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Research Article

Seaweed Diversity and Bioactive Compounds in Panjang Islands, Central Java, Indonesia

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ABSTRACT

The biodiversity of seaweed encompasses a wide array of potential bioactive compounds applicable to various industries, particularly pharmaceuticals. This study aimed to collect seaweed diversity data from Panjang Island, Jepara, Central Java, Indonesia, and to identify the bioactive compounds and biological activity of each seaweed species for preliminary screening. Random sampling was used to collect the sample. Qualitative identification of bioactive compounds was performed using the maceration method for extraction, phytochemical screening tests, and pigment identification based on Rf values on TLC. Antibacterial screening tests were performed against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, followed by testing against pathogenic fungi (*C. albicans*) using the disk diffusion method, and an antioxidant test using the DPPH method. The results showed that six species from three phyla (Chlorophyta, Phaeophyta, and Rhodophyta) exhibited distinct morphological characteristics, and the types of bioactive compounds produced by each species differed. The biological activity test results showed a low inhibitory activity. Antibacterial and antifungal biological activities were at the value of (<5 mm), and antioxidant biological activity was (>750 ppm). However, the active compounds and pigments with potential antibacterial, antifungal, and antioxidant properties can be optimized in various fields of bioindustry in the future.

1. Introduction

Seaweed diversity constitutes a vital element of coastal ecosystems. It serves as a primary producer and provides habitat for marine organisms. Additionally, seaweeds possess significant potential for bioprospecting due to the bioactive compounds they produce. These compounds hold promise in the pharmaceutical and nutraceutical sectors. (Bahammou *et al*. 2021), reported the potential of seaweed bioactive compounds as antioxidants, anti-inflammatories, antifungal, and antibacterial agents. Bioactive compounds produced by seaweed species have been considered as potential bioprospecting materials.

Bioactive compounds extracted from seaweed, including alkaloids, steroids, saponins, flavonoids, and tannins, hold significant promise for development within various health-related industries. The spectrum of bioactive compounds varies considerably among different seaweed species. Each seaweed species

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contains active compounds and pigments that differ from those of other species. Seaweed diversity at each location was linearly correlated with the active compounds produced. Previous studies by Setyati *et al*. (2024) reported seaweed diversity in the waters of Panjang Island, showing fairly high species diversity. Seaweeds from the phyla Chlorophyta, Rhodophyta, and Phaeophyta dominate these water bodies.

In addition, (Pamungkas *et al*. 2024; Santosa *et al*. 2024) successfully collected several species of seaweed from the genera *Caulerpa, Sargassum* sp., *Padina* sp., *Halimeda* sp., and *Halymenia* sp. in Panjang Island waters. Pulau Panjang has various types of substrates, such as dead coral, rubble, sand, and rocks, which support seaweed growth. This substrate diversity is directly positively correlated with the species diversity of macroalgae in these waters. Suitable substrates, such as dead coral, rubble, and rocky structures, provide strong attachment points for seaweed holdfast, enabling water growth, nutrient absorption, and resistance to wave action (Ceccarelli *et al*. 2020). Water quality parameters such as salinity, DO, and pH on Panjang Island also affect the diversity of active compounds produced. However, research on the diversity of seaweed found on Panjang Island remains limited. Further research is needed to identify the bioactive compounds and potential biological activities of several seaweed species.

Based on this background, this study aimed to assess the diversity of seaweed species and investigate the bioactive compounds, pigments, and biological activities, including antifungal, antibacterial, and antioxidant activities, of seaweeds found in the waters of Panjang Island. The results of this study are expected to facilitate further utilization in the bioprospection of bioactive compounds of seaweed.

2. Materials and Methods

2.1. Materials

Seaweed sample diversity was obtained in early March 2024 from the waters of Panjang Island, Jepara, Central Java, Indonesia, with coordinates 6˚34'40 "S and 110˚37'51 "E (Figure 1). Phytochemical screening using Mayer, Wagner, Dragendorff, Liebermann-Burchard reagent; TLC testing using n-hexane and ethyl acetate absorbent; antibacterial using *Escherichia coli* (Migula 1895) ATCC 25922 and *Staphylococcus aureus* (Rosenbach 1884) ATCC 12600 as the pathogens, antifungal using *Candida albicans* (Robin) Berkhout ATCC 10231, and antioxidant activities using DPPH (2,2-diphenyl-1-picrylhydrazyl)and methanol. This research was conducted at the Tropical Marine Biotechnology Laboratory of Diponegoro University, from March to June 2024.

