Natural Pigment Screening: Comparative Analysis of Chlorophyll and Carotenoid Content and Antioxidant Potential in Various Seaweed Species

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Abstract

Seaweeds contain a variety of photosynthetic pigments, including chlorophylls and carotenoids, which act as natural antioxidants and provide a safer alternative to synthetic additives. This research was conducted to determine the chlorophyll a, carotenoid content, and antioxidant activity of nine samples of seaweeds collected from Kartini Beach, Jepara, Central Java, Indonesia. That could be examined for the relationship between pigments and radical scavenging activity. Several seaweeds (Sargassum sp. 1, Sargassum sp. 2, Padina sp., Turbinaria sp., Halimeda sp. 1, Halimeda sp. 2, Caulerpa sp., Gracilaria sp., Eucheuma sp.) were subjected to maceration in methanol (1 g dw/25 mL) for seven days (room temperature). Pigment determination was based on UV–Vis spectra (400–750 nm), and the content of chlorophyll a and total carotenoids was calculated using absorbance equations. Antioxidant activity was analyzed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, with 20 µg/mL of ascorbic acid as the positive control. The following tests were used for data analysis: Kruskal-Wallis, Mann-Whitney, and Spearman correlation. The chlorophyll a and carotenoid contents were significantly different among the extracts. Extract type had an impact on chlorophyll a and carotenoids ($P < 0.05$), with the highest chlorophyll a (489.42 µg/g dw) and carotenoids (248.83 µg/g dw) found in Caulerpa sp. Sargassum sp.1 extract showed the strongest DPPH radical scavenging activity with an inhibition percentage of 92.59%, followed by Caulerpa sp. (76.43%), then ascorbic acid (71.21%). A strong positive correlation was found between chlorophyll a and carotenoids ($\rho = 0.770$), and a moderate positive correlation between antioxidant activity and pigment content ($\rho = 0.596$ in chlorophyll a and $\rho = 0.507$ in carotenoids), indicating that pigments contribute together with other metabolites. The study results suggest that, based on morphology, C. serrulata and S. echinocarpum have the potential to be utilized as a source of natural pigments with antioxidant activity in the food, nutraceutical, and cosmeceutical industries

Keywords: *Caulerpa serrulata*, methanol extract, natural colorant, pigment content, *Sargassum echinocarpum*

INTRODUCTION

Seaweed is a marine bioresource containing many bioactive compounds such as natural pigments, chlorophyll, and carotenoids. These pigments are essential for photosynthesis and have some health benefits (Nunes *et al*., 2017; Yalçın *et al*., 2020). The antioxidant activity of chlorophylls and their derivatives is due to their ability to quench singlet oxygen, donate electrons, and scavenge free radicals. This activity reduces oxidative stress and prevents biomolecular damage (Pérez-Gálvez *et al*., 2020). The carotenoids, i.e., β-carotene, lutein, and fucoxanthin, are efficient antioxidants that neutralize reactive oxygen species, quench singlet oxygen, and prevent lipid peroxidation in cell membranes (Kawata *et al*., 2018; Pruccoli *et al*., 2024). Therefore, the natural pigment profiles of chlorophylls and carotenoids in different seaweed species are indispensable for comparative investigation of their antioxidant activity.

The underlying issue is the increasing demand for natural antioxidant sources to replace synthetic ones, which can exhibit toxic, mutagenic, or carcinogenic effects when used over an extended period. Free radicals and reactive oxygen/nitrogen species (ROS/RNS) are crucial components of oxidative stress that damage proteins, lipids, and DNA, ultimately leading to degenerative diseases, including cardiovascular diseases, diabetes, Alzheimer's disease, Parkinson's disease, and chronic inflammation (Pooja *et al.*, 2025). Seaweed is regarded as a potential novel resource due to its bioactive pigments, such as carotenoids (fucoxanthin, astaxanthin, β-carotene, and lutein), chlorophylls, and phycobiliproteins, and other bioactive compounds, such as phlorotannin, flavonoid, and sulfated polysaccharide, which have potent antioxidant activity (Catarino *et al.*, 2020; Vieira *et al.*, 2025). These pigments can scavenge free radicals and exhibit

Diterima/Received : 08-07-2025, Disetujui/Accepted : 02-10-2025
DOI: https://doi.org/10.14710/jkt.v29i1.28463

other bioactivities, such as anti-inflammatory, neuroprotective, and metabolic regulation, making them valuable for applications in functional foods, nutraceuticals, pharmaceuticals, and cosmetics (Meirelles *et al.*, 2023; Shanaida *et al.*, 2025).

