

Bioactive Components and Dietary Fibers of the Red, Green, and Brown Seaweeds in the Garut Coast, Indonesia

Nurhidajah, Diode Yonata, Boby Pranata, Yunan Kholifatuddin Sya'di*
Department of Food Technology, Universitas Muhammadiyah Semarang, Indonesia
*Corresponding author: yunan.ks@unimus.ac.id

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ABSTRACT

Understanding the bioactive components and dietary fiber from various seaweed species is crucial as a scientific basis, especially their use in developing functional food products. This research aimed to determine the bioactive components and dietary fiber of *Gracilariopsis longissima*, *Ulva lactuca*, and *Sargassum aquifolium*, which grow in Garut Coast, Indonesia. Apart from water content, significant differences were observed in each seaweed's proximate composition (ash, lipid, crude fiber and protein). In addition, the antioxidant activity (7.74- 8.91% RSA), total phenolics (36.85- 87.37mg GAE/ 100g) and flavonoid content (7.81- 17.58mg QE/ 100g) of each seaweed were significantly different. In particular, *Sargassum aquifolium* contains significantly higher total dietary fiber (66.04%), soluble dietary fiber (26.11%) and insoluble dietary fiber (39.93%) than *Gracilariopsis longissima* and *Ulva lactuca*. In conclusion, each seaweed species growing in the Garut Coast (Indonesia) has significantly different bioactive components and dietary fiber.

INTRODUCTION

As the leading producer of the aquatic food chain, seaweed has nutritional content and potential bioactive compounds that can be applied to food and health. Seaweed has been used as food since the fourth century. East Asian countries such as Japan, China, and Korea are the primary consumers of seaweed (Bixler & Porse, 2011). Seaweed has recently received a special attention because of its cosmopolitan distribution, renewable nature, and wide application (Barbier *et al.*, 2019). Seaweed-based foods are becoming a trend, especially in the western world, and production has almost tripled between 2000 and 2019 (Zhang *et al.*, 2022).

Seaweed is a multicellular algae with a wide geographical distribution. Based on their pigments, seaweeds are classified into three main groups, *Rhodophyta* (red algae), *Chlorophyta* (green algae), and *Phaeophyta* (brown algae) (Cermeño *et al.*, 2020; Yang *et al.*, 2021). Currently, 291 seaweed species have been exploited commercially in 43 countries worldwide (Tiwari & Troy, 2015). Indonesia has a large share of the world's

seaweed market. Based on data from the International Trade Center in 2018, Indonesia's seaweed exports in raw materials reached 205.76 thousand tons and were ranked first globally (CAS, 2022).

Seaweed cultivation in Indonesia began in 1967, specifically in the Seribu Islands (van der Heijden *et al.*, 2022). Indonesian water bodies are an area with large seaweed-producing potential. Indonesia's seaweed production in 2020 was 5.01 million wet tons (CAS, 2022), with an abundant diversity of seaweed, including the largest compared to other countries (Erniati *et al.*, 2023). Many types of seaweed have been cultivated in Indonesia, such as *Caulerpa recemosa*, *Caulerpa lentillifera*, *Eucheuma cottonii*, *Eucheuma spinosum*, and *Gracilaria gigas* (Nufus *et al.*, 2017). The southern coast of Garut Regency, West Java, Indonesia, has recently developed as a seaweed agrotourism area (Rusli, 2022). Several seaweed species have been found in this area, such as *Gracilariopsis longissima*, *Ulva lactuca*, and *Sargassum aquifolium* (Pratiwi *et al.*, 2021). The types of seaweed *Gracilaria* sp., *Ulva* sp., and *Sargassum* sp. are types of seaweed that humans can consume, therefore their existence has an excellent potential for an intensive cultivation (Debbarma *et al.*, 2016).