2.2. Methods

2.2.1. Water Quality Parameter Measurement

Data collection for water quality parameters was carried out to record the values of salinity using a refractometer, a pH meter was used to measure water acidity, and a DO meter was used to measure water temperature and dissolved oxygen (DO) at the sampling location for 3 repetitions on the day of the sampling. The seaweed sampling method uses random sampling (Widyartini *et al*. 2023), based on the condition and distribution of seaweed at ±1.2 m depth in the sampling location. Seaweed sampling was executed during low tides on sunny weather conditions. Random sampling methods are used to minimize bias and ensure that every individual in the population has an equal chance of being selected.

2.2.2. Samples Preparation and Extraction

Samples were classified based on morphological and size characteristics, using the Algaebase literature guidelines as a reference. The collected samples were washed three times with running water and subsequently air-dried for seven days. The dried samples were then cut into small pieces and weighed individually. The sample extraction process was based on the method of Sobuj *et al*. (2021), namely maceration using methanol as the extraction solvent at a 1:10 ratio for 7 days, with a shaker used to support the maceration process (Taherkhani *et al*. 2024). The next step is to analyze the yield of the crude extract by calculating the weight of the evaporated extract divided by the dry weight, then multiplying by 100%.

2.2.3. Screening Bioactive Compounds

Phytochemical screening of seaweed extracts was carried out to identify secondary metabolite compounds such as flavonoids (purple, reddish-black, or yellow coloration show positive result), saponins (presence of froth show positive result), tannins (blue or greenish-black coloration show positive result), alkaloids (white precipitate show positive result), and steroids (dark blue coloration show positive result) contained in seaweed

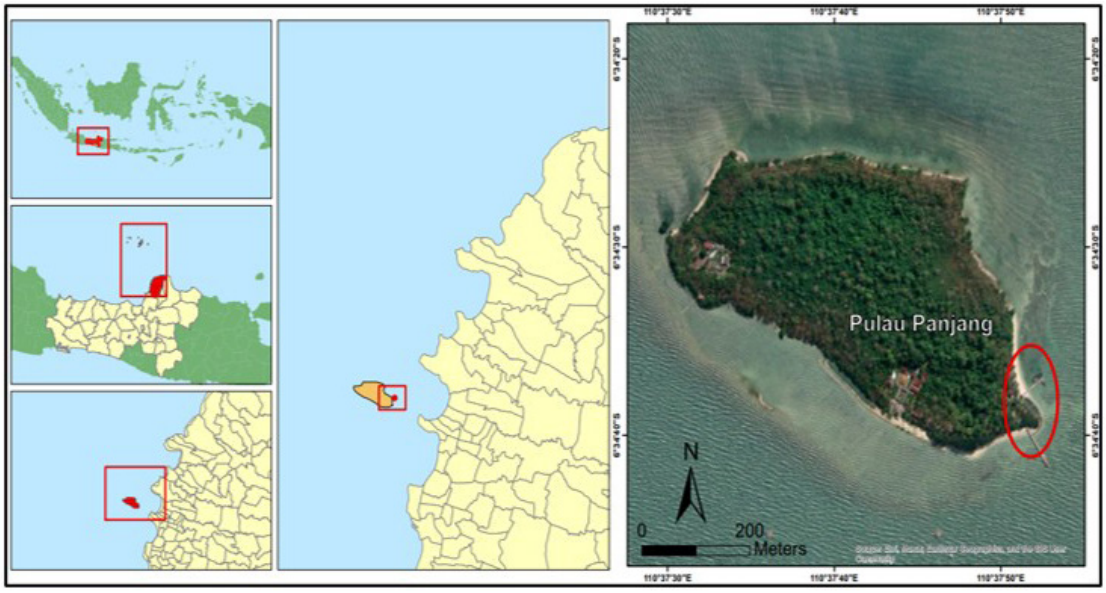

Figure 1. Location map of seaweed sample collected from Panjang Island, Jepara (Google Earth 2025)

extracts using Lieberman Burchard, $FeCl_3$ 5%, HCl 37%, Wagner and Dragendorff test reagents (Susilowati *et al*. 2024). The presence of pigments was identified based on the Rf value using the Thin Layer Chromatography (TLC) method described by Rajauria & Abu-Ghannam (2013), with a silica plate (MERCK G60 F254) as the stationary phase and a mixed solvent of n-hexane: ethyl acetate with a 6:4 ratio as the mobile phase. Plate visualization and Rf measurements were performed by observing the spots under visible light and ultraviolet light at wavelengths of 254 and 366 nm.