Variations in the pigment composition of seaweeds from various aquatic habitats have been extensively examined for a long time. For instance, for Red Sea brown seaweeds, carotenoid values ranging from 2.19 to 3.15 µg/g were reported, whereas the value of chlorophyll content ranged from 2.88 to 3.86 µg/g. Major seaweed pigments, such as fucoxanthin, violaxanthin, β-carotene, and chlorophyll a and b derivatives, have been identified (Rajauria, 2019). Among these, *Polycladia myrica* has a higher concentration of fucoxanthin (3.12 µg/g dw) and β-carotene (0.55 mg/100 g dw), which is attributed to the strong antioxidant potential of this seaweed (Ismail *et al.*, 2023). The chlorophyll a content in a study of 11 red seaweed species from the central coast of Portugal varied between 2.27 and 38.41 µg/g of fresh weight, with even lower amounts of carotenoids (0.12–1.88 µg/g). Of the 11 species, *Jania rubens* and *Porphyra umbilicalis* showed slightly higher chlorophyll a and carotenoid contents than the others, as these seaweeds had marked differences in their pigment profiles (Freitas *et al.*, 2022). In 15 species of tropical and temperate seaweeds in Asia (Indonesia and Japan), the primary chlorophyll content varies by the phylum. Chlorophyll a and b are present in green seaweeds, chlorophyll a and c are present in brown seaweeds, and predominantly chlorophyll a is present in red seaweeds. The main carotenoid identified is β-carotene, which is found in all species, with fucoxanthin predominating in brown seaweed (Susanto *et al.*, 2019). This is consistent with previous findings from Indonesia, where brown algae collected from Pulau Panjang showed chlorophyll contents of 1.73–8.84 mg/g dw and carotenoids of 0.55–4.06 mg/g dw, with fucoxanthin and β-carotene ranging from 0.43–4.11 mg/g dw and 0.16–0.78 mg/g dw, respectively (Heriyanto *et al.*, 2017). Hidayati *et al.* (2022) reported that *Padina* sp. from Bintan Island had a higher pigment profile, with chlorophyll and total carotenoid contents of 9.18 mg/g dw and 26.46 µmol/g dw, respectively. Collectively, these studies indicate that seaweed pigments differ significantly by species, growing location, and growing conditions, indicating the need for comparative studies to select better seaweed species for their potential value as natural antioxidants.

Chlorophyll and carotenoids are the primary pigments in seaweeds. Although this is documented in the existing literature, most research tends to focus on identifying individual pigments. There is a gap in studies exploring the relationship between the composition of these pigments and their functional capabilities, such as their antioxidant properties (Heriyanto *et al.*, 2017; Susanto *et al.*, 2019; Freitas *et al.*, 2022). In addition, research comparing seaweed species to identify pigment-rich seaweeds and study the correlation of various pigments, which are potentially able to serve as natural antioxidants, is limited (Rajauria, 2019; Ismail *et al.*, 2023). This study aimed to assess and compare the pigment profiles of selected seaweed species to evaluate their potential as natural pigments and as antioxidants.

MATERIALS AND METHODS

Seaweed samples (Figure 1) were collected from the waters of Kartini Beach, Jepara, Central Java, Indonesia (6°35'02.1 "S110°38'39.3" E) in February 2024. The samples were collected manually using skin diving equipment at a depth of 1 m. Subsequently, the samples were prepared for the next phase of the investigation. Images of the seaweed samples were captured before the extraction process. The seaweed samples were then rinsed with fresh water to remove impurities and salt, and dried in the shade. Once dried, the samples were cut into small pieces, approximately 1 cm in size, and stored in a Ziplock bag. Methanol (pro-analysis, Merck) was used for extraction and analysis. The reagents used were 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich) and ascorbic acid (Sigma-Aldrich).

