The phenol, flavonoid, and tannin content of *Halimeda macroloba* from Sumberkima indicates its potential as an aquaculture commodity based on local biological resources with high economic value. Phenolic and flavonoid compounds have potential as antioxidants, anti-inflammatories, and immunomodulators (Pangestuti & Kim, 2011), which can be utilized as feed additives to enhance the immune response and resistance of cultivated organisms to oxidative stress (Citarasu, 2010), while also opening up opportunities for use in the pharmaceutical and cosmetic sectors as natural anti-aging and anti-inflammatory ingredients (Holdt & Kraan, 2011).

Sumberkima's stable environmental conditions (salinity, tropical temperature, high light penetration, and suitable substrate) support phenolic and flavonoid biosynthesis (Lobban & Harrison, 1997), making this area a potential domestication location in line with the blue economy strategy (FAO, 2022) to reduce pressure on natural resources. Although the antioxidant activity of aquadest extract is very weak, this is more due to the limitations of the extraction method than the low bioactive potential of the macroalgae itself. Therefore, solvent optimization (Nurjanah et al., 2021) and regulation of light intensity and nutrients in a controlled cultivation system have the potential to improve the quality of secondary metabolites, making *Halimeda macroloba* a superior macroalgae commodity that supports the sustainable aquaculture industry in Bali.

CONCLUSION

Extraction of *Halimeda macroloba* using distilled water has proven effective for extracting polar bioactive compounds, but is ineffective as an extraction method for producing strong antioxidant activity. This indicates that solvent polarity is the main limiting factor: distilled water is capable of isolating simple phenolic compounds, but is unable to extract semi-polar and non-polar compounds, which play a significant role in free radical scavenging mechanisms. Therefore, distilled water can be concluded as an effective solvent for the initial characterization of polar compounds, but is ineffective when the primary goal is to obtain an extract with high antioxidant activity.

The phytochemical profile of *Halimeda macroloba* from the coastal area of Sumberkima Village can be concluded to be dominated by phenolic compounds (phenols, flavonoids, and tannins), while saponins, alkaloids, and terpenoids were not identified under the extraction conditions used. These findings indicate that the phytochemical characteristics of *Halimeda macroloba* in this location are specific and influenced by the relatively stable conditions of its habitat, so that the profile can be used as valid basic data for the domestication process, while also confirming that *Halimeda macroloba* from Sumberkima has the potential to be developed as a high economic value aquaculture commodity based on the content of phenolic compounds, not as a source of strong antioxidants in the form of its aqueous extract.

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REFERENCES

Adawiah, A., Sukandar, D., & Muawanah, A. (2015). Aktivitas Antioksidan dan Kandungan Komponen Bioaktif Sari Buah Namnam. Jurnal Kimia VALENSI, 1(November), 130–