2.2.4. Antibacterial and Antifungal Activity Test

The seaweed extracts were tested for antibacterial and antifungal activity against the Gram-negative pathogenic bacterium *E. coli*, the Gram-positive pathogenic bacterium *S. aureus*, and the pathogenic fungus *C. albicans*. The pathogens were inoculated into broth media for 1 × 24 hours, then standardized using McFarland 0.5 (1.5×10^8 CFU) (Selvi *et al*. 2023; Küçükçiftci *et al*. 2025). The concentrations of the extract used in the antibacterial and antifungal assays were 1000 ppm. Dimethyl sulfoxide (DMSO) 1% was used as the negative control in both assays (Ainane *et al*. 2014), while chloramphenicol served as the positive control for the antibacterial assay that contained 10µg/disk for all bacterial pathogens (O'keeffe *et al*. 2019) and nystatin for 5µg/disk for the antifungal assay (Peres *et al*. 2012; Puškárová *et al*. 2017). Antibacterial and antifungal testing was performed using an *in vitro* disk diffusion assay.

2.2.5. Antioxidant Activity Test

Antioxidant activity was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as described by Cho *et al*. (2007), with modifications. The free radical capture activity was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) at a wavelength of 520 nm (Nazarudin *et al*. 2022). The antioxidant activity of the sample extract was evaluated at concentrations of 125, 250, 500, and 1000 ppm. A DPPH solution (4 × 10-4 M) was prepared by dissolving 0.004 g of DPPH powder in methanol solvent (Afrin *et al*. 2023). Ascorbic acid was used as the reference standard at concentrations of 1.25, 2.5, 5, 10, and 20 ppm.

3. Results

3.1. Water Quality Parameter Measurement

The measured water quality parameters included salinity, pH, temperature, and dissolved oxygen (DO). These measurements were conducted at the sampling site on Panjang Island. The results of the water quality measurements are presented in Table 1 below.

3.2. Identification of Seaweed Samples

Based on the identification of seaweed samples collected from the waters of Panjang Island, six species

of seaweed were found. These seaweeds belong to the green (Chlorophyta), brown (Phaeophyta), and red (Rhodophyta) algae. The results of the identification and detailed morphology are presented in Figure 2 below.

3.3. Analysis of Crude Extract Yield

The extraction process was carried out using the maceration method, which aims to extract the components of active compounds in the six species of seaweed. Maceration of seaweed samples was conducted using methanol. Each seaweed sample had a different dry weight depending on the species. The crude extract yield is calculated as the weight of the evaporated extract divided by the dry weight, then multiplied by 100%. The percentage of crude extract yield is shown in Table 2 below.

Table 1. Water quality parameters of Panjang Island Waters

Parameters	Result
Dissolved Oxygen	9.27±0.78 mg/L
pH	8.51±0.18
Temperature	30±2.08°C
Salinity	31±0.58 ppt

3.4. Screening Bioactive Compounds

Phytochemical screening and TLC tests of extracts from *H. micronesica, H. macroloba, C. serrulata, P. australis, S. binderi*, and *H. durvillei* were conducted to qualitatively determine the groups of secondary metabolite compounds and the variation of predicted pigments. The screening results revealed that the seaweed extracts contained various secondary metabolites, including saponins, steroids, tannins, alkaloids, and flavonoids. In addition, the TLC test showed various Rf values, indicating various pigments. The results of the phytochemical screening and TLC tests are shown in Table 3 below.