The extraction process involved maceration with methanol. Pigment extraction procedures using methanol have shown high recovery rates, making them a quick and efficient choice for some

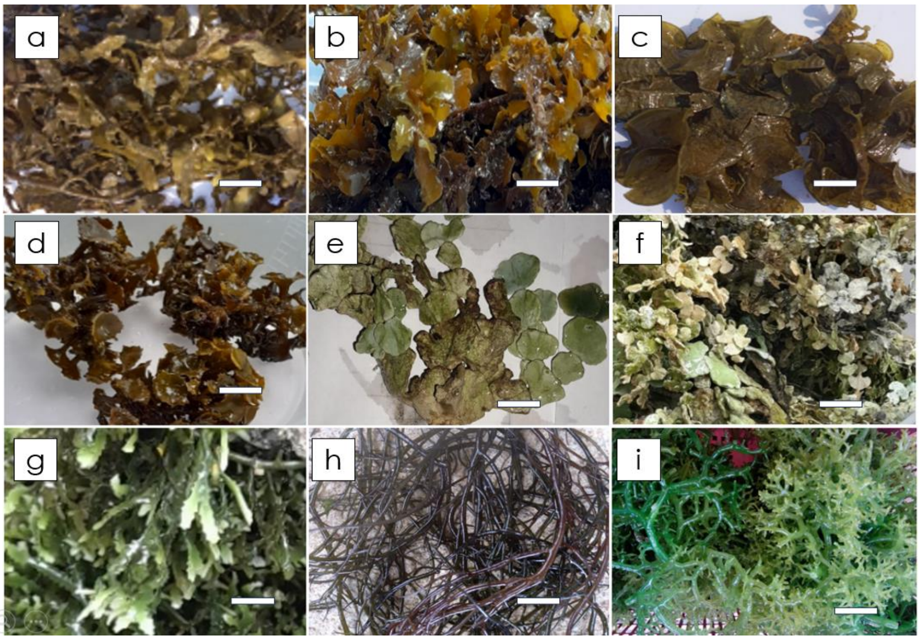

Figure 1. Seaweed samples from Kartini Beach Waters, Jepara, Central Java, Indonesia (white bar = 2 cm): (a) *Sargassum* sp. 1, (b) *Sargassum* sp. 2, (c) *Padina* sp., (d) *Turbinaria* sp., (e) *Halimeda* sp. 1, (f) *Halimeda* sp. 2, (g) *Caulerpa* sp., (h) *Gracilaria* sp., and (i) *Eucheuma* sp.

analytical methodologies (Vendruscolo *et al.*, 2021). This procedure was carried out for seven days at room temperature in the dark. One gram of seaweed dry weight (dw) was macerated with 25 mL of methanol. The mixture was filtered through Whatman No. 1 filter paper. Long maceration can increase the amount of secondary metabolite compounds and antioxidant activities, which are important for the bioavailability of the final product (Mousavi *et al.*, 2022). The macerates were then diluted to a total volume of 25 mL (100% concentration) for further analysis.

The qualitative pigment content was estimated using spectral patterns in the visible light region. The main target pigments are the most abundant produced by seaweeds, namely chlorophyll a and carotenoids. A UV-Vis Spectrophotometer (Shimadzu 1280) and UV-Probe 2.70 software were used to analyze the spectral characteristics of each seaweed extract. For the initial analysis, the seaweed extract was diluted by a factor of two. The absorbance spectra of the extract in the UV region were recorded at different wavelengths (λ), and the absorbance in the visible light region was measured at wavelengths between 400 and 750 nm. The absorbance values at certain wavelengths were evaluated using the equations for chlorophyll a (chl-a) and carotenoids described by Osório *et al.* (2020). The values were computed and expressed in terms of μg/g dry weight (dw) for pigment content.