Gracilariopsis longissima, *Ulva lactuca*, and *Sargassum aquifolium* contain large amounts of dietary fiber components (Rasyid, 2017; Arguelles & Sapin, 2021; Ullah *et al.*, 2023). Apart from that, it also contains various bioactive compounds with antioxidant properties that can prevent and treat various degenerative diseases (Sánchez *et al.*, 2020; Wu *et al.*, 2020; Arguelles & Sapin, 2021; Agarwal *et al.*, 2023). Each type of seaweed has varying nutritional content and biochemical components. This variation is affected by differences in species, developmental stages, environmental factors, geographical factors, habitat, and harvest season (Pangestu & Kim, 2015; Chan & Matanjun, 2016; Viera *et al.*, 2018). Information regarding bioactive components and dietary fiber from seaweed that grows on the southern coast of West Java is currently not available. Therefore, it is necessary to characterize the bioactive components and dietary fiber from the red (*Gracilariopsis longissima*), green (*Ulva lactuca*), and brown (*Sargassum aquifolium*) seaweeds growing on the southern coast of Garut Regency, West Java, Indonesia, before further utilization, especially its use as a functional food material.

MATERIALS AND METHODS

1. Raw materials and reagents

The red (*Gracilariopsis longissima*), green (*Ulva lactuca*), and brown (*Sargassum aquifolium*) seaweeds were obtained from the coast of Garut, West Java, Indonesia (Latitude 7°13'40"S and Longitude 107°54'31"). The size and age of seaweed are not considered when having a collection method. After collection, the grass is immediately washed with seawater in the field and sent to the laboratory in a sealed bag. All solvents

and chemicals are of an analytical grade level purity originating from Himedia, Merck, and Sigma-Aldrich.

2. Sample preparation

Seaweed is cleaned using distilled water to remove sand, epiphytes, and pollutants and then drained. The seaweed is then dried using a dryer cabinet. Each fresh, clean seaweed was placed on a tray, distributed evenly in one layer, and dried at 60°C for 24 hours. The dried seaweed is then crushed until smooth and sieved through a 100-mesh sieve. Each seaweed powder was packaged in an airtight plastic bag and stored in a glass bottle at 4°C until analysis.

3. Proximate composition

The proximate composition of each seaweed was determined based on a protocol developed by the Association of Official Analytical Chemists (AOAC, 2005). The water and ash content of seaweed was measured gravimetrically. Protein content was obtained using the kjedhal micro procedure ($N \times 6.25$). The Soxhlet extraction procedure with petroleum ether solvent obtained fat content data. Each measurement was carried out in triplicate.

4. Dietary fiber

The enzymatic hydrolysis method described by Asp *et al.* (1983) was used to determine the levels of total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF). A total of 1g (W) of seaweed was used, and 25mL of 0.1M phosphate buffer with a pH of 6.0 and 0.1mL of termamyl solution were added. The next stage was heated at 99°C for 15 minutes, then 20mL of distilled water was added and cooled. The cooled mixture was then adjusted to the pH value until it reached 1.5 using 4 M HCl. 100mg of pepsin enzyme was added and heated at 40°C for 60 minutes. Then, 20mL of distilled water and 4 M NaOH were added until the pH value reached 6.8. The mixture was then added with 100mg of pancreatin enzyme and heated at 40°C for 60 minutes. Subsequently, 4 M HCl was added until pH 4.5 was reached, and 280mL of 95% ethanol was heated and incubated for 60 minutes at room temperature. The resulting precipitate was filtered (Ws), and the resulting residue was washed in each solution twice using 10mL of distilled water, 10mL of 95% ethanol, and 10mL of acetone. The resulting residue was dried at 105°C and weighed after cooling (Wd). The sample was placed in a furnace at 525°C and weighed after cooling (Wa). Other samples were determined for protein content (Wp), and blanks were prepared to determine the weight of contaminants (Wb).

5. Antioxidant activity

The antioxidant activity of seaweed was determined using the DPPH method (Waewkum & Singthong, 2021). Sample preparation was carried out by extracting several samples for 24 hours. 1mL of sample solution was reacted with 1mL of DPPH, then 2mL of ethanol was added. The mixture was shaken until homogeneous and then incubated at 37°C for 30 minutes. Absorption was measured at a wavelength of 517nm.

6. Total phenolic

The total phenolic compound content of seaweed was determined using the Folin-Ciocalteu phenol reagent described by El-Shenody *et al.* (2019). The total phenolic content of grasses is expressed in mg gallic acid equivalents per 100 grams (mg GAE/100g).