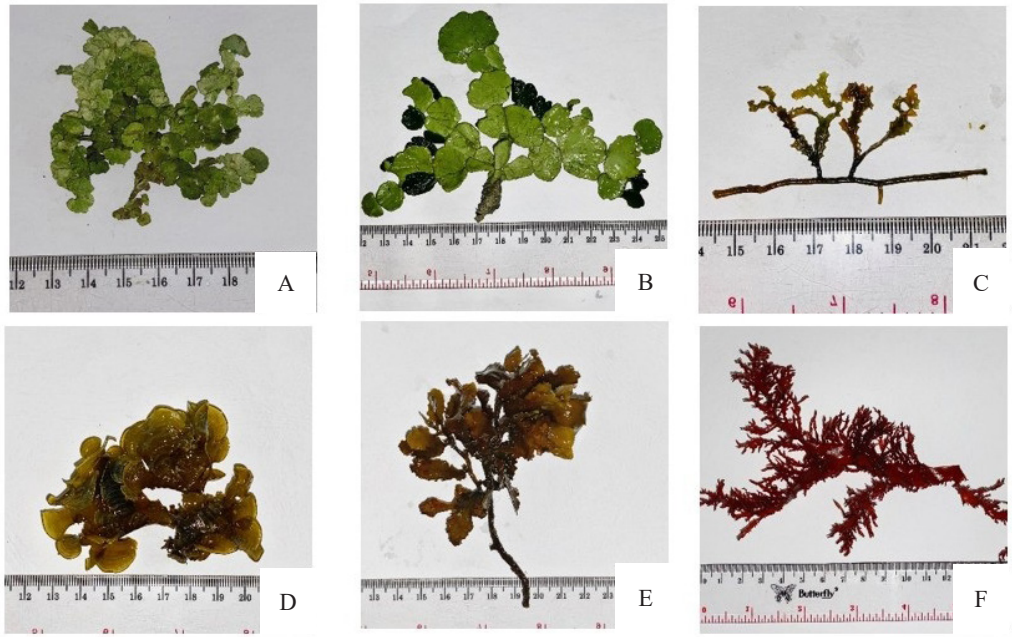

Figure 2. Collection of seaweed samples from Panjang Island waters, Jepara, Indonesia: A) *Halimeda micronesica* (Yamada 1941); B) *Halimeda macroloba* (Decaisne 1841); C) *Caulerpa serrulata* (Agardh 1837); D) *Padina australis* (Hauck 1887); E) *Sargassum binderi* (Agardh 1848); F) *Halymenia durvillei* (Vincent 1828) (Private Documentation: March 2024)

Table 2. Percentage of crude seaweed extract yield

Species	Dry weight (g)	Wet weight (g)	Extra volume (mL)	Evaporation total results	Extraction yield (%)
H. micronesica	100	186.87	1000	0.67	0.67
H. macroloba	50.18	123.97	500	0.33	0.66
C. serrulata	35	46.63	570	0.23	0.66
P. australis	15	21.31	590	0.1	0.67
S. binderi	100	121.11	1800	0.67	0.67
H. durvillei	281.42	200	2268	1.88	0.67

3.5. Antibacterial and Antifungal Activity

The antibacterial activity of the samples was tested against *E. coli* and *S. aureus*, and their antifungal activity was tested against *C. albicans*. The extent of the clear zone that appeared on the test media indicated the ability of the crude seaweed extract to act as antibacterial or antifungal agents (Table 4). The results showed that four out of six seaweed species were active as antibacterial agents against the pathogen *E. coli*. In comparison, three out of six seaweed species were active as antibacterial agents against the pathogen *S. aureus*. Antifungal testing showed that three of six species were active against the pathogen *C. albicans.*

3.6. Antioxidant Activity Test

Antioxidant activity was assessed using the DPPH method with an ELISA reader. The ratio used was 4:1 for the sample-to-DPPH solution. The results of the antioxidant assay, including the percentage of inhibition, IC_{50} values, and the inhibition percentage graph, are presented in Table 5 and Figure 3 below.

The antioxidant activity of each seaweed extract varies in its inhibition percentage. IC_{50} value of all seaweeds extract was determined from the average inhibitory concentration with 3 repetitions. The antioxidant activity is shown in Table 5.

Table 3. Diversity of bioactive compounds

Species	Phytochemical screening results	TLC test (Rf Value)	Reference
H. micronesica	Flavonoids, tannins & steroids	0.20 (Carotenoid) 0.60 (Chlorophyll b) 0.70 (Carotenoid) 0.78 (Chlorophyll b) 0.87 (Chlorophyll a)	(Sathya 2017)
H. macroloba	Alkaloids	010 (Chlorophyll a) 0.77 (Pheophytin α) 0.97 (β-carotenoid)	(Sathya 2017)
C. serrulata	Tannin, steroids & alkaloids	0.13 (Chlorophyll b) 0.27 (Carotenoid) 0.88 (Carotenoid)	(Gomes *et al.* 2024)
P. australis	Alkaloids	0.13 (Chlorophyll -B) 0.27 (Xanthophyll) 0.88 (Pheophytin α)	(Rajauria & Abu-Ghannam 2013)
S. binderi	Tannins, steroids, saponins & alkaloids	0.58 (Carotenoid) 0.36 (Fucoxanthin) 0.70 (Carotenoid)	(Rajauria & Abu-Ghannam 2013)
H. durvillei	Flavonoids, saponins & alkaloids	0.17(Canthaxanthin) 0.27 (Chlorophyll b)	(Gomes *et al.* 2024)

Table 4. Antibacterial and antifungal activity

Species	Antibacterial screening		Antifungal screening
	E. coli (mm)	*S. aureus* (mm)	*C. albicans* (mm)
H. micronesica	±3.54	-	-
H. macroloba	-	±1.5	-
C. serrulata	±2.51	±2.4	±1.7
P. australis	±2.45	-	-
S. binderi	-	±2.3	±1.8
H. durvillei	±2.23	-	±2.9
Chloramphenicol (+)	±3.46	±3.5	-
Nystatin (+)	-	-	±2.9
DMSO (-)	-	-	-