$$\text{Chl-a } (\mu g/mL) = -2.0780 \times (A_{632} - A_{750}) - 6.5079 \times (A_{652} - A_{750}) + 16.2127 \times (A_{665} - A_{750}) - 2.1372 \times (A_{696} - A_{750})$$

$$\text{Carotenoids } (\mu g/mL) = 4 \times (A_{480} - A_{750})$$

The synthetic free radical used to measure the antioxidative activity is 2,2-diphenyl-1-picrylhydrazyl (DPPH). Ascorbic acid was used as a positive control for standard antioxidant activity. We used the method described by Sihono *et al.* (2018), with a few changes, to confirm the antioxidant activity of the seaweed extracts. DPPH was prepared as a 0.1 mM solution in methanol (MeOH). A total of 160 μL of sample extract (100% concentration) and ascorbic acid (20 μg mL-1) were added to each well of a 96-well microplate, followed by the addition of 40 μL of DPPH (A). For the sample controls, 160 µL of seaweed extract and 40 µL of MeOH (B) were added, for the negative control, 160 µL of MeOH and 40 µL of DPPH (C) were added, and for the blank, 200 µL of MeOH (D) was added. The mixture was incubated for 30 minutes at room temperature (25 – 28 °C). The absorbance of each well was recorded at 520 nm using a microplate reader (Accuris SmartReader 96). The following equation was used to determine the % of DPPH inhibition:

$$\text{DPPH Inhibition (\%)} = [(C\text{-}D) - (A\text{-}B)]/(C\text{-}D) \times 100$$

Data are reported as mean (n = 3) with standard deviation (SD). Statistical analyses were conducted using a non-parametric approach, specifically the Kruskal–Wallis test, to evaluate the differences in pigment content and antioxidant activity among the extracts from various seaweed species. Furthermore, the Mann-Whitney U test was used to determine the significance of differences between the treatment groups. The correlation between pigment content and antioxidant activity was determined using Spearman's correlation test. Statistical significance was set at a 95% confidence interval.

RESULTS AND DISCUSSION

Seaweed produces the main pigment chlorophyll and some accessory carotenoids, such as xanthophyll and carotene. The absorption spectrum of chlorophyll consists of two major peaks. Chlorophyll a has peak absorption at approximately 430 nm (blue light) and 662 nm (red light), whereas chlorophyll b has peak light absorbance at approximately 450 nm and 640 nm (Zhang *et al.*, 2019). However, carotenoids are accessory pigments that absorb light in the blue-green range of the light spectrum (450-550 nm) (Hashimoto *et al.*, 2018). As shown in the pigment spectral profiles in Figure 2, all seaweed methanol extracts demonstrated the presence of chlorophyll a and carotenoids in their absorbance profiles. The amount of pigment in the nine samples is quantitatively presented in Figure 3. Seaweed extract type had a significant effect on chlorophyll a ($P < 0.05$) and carotenoid ($P < 0.05$) contents. The extract from *Caulerpa* sp. exhibited the highest chlorophyll a concentration at 489.42 µg/g, followed by the *Padina* sp. extract at 411.17 µg/g and the *Sargassum* sp. 1 extract at 408.77 µg/g, respectively. Similarly, the highest carotenoid concentration was observed in *Caulerpa* sp. extract at 248.83 µg/g, followed by *Halimeda* sp. 1 and *Padina* sp. extracts at 200.37 µg/g and 149.37 µg/g, respectively.