7. Flavonoid content

The total flavonoid content of seaweed refers to the method of Praveen *et al.* (2019a), using quercetin as a standard. Flavonoid content was expressed in mg quercetin, equivalent to 100 grams of seaweed (mg QE/100g).

8. Statistical analysis

All experiments were carried out in triplicate, and the results were expressed as mean \pm standard deviation. Significant differences between seaweed parameters were statistically analyzed using the analysis of variance (one-way ANOVA) method using SPSS for Windows software. When the F value shows significance, a further test was carried out using the Duncan multiple range test (DMRT) method to record data that have significant differences at $P < 0.05$.

RESULTS AND DISCUSSION

1. Proximate composition

The proximate content from the red (*Gracilariopsis longissima*), green (*Ulva lactuca*) and brown (*Sargassum aquifolium*) dried seaweeds is summarized in Table (1). Apart from the water content (9.04-9.19%), all proximate compositions (ash, lipid, crude fiber, and protein) of seaweed showed significant differences between species ($P < 0.05$). Among the three seaweed species, the highest proximate composition was detected in the *Sargassum aquifolium*.

Table 1. Proximate composition of seaweed from Garut Coast, Indonesia

Parameter	Type of seaweed		
	<i>Gracilariopsis longissima</i>	<i>Sargassum aquifolium</i>	<i>Ulva lactuca</i>
Moisture content (%)	9.17 ± 0.17 ^a	9.04 ± 0.26 ^a	9.19 ± 0.12 ^a
Ash (% dw)	23.18 ± 0.48 ^b	26.94 ± 0.38 ^c	19.28 ± 0.64 ^a
Lipid (% dw)	2.01 ± 0.36 ^b	2.75 ± 0.73 ^c	1.36 ± 0.19 ^a
Crude fiber (% dw)	14.38 ± 0.27 ^b	18.92 ± 0.41 ^c	7.94 ± 0.83 ^a
Protein (% dw)	7.93 ± 0.38 ^a	9.27 ± 0.61 ^c	8.13 ± 0.45 ^b

Values are the average ± standard deviation of 3 replicates. Different superscripts in the same line show statistically significant differences ($P < 0.05$), as determined by the LSD test.

Ash content is one of the most abundant elements in seaweed; as shown in this study, seaweed contains around 19.28 to 26.94% ash. Differences in ash content are associated with salt in fronds and mineral accumulation due to salinity (El-Manawy *et al.*, 2019). Variations in lipid components between seaweed species are associated with taxonomic entities, the extraction process and solvent polarity can also influence the lipids extracted from seaweed (Li *et al.*, 2013). The lipid content of the seaweed investigated was low, only 1.36- 2.75%. The protein content of the seaweed investigated ranged from 7.93 to 9.27%, higher than seaweed in the Red Sea, Egypt, which is only 4.8- 7.1% (El-Manawy *et al.*, 2019). The high protein content of the brown seaweed compared to the green seaweed has previously reported (Ahmed *et al.*, 2015), while other studies report that the red seaweed contains higher protein than the brown seaweed (Osman *et al.*, 2011). Proteins in seaweed are strongly influenced by taxonomy and species, even within the same genus (El-Manawy *et al.*, 2019). Finally, significant variations were also seen in seaweed's crude fiber content, ranging from 7.94 to 18.92%.

The proximate composition of *Sargassum aquifolium* is slightly different from that reported in Quezon, the Philippines, which contains 6.74% water, 30.19% ash, 3.86% fat, 10.03% crude fiber and 16.89% protein (Arguelles & Sapin, 2021). The proximate composition of the *Gracilariopsis longissima* in this study is also different from *Gracilariopsis longissima*, which grows in Cox's Bazar, Bangladesh, including 27.83% protein, 1.26% lipid and 24.71% ash (Ullah *et al.*, 2023). Significant differences were also reflected in the proximate composition of *Ulva lactuca* in this study compared to that grown on Qheshm Island, southern Iran, with a water content of 5.96%, ash 18.03%, lipid 0.99%, crude fiber 5.96%, and protein 10.69% (Tabarsa *et al.*, 2011). Each growing location has different seasonal changes and environmental conditions, including tidal variations, which play an important role in determining the proximate composition of seaweed (Praveen *et al.*, 2019a). In this study, the type of seaweed species also influences differences in proximate composition. These results align with previous research regarding the evaluation of the proximate composition of various seaweed species growing in the Red Sea (El-Manawy *et al.*, 2019).