4. Discussion

4.1. Water Quality Parameter

This study reports the results of three replicate measurements of parameter quality in Panjang Island waters, including DO, pH, temperature, and salinity. The results of the water parameter measurements were calculated based on the average and standard deviation values (Table 1). Measuring the water quality on Panjang Island aims to determine the environmental conditions of seaweed habitats. In addition, water quality parameters affect the quantity and quality of secondary metabolites produced by seaweed (Lomartire *et al*. 2021). The water quality parameters of live seaweeds affect the quality of the active compounds produced. Abiotic environmental factors, such as salinity, pH, temperature, and nutrient composition, affect the profiles of bioactive compounds. This is because seaweed produces active compounds when environmental conditions exert pressure on its growth and development, which then respond by producing secondary metabolites. (Cotas *et al*. 2020), reported that the parameters that can affect the production of active phenol compounds from seaweed depend on seasonal conditions, location, and speciation of an organism. However, when water parameters exceed the normal threshold of water conditions, seaweeds directly employ cellular mechanisms to produce bioactive compounds to survive. In addition to water

Table 5. Antioxidant activity test

Species	Antioxidant activity (IC_{50}) (ppm)
H. micronesica	±904
H. macroloba	±753.3
C. serrulata	±1100.6
P. australis	±902
S. binderi	±892.6
H. durvillei	±862.4

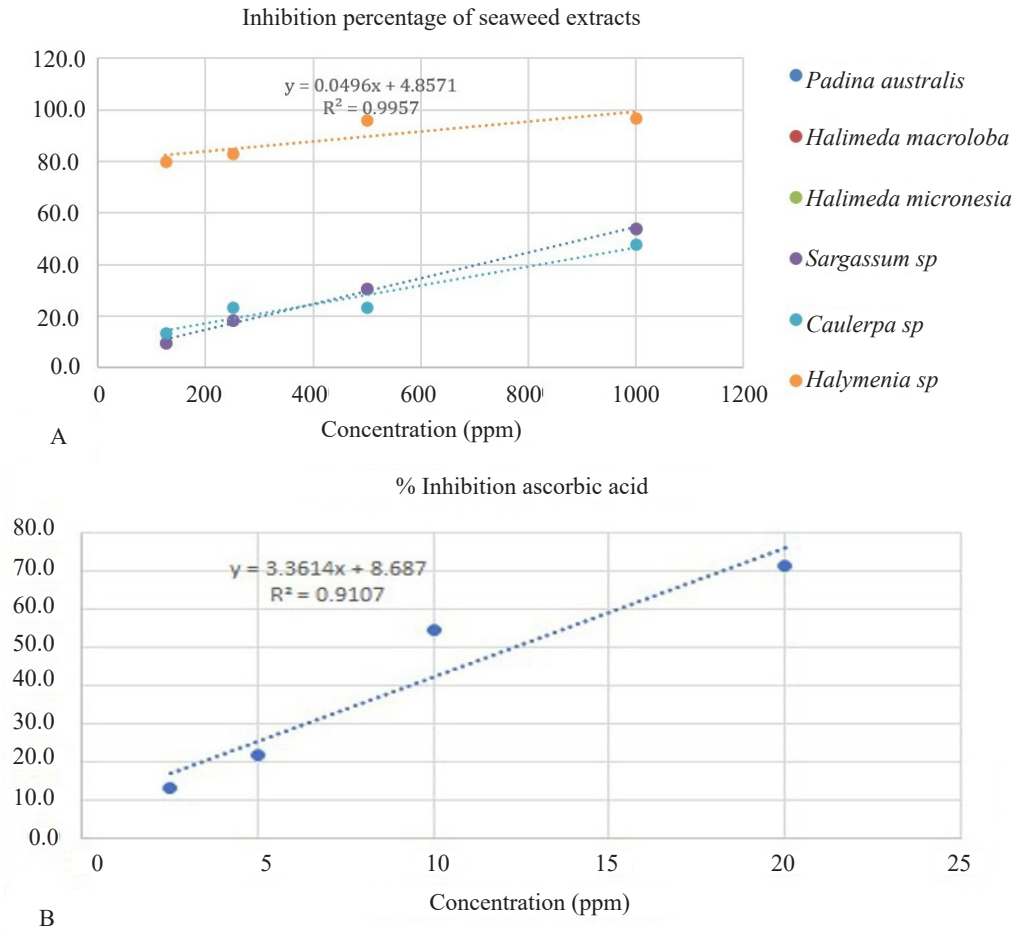

Figure 3. Antioxidant activity test curve with ELISA. A) seaweed extract inhibition curve, and B) ascorbic acid standard solution inhibition curve

parameters, the conditions of polluted materials that contaminate the environment also affect seaweeds' production of secondary metabolites, such as heavy metals (cadmium, chromium, and zinc) (Connan & Stengel 2011).