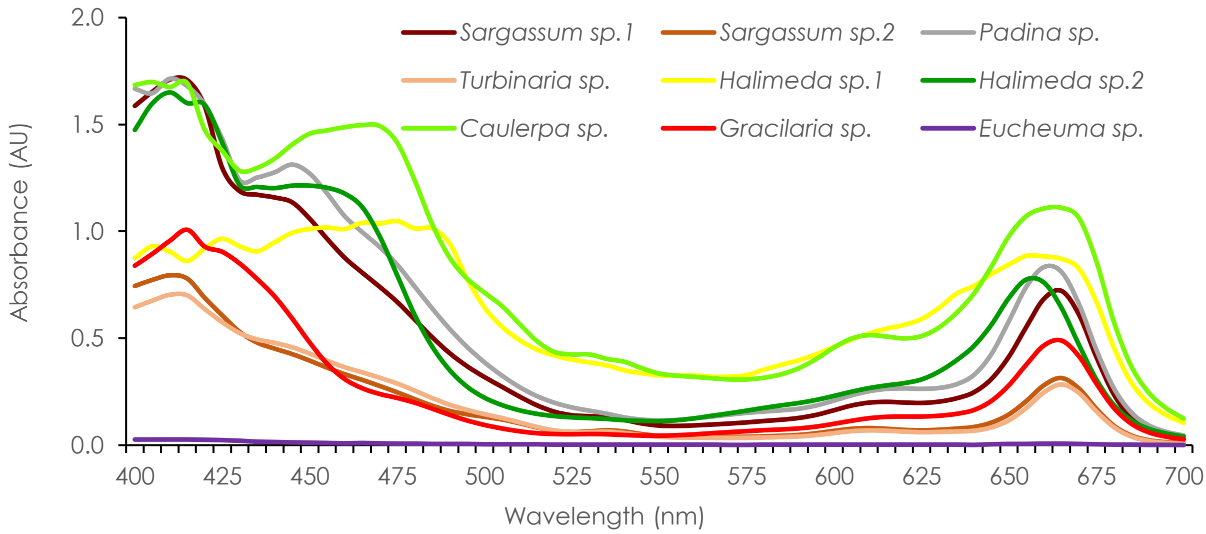

Figure 2. Spectral pattern of pigments contained (λ 400 – 700 nm) in the methanol extract of seaweed samples

Figure 4 illustrates the antioxidant properties of seaweed extracts, which are abundant in chlorophyll and carotenoids and range from low to high activity levels. The seaweed extract species significantly influenced the antioxidant potential (P < 0.05). The extract of *Sargassum* sp.1 exhibited the highest antioxidant activity, with a DPPH inhibition rate of 92.59%. This was followed by extracts from *Sargassum* sp.2 and *Caulerpa* sp. at the second level, which showed inhibition rates of 76.88% and 76.43%, respectively (P > 0.05). All three extracts surpassed the DPPH inhibition level of 20 µg/mL ascorbic acid, which served as the positive control, resulting in a DPPH inhibition of 71.21%.

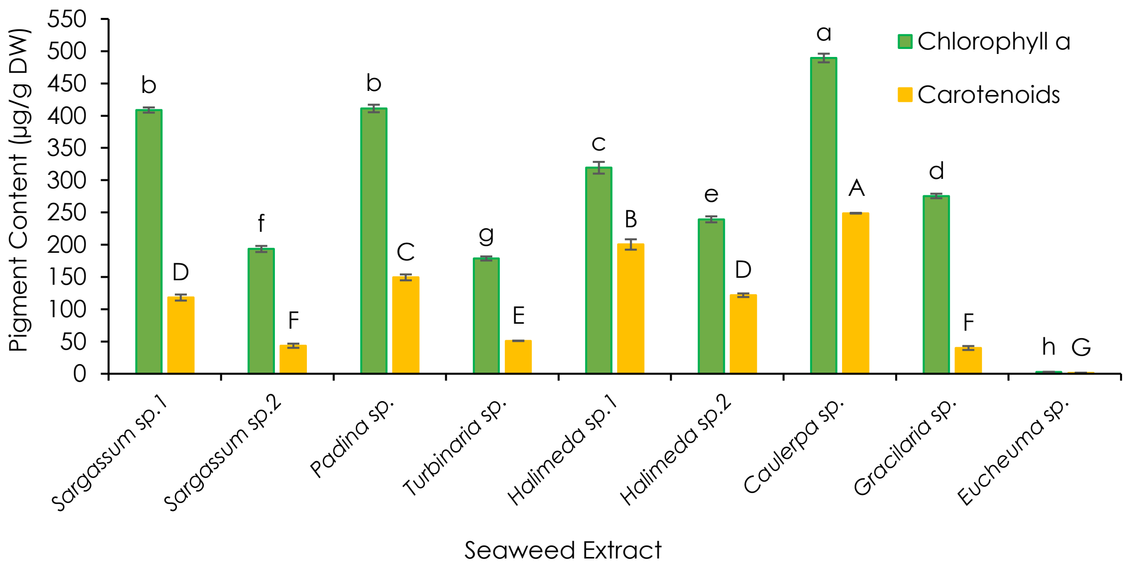

Figure 3. Chlorophyll a and carotenoid pigment contents of methanol extracts of seaweed samples (n = 3, different lower/uppercase letter notations on bars indicate significant difference at a = 0.05)

