

# Nutritional composition and antioxidant potential of brown seaweed (*Padina minor*) from Bengkulu, Indonesia

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**Abstract.** Seaweed is a popular food that has potential as a new source of bioactive compounds for humans. Bengkulu Province has abundant natural seaweed resources, one of which is *Padina minor*. Despite that, no research has been conducted on its nutritional composition and antioxidant potential thus far. Understanding this information is critical for utilizing *P. minor*, particularly in the food industry. This research was carried out to examine the nutritional composition and antioxidant activity of *P. minor* to provide useful information, especially for coastal communities. The seaweed powder were characterized for their nutritional composition (water, ash, protein, lipid, crude fiber and carbohydrates), while the fresh seaweeds were extracted using methanol. The methanol extract was analyzed to determine its antioxidant activity using DPPH solution. The results showed that the largest nutritional components in *P. minor* are water, carbohydrates and ash. The IC<sub>50</sub> value of *P. minor* was 11.26 ppm, exhibited very strong antioxidants, similar with ascorbic acid (IC<sub>50</sub>= 3.27 ppm). Based on this, *P. minor* from Bengkulu has the potential to be developed as an alternative source of minerals, dietary food and a source of antioxidant.

## 1 Introduction

High health concerns, particularly those associated with degenerative diseases, raise consumer awareness of lifestyle and healthy eating habits. This impacts the establishment of food consumption habits that are beneficial to health. Food with added health benefits, such as antioxidants, anti-cholesterol, and antidiabetic properties, is known as functional food. High demand for functional food generates a wide range of food products, one of which is derived from marine products.

Seaweed is an abundant fishery and marine commodity in Indonesia, both naturally and cultivated. According to certain studies, seaweed has numerous health benefits. Seaweed is rich in polysaccharides, proteins with complete essential amino acid content, unsaturated lipidty acids, and various vitamins and minerals [1]. Nowadays, seaweed gets a lot of attention because of its bioactive compounds, which have diverse biological and chemical activities like antioxidant, anti-inflammatory, anti-dyslipidemic, antitumor, and anti-microbial and can reduce the risk of cardiovascular disease.

Bengkulu Province has a relatively high diversity of marine resources, including seaweed. A preliminary study showed that Bengkulu has 20 species of seaweed; one of them was *P.*

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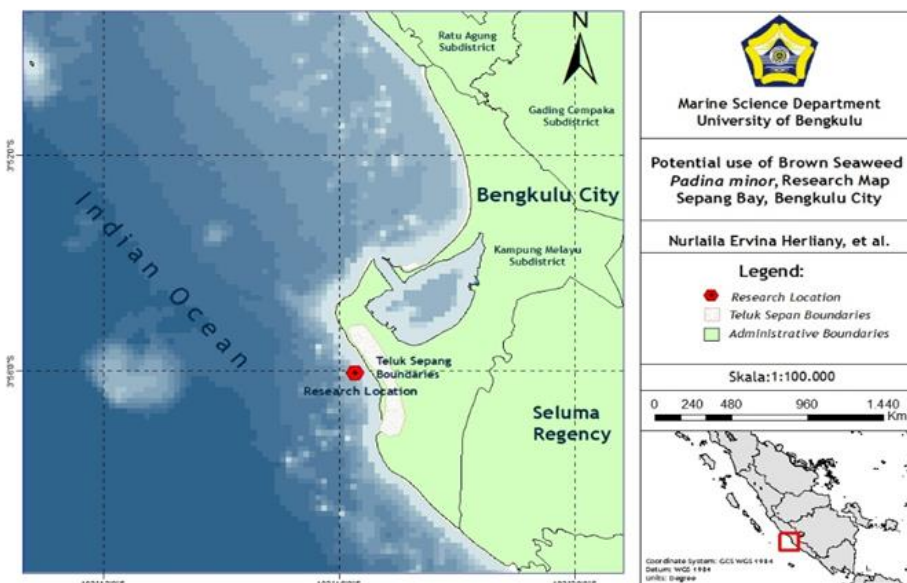
*minor*. Unfortunately, this species is not utilized by local communities due to the lack of scientific data regarding its nutritional content. There is a knowledge gap on the nutritional composition of *P. minor* from the coastal waters of Bengkulu. As a result, the purpose of this study was to gather such information to favour potential development of *P. minor* utilization in Bengkulu. The present study is the first published data on the proximate composition and antioxidant activity of *P. minor* from Bengkulu. Based on these preliminary findings, we investigated the proximate composition and antioxidant activity of *P. minor* collected from Bengkulu to provide scientific information, particularly for coastal communities.