The relatively low bioactivity observed in seaweed extracts from Panjang Island may be closely related to the prevailing environmental conditions at the sampling site. As shown in Table 1, the waters exhibited stable and favorable parameters, including high dissolved oxygen levels (9.27±0.78 mg/L), slightly alkaline pH (8.51±0.18), normal tropical temperatures (30±2.08°C), and typical marine salinity (31±0.58 ppt). Such conditions indicate a low-stress environment for seaweed growth. Previous studies have demonstrated that the production of secondary metabolites in marine macroalgae is often enhanced under environmental stress, such as extreme pH, salinity fluctuations, or nutrient limitation, as these compounds function as chemical defenses against biotic and abiotic pressures (Lopes *et al*. 2024; Macías-de la Rosa *et al*. 2024). In contrast, under stable, optimal conditions, seaweeds tend to prioritize primary metabolic processes and growth over secondary metabolite synthesis. Therefore, the relatively low bioactivity detected in this study may reflect limited induction of bioactive compound production under the low-stress environmental conditions of Panjang Island waters.

4.2. Identification of Seaweed Samples and Analysis of Crude Extract Yield

The diversity of seaweeds collected from the waters of Panjang Island came from the phyla: Chlorophyta, Rhodophyta, and Phaeophyta (Figure 2). Seaweed species include *Halimeda micronesica, Halimeda macroloba, Caulerpa serrulata, Padina australis, Sargassum binderi,* and *Halymenia durvillei.* Seaweed species exhibit different characteristics and morphologies. The seaweed *H. micronesica* has a smooth thallus with creepers that can reach a height of 10 cm (Pongparadon *et al*. 2015), growing on rock coral substrates attached to the sides of living corals. The seaweed species *H. macroloba* has a thallus with a high lime content of $CaCO_3$. The fan-shaped thick segmentation measured 15 mm in length and 21 mm in width. Habitat thrives on sand substrates and sand-mud. Different species of *C. serrulata* seaweed, which belong to the same phylum, have elongated, flat, and spiral ramuli with jagged or wavy edges (Shams & Amini 2017). It grows on sand and rock substrates across the reef. Another seaweed species from the *Phaeophyta phylum, P. australis*, has a thallus shape similar to that of a fan, with thin, lobed segments, radial hairlines, and calcification on its surface. It attaches to rocks across the average area of the reef, both in sheltered areas and in areas exposed to direct waves (Prathep 2005). The seaweed species *S. binderi* has a flat shape with a smooth/slippery texture, with regular alternate branching, opposite main branches close together, and arising above the main stem (Noiraksar & Ajisaka 2008). The seaweed species *H. durvillei*, which also belongs to the phylum Rhodophyta, was successfully collected with the characteristics of a flat thallus with hairy edges. In contrast, others were branched, with a flat, wide central stem and a wrinkled edge. The habitat in which it grows is attached to corals or other hard substrates (Mon 2018).

The collected seaweeds have different morphological characteristics and habitats. These differences may be in the form of size, thallus shape, branching type, and habitat differences. In addition, seaweed diversity is correlated with the variety of bioactive compounds produced by each seaweed (Ahmed *et al*. 2024). Each seaweed species has a distinct secondary metabolite profile due to its environmental adaptation. Variations in the production of secondary metabolites by each seaweed species are influenced by environmental pressure. Studies by Lomartire & Gonçalves (2022) reported that seaweed species from the phylum Chlorophyta produce bioactive compounds from the terpenoids and alkaloid compound classes in response to environmental stress. Variations in these compounds are a form of adaptation to ecological interactions. Seaweed diversity and bioactive compound profiles are influenced by geographic variations and the distribution of seaweed habitats (Nurhidajah *et al*. 2024). (Park *et al*. 2023) reported that seaweeds of the same species but from different locations showed significant differences in the bioactive compounds produced. Differences in the variation of active compounds in several seaweed species collected from the waters of Panjang Island are shown in Figure 2.

In the extraction process, all seaweed samples were macerated using methanol, a polar solvent. The use of methanol solvents aims to optimize the process of extracting active compounds from seaweed (Riyadi *et al*. 2023). The percentage yield of the evaporated seaweed crude extract was determined. Based on the results of the calculation of the percentage yield (Table 2), it was concluded that the six types of seaweed tested. However, the initial dry weight and the amount of solvent used were different; the final result (yield) was very similar,

in the range of 0.66-0.67%. This indicates that with the maceration method used in this experiment, all types of seaweed produced a pure extract with almost the same percentage of dry weight. (Zarrinmehr *et al*. 2022), reported that the yield of an extract can be influenced by several factors, namely the concentration of the solvent and the polarity of the solvent used. The similarity in extraction yield among the six seaweed species is likely attributable to the use of identical extraction methods and controlled experimental conditions, including solvent type, extraction time, temperature, and solvent-to-sample ratio. These consistent conditions minimized variability during the extraction process, resulting in comparable yields. In addition, the six seaweed species may possess relatively similar compositions of extractable compounds, particularly those soluble in the solvent used, leading to nearly identical percentages of dry extract (Holdt and Kraan 2011). The higher the solvent concentration and polarity of the solvent used, the higher the percentage of yield.