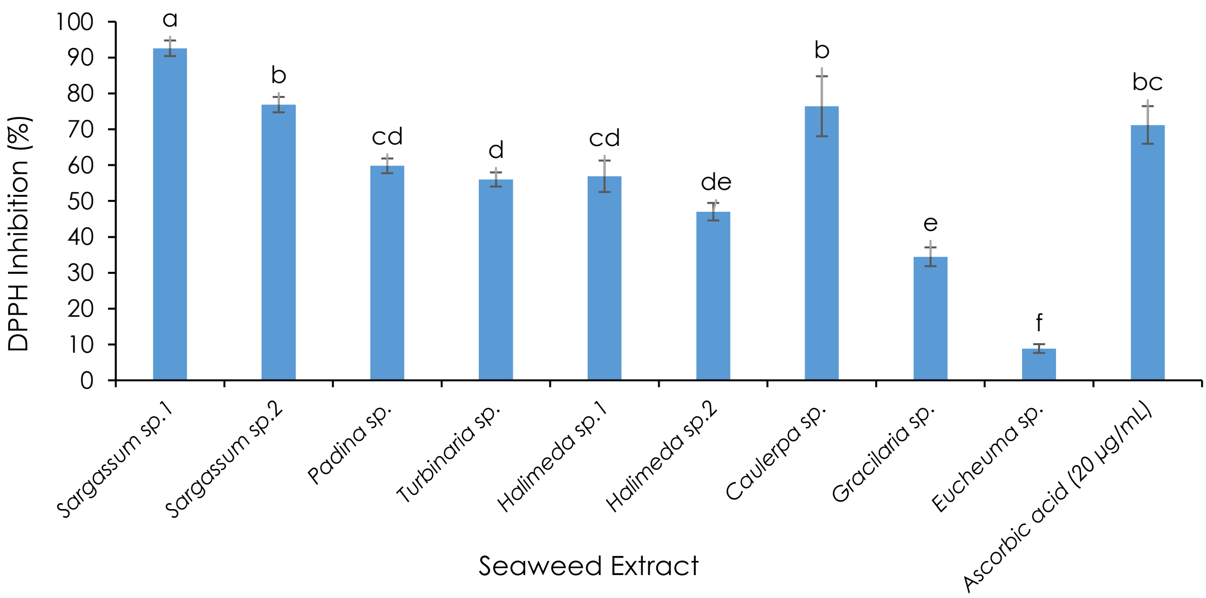

Figure 4. Antioxidant activity based on percentage DPPH inhibition of methanol extracts of seaweed samples and ascorbic acid (n = 3, different lower/uppercase letter notations on bars indicate significant difference at a = 0.05)

Table 2. Spearman's Rank Correlation Coefficient (ρ) between chlorophyll a, carotenoid, and antioxidant activity

Variable	Chlorophyll a	Carotenoid	Antioxidant Activity
Chlorophyll a	–	0.770 (p = 0.000)	0.596 (p = 0.001)
Carotenoid		–	0.507 (p = 0.007)
Antioxidant Activity			–

Notes: Values in the table are Spearman's coefficient ρ (n = 27).

Table 2 lists the results of the Spearman correlation test, which was calculated using the rho (ρ) value. The test found a relationship between the content of chlorophyll a, carotenoids, and the level of antioxidant activity. The chlorophyll a content showed a strong positive correlation with the carotenoid content (ρ = 0.770). In contrast, the correlations between chlorophyll a and antioxidant activity levels, as well as carotenoids and antioxidant activity levels, showed moderate correlations (ρ = 0.596 and ρ = 0.507, respectively). Photosynthetic pigments are key drivers of the antioxidant activity in seaweed extracts. Nevertheless, the correlation between pigment content and antioxidant activity is not completely direct. For instance, *Gracilaria* sp. exhibited moderate pigment levels but lower antioxidant activity than *Caulerpa* sp. These correlation values suggest that chlorophyll a and carotenoids alone are insufficient to account for the total antioxidant capacity. Other compounds that possess antioxidant activity, such as phenolics, flavonoids, terpenoids, and polysaccharides, may contribute to the overall antioxidant activity (Begum *et al.*, 2021; Sobuj *et al.*, 2021). Some recent reviews have highlighted the pigments (chlorophyll and carotenoids) of algae as part of the associated antioxidant activity, which synergistically acts with phenolics and other secondary metabolites to ensure that algae have a high level of antioxidant activity (Budzianowska *et al.*, 2025).