2. Antioxidant activity

The antioxidant activity of the three seaweeds is presented in Table (2), which differs significantly between species ($P < 0.05$). *Sargassum aquifolium* showed the highest antioxidant activity (8.91% RSA), followed by *Gracilariopsis longissima* (8.36% RSA) and *Ulva lactuca* (7.74% RSA), respectively. Seaweed is known to contain various bioactive compounds with antioxidant properties. Antioxidant activity in seaweed generally comes from high levels of phenolic compounds, as **Booochum *et al.* (2011)** reported. These compounds include the main phenolic compounds, polysaccharides, pigments, vitamins, and proteins, which contribute to the antioxidant activity of seaweed (**Michalak *et al.*, 2022; Ismail *et al.*, 2023**). Variations in the antioxidant activity of seaweed are affected by the composition of bioactive components and the availability of different nutrients. These antioxidants are produced from environmental conditions, such as exposure to UV radiation and pollutants in addition to changes in temperature and salinity (**Michalak *et al.*, 2022**). Additionally, antioxidant activity is also strengthened by other bioactive components, viz. phlorotannin, sulfated polysaccharides, and carotenoids, which have antioxidant properties (**Hermund, 2018**).

Table 2. Bioactive components of seaweed from Garut Coast, Indonesia

Type of seaweed	Parameters		
	Antioxidant activities (% RSA)	Total phenolics (mg GAE/ 100 g)	Flavonoids (mg QE/ 100 g)
<i>Gracilariopsis longissima</i>	8.36 ± 0.15 ^b	87.37 ± 2.28 ^b	17.58 ± 0.66 ^c
<i>Sargassum aquifolium</i>	8.91 ± 0.69 ^c	97.98 ± 2.77 ^c	7.81 ± 0.11 ^a
<i>Ulva lactuca</i>	7.74 ± 0.16 ^a	36.85 ± 0.98 ^a	9.06 ± 0.33 ^b

Values are the average ± standard deviation of 3 replicates. Different superscripts in the same column show statistically significant differences ($P < 0.05$), as determined by the LSD test.

3. Flavonoid

Flavonoids are polyphenolic compounds found in various plants including seaweed. Several types of seaweed contain flavonoid compounds such as *Sargassum* sp., *E. cottonii*, *Gracilaria salicornia*, and others (**Pangestuti *et al.*, 2017; Helena & Sanjayasari, 2018; Loho *et al.*, 2021**). This study found that the *Gracilariopsis longissima*, *Sargassum aquifolium*, and *Ulva lactuca* also contain flavonoids. *Gracilariopsis longissima* contains the highest levels of flavonoids with 17.58mg QE/ 100g ($P < 0.05$). Various species of seaweed are known to contain different flavonoid compounds. The composition of bioactive compounds including flavonoids can vary depending on species, geographic location, and environmental conditions (**Øverland *et al.*, 2019**). Moreover, seaweed contains other bioactive compounds, such as phenolics and saponins (**Diachanty & Nurjanah, 2017**). The flavonoid content in seaweed shows the potential to act as an antioxidant, which can benefit human health.

4. Phenolics total

The total phenolics in seaweed used in this study ranged from 36.85- 97.98mg GAE/100g and were different significantly between species ($P < 0.05$). The *Sargassum aquifolium* has the highest level (97.98). The total phenolic content of *Sargassum aquifolium* has been reported in several studies. The total phenolic compounds of *Sargassum aquifolium* extract were 574.00mg GAE/ 100g in the reported study of **Arguelles and Sapin (2021)**. In another study, the *sargassum* sp. type contained up to 12.70mg GAE/ g (**Hidayati et al., 2019**). These indicate that *Sargassum aquifolium* and related species contain large amounts of phenolic compounds with an antioxidant potential. Notably, different seaweeds' phenolic content is influenced by species, life stage, size, age, location, and environmental conditions (**Cotas et al., 2020; Purbosari et al., 2020**). It is noteworthy that, phenolic compounds are bioactive molecules found in seaweed with various potential applications. Phenolic compounds derived from seaweed have been studied for their potential use as natural antioxidants (**Gunathilake et al., 2022**). Based on its potential natural antioxidant activity in various applications, they have been used in food, cosmetics, pharmaceutical, and biomedical industries (**Hidayati et al., 2019; Arguelles & Sapin, 2021**).