4.3. Screening Bioactive Compounds

Six seaweed species were collected, and phytochemical screening tests were performed using test reagents to detect the bioactive compounds in the seaweed methanol extracts. The results of this study showed that five seaweed species were positive for alkaloid-active compounds, except *H. micronesica*. However, Lubis *et al*. (2020) reported that *H. micronesica* seaweed obtained from the waters of Maspari Island, South Sumatra, contains alkaloids. This is in accordance with the statement by Nurhidajah *et al*. (2024), who stated that differences in location are one of the factors that affect the type of bioactive compounds produced by seaweed. The five seaweed species that were positive for bioactive alkaloid compounds are thought to be the result of the interaction of seaweed diversity distribution, which creates the same defense mechanism as other species in the same location. The diversity of seaweeds at the same location shows that the secondary metabolite compounds produced are the same as those produced by the seaweed species distributed at the same location (Park *et al*. 2023). This pattern demonstrates seaweed's ability to interact specifically with its environment and surrounding organisms.

Other results in this study show that seaweed diversity is also reflected in variation in pigments successfully identified by Rf values in the TLC test. Previous studies have determined the Rf values and types of pigments detected. This study successfully identified several types of pigments in each type of seaweed. The Rf value in the TLC test indicates the ability of each pigment to travel a distance on the TLC plate as a stationary phase. The mobile phase used was n-hexane/ethyl acetate at a ratio (v/v) of 6:4. The spots that appeared on the TLC plate were observed visibly and non-visibly using UV light at different wavelengths. The results showed that the seaweed extracts contained diverse types of pigments. Seaweeds from the phylum Chlorophyta dominate the type of chlorophyll a and b pigments, whereas seaweeds from the *Phaeophyta phylum* dominate the type of pigment produced, namely, carotenoid pigments. Based on the Rf value in the TLC test, *H. durvillei* from the phylum Rhodophyta contained canthaxanthin pigment at an Rf value of 0.17-0.42. It is a pigment from the red-colored ketocarotenoid group that can be used in the pharmaceutical industry (Yordi *et al*. 2024). Seaweeds also produce diverse pigments in response to their biological activities and environmental pressures.

4.4. Antibacterial and Antifungal Activity

The bioactive compounds produced by seaweeds play a role in their biological activities in marine waters. These bioactive compounds have the potential to be used in seaweed defense against environmental conditions. Bioactive compounds also serve as a defense against pathogenic bacteria, fungi, and UV radiation from sunlight. In this study, antibacterial and antifungal screening tests were successfully conducted against *E. coli, S. aureus,* and *C. albicans* (Table 4). Antibacterial screening of seaweed extracts of *H. micronesica, C. serrulata, P. australis,* and *H. durvillei* against the gram-negative bacteria *E. coli* showed a weak antibacterial inhibition zone (< 5 mm). (Sanam *et al*. 2022) reported that the antimicrobial potency was evaluated using inhibition zone diameter into four classifications: weak (5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (20-30 mm). Antibacterial screening against *S. aureus* showed that the seaweed extracts of *H. macroloba, C. serrulata,* and *S. binderii* exhibited weak antibacterial activity. The antifungal screening test showed that three out of six seaweed species exhibited antifungal activity against the pathogen *C. albicans.* The antibacterial and antifungal activities of seaweed extracts demonstrate the ability of seaweed bioactive compounds to act as antibacterial and antifungal agents, as indicated by the clear zone that appears on the test media. Antibacterial and antifungal activities are linear to the mechanism of bioactive compounds produced by seaweed in destroying bacterial cells and inhibiting the

growth of pathogenic bacteria and fungi. (Pérez *et al*. 2016) reported that active compounds from the terpenoid, alkaloid, steroid, and saponin groups in seaweed extracts have antibacterial and antifungal activities. Alkaloid and flavonoid bioactive compounds in seaweed extracts inhibit antibacterial activity by damaging bacterial cell walls and membranes (Kalasariya *et al*. 2021). In addition, Karpiński & Adamczak (2019) reported that the dominant fucoxanthin pigment in seaweeds of the phylum Phaeophyta has antimicrobial properties. Based on (Table 4), several seaweed extracts exhibited measurable antibacterial and antifungal activities, although not with a broad spectrum, with inhibition zones against *E. coli, S. aureus,* and *C. albicans* ranging from ±1.5 to ±3.54 mm. The positive controls, chloramphenicol and nystatin, produced inhibition zones ranging from ±2.9 to ±3.5 mm. Several factors, including bacterial density, media consistency and thickness, disc size, breakpoints, temperature, and more, could influence these varying results of positive control inhibition zone diameters. A slight difference in the contributing factors can lead to variability in results, since the zone inhibition diameters also rely on the diffusion of the antimicrobial into the media (Sahoo *et al*. 2024). The distribution of bacterial colonies throughout the media may also influence zone development; a high bacterial density can decrease the size of the inhibition zone. Additionally, pathogen efficacy may influence the inhibition zone diameter: as the resistance of the pathogen strain's resistance increases, the inhibition zone diameter decreases.