Seaweeds contain photosynthetic pigments, including chlorophyll a and carotenoids, which contribute to their biological activities, such as antioxidant ability. The structure of chlorophyll is unique, allowing it to undergo beneficial redox reactions. The molecular structure of chlorophyll, which contains a porphyrin ring with a central Mg^{2+} atom, may play a key role in electron transfer and, consequently, in the prevention of oxidative stress induced by reactive oxygen species (ROS) (Chen *et al.*, 2024). Carotenoids are also instrumental in potentiating the antioxidant-related effects of chlorophyll, mainly due to their strong antioxidant activity and capacity to quench singlet oxygen (Darvin *et al.*, 2022). They exhibit strong antioxidant effects and scavenge free radicals. Carotenoids are powerful antioxidants that scavenge peroxyl radicals with high efficiency, and the resulting carotenyl radicals can be resonance-stabilized (Mordi *et al.*, 2020). Their antioxidant activity increased with the length of the conjugated chain structure.

In this study, *Sargassum* sp. and *Caulerpa* sp. had comparatively high amounts of chlorophyll a and carotenoids, which were in agreement with their strong DPPH radical scavenging activity. This indicates that photosynthetic pigments play a major role in radical quenching. In addition, the low levels of pigments and weak antioxidant activity observed in *Eucheuma* sp. and *Gracilaria* sp., which exhibited the same trend, further confirmed the association between pigment content and antioxidant potential. The extract of the green seaweed *Caulerpa* sp. had the highest pigment yield but not the highest antioxidant activity. In contrast , the highest antioxidant activity was demonstrated by the brown seaweed *Sargassum* sp.1. Although a positive correlation was found between chlorophyll a and carotenoid content and antioxidant activity, this association was only moderately significant. Therefore, it can be concluded that factors other than pigment concentration also influence the antioxidant activity other than the pigment concentration in seaweeds. Phenolic compounds, particularly polyphenols and phlorotannins, are the main contributors to the antioxidant activity of brown seaweeds (Huang *et al.*, 2020; Sobuj *et al.*, 2021). These compounds also possess free radical-scavenging activity and reducing power.

Carotenoids in green seaweed differed distinctly from those in brown seaweed. Notably, the green seaweed *Caulerpa* contains carotenoids, such as β-carotene, lutein, and zeaxanthin (Kurniawan *et al.*, 2023). Conversely, brown seaweeds are rich in carotenoids, with fucoxanthin being the most prevalent carotenoid in numerous species (Balasubramaniam *et al.*, 2020; Yalçın *et al.*, 2020). *Sargassum* is characterized by six unique pigments: β-carotene, pheophytin, chlorophyll a, chlorophyll c, fucoxanthin, and xanthophyll. The antioxidant efficacy of these pigments is hierarchically ordered as follows: fucoxanthin, β-carotene, xanthophyll, chlorophyll c, chlorophyll a, and pheophytin (Kusmita *et al.* 2023). Variations in carotenoid pigment composition in different seaweed species significantly affect their antioxidant activity. The *Sargassum* genus, which produces fucoxanthin, is thought to be responsible for the high percentage of DPPH radical inhibition by its extract, whereas the Caulerpa extract does not contain fucoxanthin.