5. Dietary fiber

Seaweed is an aquatic food source of dietary fiber (DF) and provides more excellent nutritional value than land plants (**Praveen et al., 2019b**). The dietary fiber (TDF) available in seaweed generally ranges between 25- 70% (**Raposo et al., 2016**), of which 12 to 40% is classified as a soluble dietary fiber (IDF) (**Sanz-Pintos et al., 2017; Uribe et al., 2018**). In this study, seaweed TDF ranged from 39.66 to 66.04%, and 37.71 to 39.93% of the TDF and IDF, respectively (Fig. 1).

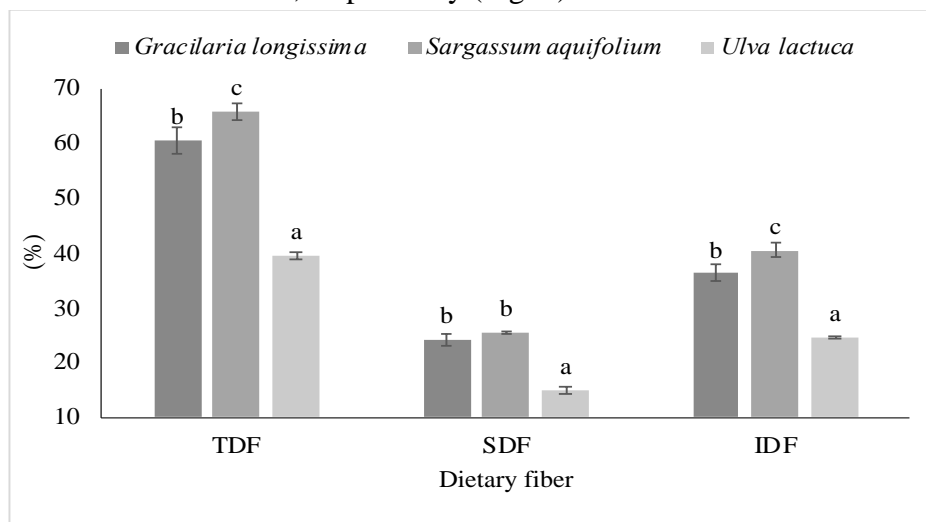


Fig. 1. Seaweed dietary fiber from the Garut Coast, Indonesia

Sargassum aquifolium generally has the highest TDF, SDF, and IDF components. Types of SDF from seaweed are alginate, laminarin and fucoidan in the brown seaweed, carrageenan and agarose in the red seaweed, and ulvan in the green seaweed. Types of IDF in the seaweed are generally found in the form of cellulose, lignin, hemicellulose, and a small amount of starch, most of which have a bioactive activity (**Praveen *et al.*, 2019b; Huang *et al.*, 2022**). DF from seaweed has been confirmed to have important functional properties such as antioxidant, anti-inflammatory, anticoagulant, anticarcinogenic, and antiviral (**Tanna & Mishra, 2019**). The DF seaweed also has potential probiotic effects on human health. Several studies show that DF provides good benefits for the body health, such as maintaining metabolic homeostasis, improving inflammation, and preventing intestinal microbiota from dysbiosis (**Holscher *et al.*, 2017**).

CONCLUSION

This study succeeded in achieving its aim to determine the bioactive components and dietary fiber from the seaweed *Gracilariopsis longissima*, *Ulva lactuca*, and *Sargassum aquifolium*, which grow in Garut Coast, Indonesia. Significant differences were observed in each seaweed's ash, lipid, crude fiber and protein levels. Although the flavonoid content of *Sargassum aquifolium* is lower, the total phenolics and antioxidant activity are significantly higher than those of *Gracilariopsis longissima* and *Ulva lactuca*. In addition, the dietary fiber profile (TDF, SDF and IDF) of *Sargassum aquifolium* is better than that of other species. Therefore, based on its bioactive components and dietary fiber, *Sargassum aquifolium* is highly recommended for development in functional food products.

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