4.5. Antioxidant Activity

The antioxidant activity test is a quantitative test used to determine the inhibitory effect of free radical scavengers in the extract. One of the antioxidant test methods is the DPPH method, in which the calculated value is expressed in the form of the IC_{50}. The antioxidant test in this study used DPPH powder as the free-radical reagent. Based on the results of the antioxidant activity tests (Figure 3) on seaweed extract samples, the average percentage of inhibition at different concentrations (125, 250, 500, and 1000 ppm) was obtained for each type of seaweed extract. Ascorbic acid was used as a positive control at concentrations of 1.25, 2.5, 5, 10, and 20 ppm. Based on the data processing results, the IC_{50} values of 6 seaweed species were >750 ppm, indicating a very weak category (Table 5). Specifically, a compound can be categorized as a very strong antioxidant if its IC_{50} value is <50 ppm, a strong antioxidant if 50 ppm<IC_{50}<100 ppm, a moderate antioxidant if 100 ppm<IC_{50}<150 ppm, and a weak antioxidant if its IC_{50} value is >200 ppm (Prayitno *et al*. 2025). These results are thought to be due to the fact that the seaweed sample extracts tested are still in the form of a mixture of several compounds. In contrast, ascorbic acid, which serves as a positive control test, is already a pure compound that has a specific function as an antioxidant, so the working process of ascorbic acid is optimal. Phytochemical screening is qualitative and only indicates the presence or absence of compound classes. Therefore, although flavonoids and tannins were detected, their concentrations may be insufficient to produce significant DPPH radical-scavenging activity, as antioxidant capacity has been widely reported to depend on total phenolic and total flavonoid contents rather than on qualitative detection alone (Shahidi and Ambigipalan 2015; Altemimi *et al*. 2017). Furthermore, the decrease in antioxidant activity measured in this study can be attributed to the intrinsic metabolic composition of the investigated seaweed species, particularly the lower abundance of phenolic compounds and other electron-donating metabolites, which are widely recognized as key contributors to radical-scavenging activity in macroalgae (Carpena *et al*. 2024). Such compositional differences are well documented among macroalgal taxa and are strongly influenced by species-specific metabolism as well as physiological and ecological factors. Therefore, the observed activity reflects natural biochemical variation among macroalgae and should not be construed as a limitation of the sample quality.

The free radical inhibition activity of seaweed extracts varies with sample handling methods, the type of solvent used in the extraction process, and environmental factors (Nazirah *et al*. 2023; Riwanti & Juniar 2024). (Soliman *et al*. 2024) reported that carotenoid pigments and phycocyanin are bioactive compounds with strong antioxidant properties. However, all seaweed species from the waters of Panjang Island are still classified as having biological activity in counteracting free radicals. Various pigments and bioactive compounds present in seaweed extracts exhibit free radical-scavenging activity. Compared to synthetic compounds, natural bioactive compounds from seaweed support sustainability and are environmentally friendly. The extraction solvent strongly influences antioxidant activity. If the solvent polarity is not appropriate for extracting phenolic or carotenoid compounds, the resulting extract may exhibit weak radical-scavenging ability. Processing methods such as sun-drying, heating, or prolonged extraction can degrade heat-sensitive antioxidants, including phenolics, carotenoids, and phycobiliproteins, thereby reducing

DPPH scavenging activity (Chan *et al*. 2015). Thus, the diversity of bioactive compounds in seaweed is a natural resource with antibacterial, antifungal, and antioxidant activities for bioprospection applications, which must be optimized in the future.

In conclusion, the six seaweed species identified from the waters of Panjang Island exhibit distinct morphological characteristics and differences in chemical composition. Variations in secondary metabolites and pigments among species reflect differences in species-specific traits as well as environmental influences and natural defense mechanisms. Although the bioactivity assays revealed relatively low activity levels, the presence of diverse classes of bioactive-related compounds indicates underlying chemical diversity within these seaweeds. The findings of this study contribute to baseline knowledge on seaweed diversity and metabolite composition in Panjang Island waters and provide an initial reference for future ecological and biochemical studies. Further research involving targeted compound isolation, quantitative analysis, and controlled stress-induced experiments is required to better evaluate the bioactive potential of these seaweed species.

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