The *Caulerpa* sp. examined in this study exhibited thalli that were dark green with a yellowish hue at the apex and stolons that extended horizontally. Branches arise from terete ramuli and feature blades that are elongated, flattened, and serrated. The branching pattern was predominantly single but could occasionally be dichotomous, with a range of 2-4 branches. There are 8-9 ramuli and 5-11 rhizoids situated on the underside of the stolon. These morphological characteristics are indicative of *C. serrulata* (Kader & Gerung, 2020; Destikawati *et al.*, 2024). The *Sargassum* sp. 1 sample, whose extract exhibited significant antioxidant potential, was tentatively identified as *S. echinocarpum*. Its morphological characteristics align with those documented by Triastinurmiatiningsih *et al.* (2011) and Achmadi & Arisandi (2021). The cauloid is cylindrical and displays a regularly alternating branching pattern. The phylloids are oval-shaped with serrated edges and pointed tips, and the midrib and cryptostomata are not distinctly visible. The vesicles were oval, possessed pointed tips, and were stalked. The receptacles are mixed on a single stalk with flattened phylloids that are densely clustered.

The pigment content and antioxidant activities of *C. serrulata* and *S. echinocarpum* extracts also indicate their potential as sources of natural pigments. Caulerpa and Sargassum extracts produce light green and brown colors, respectively. In a prior study, natural dye from *Caulerpa* sp. microcapsules was coated on jelly drinks to impart a greenish color and increased quality (Dewi & Purnamayati, 2023). An additional investigation examined the impact of various concentrations of *C. racemosa* extract mixed with water on row noodles. The extract of *C. racemosa* has a significant effect on color, total phenolic content, total chlorophyll content, protein content, and consumer preference (Sholicha *et al.*, 2021). The application of seaweed pigments in the food industry has evolved beyond their traditional role of adding color, with an increasing number of studies exploring their potential as functional ingredients in recent years. These pigments are incorporated into the development of functional properties and are utilized in a diverse array of products, including meat, dairy, bakery, and flour products, and serve as food additives and components in beverages (Alloyarova *et al.*, 2024). On the other hand, the exploration of the *Sargassum* genus, specifically *S. fulvellum*, a type of brown seaweed, reveals its potential as a source of natural pigments for cosmetic applications. This species generates a diverse array of compounds, including carotenoids, which serve as natural colorants in cosmetic products (Liu *et al.*, 2020). The incorporation of these pigments meets the increasing demand for natural alternatives to synthetic dyes in the cosmetics industry, a trend driven by consumer preferences for products that are safer and more environmentally sustainable (Kiki, 2023). Moreover, chlorophylls and carotenoids extracted from seaweeds are considered important nutraceuticals. These compounds are known for their powerful antioxidant and anti-chronic disease properties. They also promote the maintenance and protection of physiological functions and have substantial nutritional benefits when consumed as part of a healthy diet (Cadar *et al.*, 2025).

These results demonstrate that the seaweeds from Kartini Beach, Jepara, namely *C. serrulata* and *S. echinocarpum*, are potential sources of natural pigments, including chlorophylls and carotenoids. These compounds play an important role in antioxidant activity, which contributes to scavenging singlet oxygen and free radicals that protect biomolecules from oxidative damage. The reported slight positive correlation between pigment content and antioxidant activity suggests that they may function as natural defenses against oxidative stress. Beyond their biological functions, these pigments have great potential as safe and sustainable natural colorants in food additives, nutraceuticals, and cosmeceutical products. Therefore, the extraction and application of seaweed pigments not only offer a natural and more efficient alternative to synthetic pigments in oxidative processes, coloring, and other applications. There is also a promising path for industrial and scientific research.

CONCLUSION

Caulerpa serrulata, a green seaweed, had a maximum chlorophyll a (489.42 µg/g dw) and carotenoids (248.83 µg/g dw). However, *Sargassum echinocarpum*, a brown seaweed, yielded better antioxidant activity than the *C. serrulata* extract (76.43%), with the highest value of 92.59%. These extracts exhibited higher DPPH radical-scavenging activity than ascorbic acid at 20 µg/mL (71.21%). Pigment content and antioxidant activity were slightly positively correlated. The present investigation demonstrates the feasibility of using extracts from *C. serrulata* and *S. echinocarpum* as natural colorants with antioxidant activities. Further investigations should focus on the utilization of seaweed pigment extracts as natural antioxidants/colorants in food, nutraceutical, and cosmeceutical products, as well as in various industrial applications.

ACKNOWLEDGMENT

The authors would like to thank the Faculty of Fisheries and Marine Sciences, Diponegoro University. This work was financially supported in part by the Institute Research Grant number: 13/UN7/ F10/PP/III/2025.

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