136. https://doi.org/10.15408/jkv.v0i0.3155
Agustina, R., et al. (2021). Growth Performance and Survival Rate of Halimeda macroloba in Different Substrates of Coastal Waters. Journal of Marine Science and Management.
Badarinath, A. V., Rao, K. M., Chetty, C. M. S., Ramkanth, S., Rajan, T. V. S., & Gnanaprakash, K. (2010). A review on in-vitro antioxidant methods: comparisions, correlations and considerations. International Journal of PharmTech Research, 2(2), 1276–1285.
Bina, K., Di, K., Patas, D., & Buleleng, K. (2019). Studi Kelayakan Usaha Budidaya Rumput Laut Pada. 11(2), 573–582.
Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International, 18, 403–414.
FAO. (2022). The State of World Fisheries and Aquaculture 2022. Rome: Food and Agriculture Organization.
Gazali, M., Nurjanah, & Zamani, N. P. (2019). The Screening of Green Algae Halimeda opuntia (Linnaeus) as an Antioxidant from the Coast of West Aceh. Jurnal Ilmu Pertanian Indonesia, 24(3), 267–272. https://doi.org/10.18343/jipi.24.3.267
Guiry, M.D. & Guiry, G.M. (2024). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway.
Gunawan, D. H. (2018). Penurunan Senyawa Saponin pada Gel Lidah Buaya dengan Perebusan dan Pengukusan. Jurnal Teknologi Pangan, 9(1), 2597–436.
Harahap, K. S., Ratrinia, P., & Press, G. (2025). Senyawa Bioaktif dan Metode Ekstraksi Ramah Lingkungan Pada. March.
Harborne, J. B. (1987). Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan. Bandung: ITB Press.
Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: Functional food applications and legislation. Journal of Applied Phycology, 23, 543–597.
Lantah, P. L., Montolalu, L. A., & Reo, A. R. (2017). Kandungan Fitokimia dan Aktivitas Antioksidan Ekstrak Metanol Rumput Laut Kappaphycus alvarezii. Media Teknologi Hasil Perikanan, 5(3), 73. https://doi.org/10.35800/mthp.5.3.2017.16785
Leibo, R., Mantiri, D., & Gerung, G. (2017). Uji Aktivitas Antioksidan dari Ekstrak Total Alga Hijau Halimeda opuntia Linnaeus dan Halimeda macroloba Decaisne dari Perairan Teluk Totok. Jurnal Pesisir Dan Laut Tropis, 4(2), 30. https://doi.org/10.35800/jplt.4.2.2016.14081
Lobban, C. S., & Harrison, P. J. (1997). Seaweed Ecology and Physiology. Cambridge University Press.
Manuhara, G. J., Praseptiangga, D., & Riyanto, R. A. (2016). Extraction and Characterization of Refined K-carrageenan of Red Algae [Kappaphycus Alvarezii (Doty ex P.C. Silva, 1996)] Originated from Karimun Jawa Islands. Aquatic Procedia, 7, 106–111. https://doi.org/10.1016/j.aqpro.2016.07.014
Martiningsih, N. W., Agus, G., Widana, B., Lilik, P., & Kristiyanti, P. (2016). Skrining Fitokimia dan Uji Aktivitas Antioksidan Ekstrak Etanol Daun Matoa (Pometia pinnata) dengan Metode DPPH.
Muzaki, A. F., Setyati, W. A., Subagiyo, S., & Pramesti, R. (2018). Aktivitas Antioksidan Ekstrak Rumput Laut Halimeda macroloba dari Pantai Teluk Awur, Jepara, Jawa Tengah. Jurnal Enggano, 3(2), 144–155. https://doi.org/10.31186/jenggano.3.2.144-155
Nurjanah, Nurilmala, M., Hidayat, T., & Sudirjo, F. (2021). Karakteristik senyawa bioaktif dan aktivitas antioksidan makroalga tropis Indonesia.
Pangestuti, I. E., Sumardianto, S., & Amalia, U. (2017). Phytochemical Compound Screening of Sargassum sp. and Its Activity as Antibacterial Against Staphylococcus aureus and Escherichia coli. SAINTEK PERIKANAN: Indonesian Journal of Fisheries Science and

Technology, 12(2), 98.
Pangestuti, R., & Kim, S. K. (2011). Biological activities and health benefit effects of natural pigments derived from marine algae. *Journal of Functional Foods*, 3(4), 255–266. https://doi.org/10.1016/j.jff.2011.07.001
Prasetiyo, H., Sasongko, A. S., Fahira, D. D., & Ayuningsih, T. (2023). Skrining Fitokimia dan Uji Aktivitas Antioksidan Secara Kualitatif pada Ekstrak Rumput Laut Eucheuma cottonii. Jurnal Kemaritiman: Indonesian Journal of Maritime, 4(1), 30–42. https://doi.org/10.17509/ijom.v4i1.60288
Pratama, R. et al. (2022). Aktivitas antioksidan ekstrak makroalga berdasarkan variasi pelarut ekstraksi.
Putri, D., Ningsih, N., Hidayati, J. R., & Nugraha, A. H. (2024). Antioxidant Activity and Bioactive Compounds Extract Macroalgae Halimeda sp. Marinade, 07(02), 28–40.
Saifudin, A. (2014). Senyawa Alam Metabolit Sekunder: Teori, Konsep, dan Teknik Pemurnian. Yogyakarta: Deepublish.
Sari, D. P., et al. (2023). Stabilitas senyawa fenolik pada proses pengolahan bahan alam.
Sinutok, S., et al. (2022). Long-term monitoring of macroalgal communities and their resilience to bleaching events. Marine Ecology Progress Series.
Sitorus, R., & Hutabarat, S. (2024). Kajian alkaloid sebagai metabolit sekunder pada organisme laut dan tumbuhan.
Soamole, H. H., Sanger, G., & Harikedua, S. D. (2018). Rumput Laut Segar (Turbinaria sp., Gracilaria sp., dan Halimeda macroloba). 94–98.
Soumokil, I. J. (2024). Analisis kualitas air Teluk Pegametan, Desa Sumberkima, Kecamatan Gerokgak, Kabupaten Buleleng dalam mendukung pengembangan perikanan budidaya berbasis keramba jaring apung (KJA) ramah lingkungan [Skripsi, Universitas Pendidikan Ganesha]. http://repo.undiksha.ac.id/22002/
Subyakto, S., et al. (2016). Diversifikasi komoditas akuakultur berbasis biota lokal potensial untuk meningkatkan ketahanan pangan dan resiliensi ekonomi masyarakat pesisir. Jurnal Riset Akuakultur, 11(2), 145–158.