Futhermore, the biochemical composition of seaweed vary according to species, location, season, and growth. Since the Bengkulu shoreline is oriented directly towards the Indian Ocean, it leads to relatively strong currents. Besides that, Bengkulu coastal water exhibits semidiurnal tides, with the lowest low tide reaching up to 50 meters from the shoreline. This exposes the seaweed to direct sunlight, causing it to dry out. This combination of environmental conditions can affect its nutritional content and antioxidant activity, so we hypothesized that *P. minor* from Bengkulu exhibits distinct nutritional and antioxidant profiles due to its unique local environment.

## 2 Materials and Method

### 2.1 Sampling procedures

Seaweed was collected from Teluk Sepang Beach, Bengkulu (3° 54' 57.23" S, 102° 16' 5.20" E) (Fig. 1). The psychochemical characteristic of Teluk Sepang Beach could be seen in Table 1. Random sampling method was carried out at a single location. Fresh *P. minor* was collected during low tide by hand picking and then identified in the Laboratory of Fisheries, Faculty of Agriculture, Bengkulu University. The *P. minor* samples was cleaned with water and shade-drying at room temperature then oven dried at 60°C for 12 hours. After drying, the samples were pulverized in the grinder to obtain seaweed powder. Seaweed powder was kept in plastic at room temperature for further analysis [2].



**Fig. 1.** The sampling site in Teluk Sepang Beach, Bengkulu.

**Table 1.** Psychochemical characteristic of Teluk Sepang Beach.

Parameters	Content
Salinity (ppt)	34.67
Temperature (°C)	35.67
pH	8.29
Current (m/s)	0.53
Nitrate (mg/L)	0.06
Phosphate (mg/L)	0.004

## 2.2 Proximate analysis

Seaweed powder was analyzed for its proximate composition following by procedur of [3], including moisture content, ash content, lipid content, protein content, and crude fiber. The carbohydrate content was obtained by difference method following the equation below:

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ lipid}) \quad (1)$$

The analysis was repeated two times (duplo).

## 2.3 Seaweeds extraction

Extraction was carried out using the maceration method using a single solvent, methanol. Fresh *P. minor* (150 g) was cut into small pieces and soaked in methanol solvents for 72 hours at room temperature, with the ratio of seaweed: methanol of 1:4. The solution was filtered using Whatman filter paper No. 42, then evaporated with a rotary evaporator at 40 °C to obtaine paste. The paste was then washed using ethanol to remove salts (desalting). After washing, the paste then centrifuged at 4,200 rpm for 10 minutes. The supernatant was evaporated using the rotary evaporator at 40 °C to obtain methanol extract. Then, a stock solution with a concentration of 100 mg/ml was made using ethanol solvent and stored in the refrigerator for further analysis [2].

## 2.4 Determination of yield

The yield is obtained from the ratio of the weight of the seaweed extract to the weight of the seaweed powder. The yield value is calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of seaweed powder}} \times 100\% \quad (2)$$

## 2.5 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The scavenging activity of *P. minor* extract was analyzed using DPPH solution, following the procedure of [3]. Briefly, a total of 2 ml of sample solution at various concentrations (50, 100, 150, 200, and 250 ppm) was placed in a test tube and added with 2 ml DPPH 0.1 mM solution. The solution was mixed for 5 seconds using a vortex before being stored in a dark room for 30 minutes at room temperature. The absorbance of the solution was read using spectrophotometer at wavelength of 515 nm. As a control solution, the absorbance of a blank solution containing 2 mL DPPH and 2 mL ethanol was read. Ascorbic acid was used as a positive control. The percentage of reduced DPPH color can be used to calculate free radical scavenging activities using the following formula :

$$\text{DPPH Inhibition (\%)} = \frac{(\text{blank abs} - \text{sample abs})}{(\text{blank abs})} \times 100 \% \quad (3)$$

Where blank abs is absorbance of control and sample abs is absorbance of sample.

Antioxidant activity is expressed in IC<sub>50</sub> (Inhibition Concentration 50), the concentration of antioxidants needed to inhibit 50% free radicals. The IC<sub>50</sub> value was calculated from the regression equation,  $y = a + bx$ , where Y is the percent inhibition and X is sample concentration (ppm).

## 2.6 Data analysis

The proximate data are analyzed descriptively and presented as means  $\pm$  standard deviations of two assay replicates. The regression analysis was analyzed using MS Office Excel 2013.

## 3 Results

### 3.1 Morphological of *P. minor*

*P. minor* is a brown seaweed with a fan-like thalii and a yellowish brown to white in color (Fig. 2.). The blades surface has concentric lines that are evenly spaced. Blades up to 10 cm in height and 4 cm broad. This seaweed is found attached to rocks and dead coral in the intertidal zone of Teluk Sepang Beach, Bengkulu.



Fig. 2. Morphological of *P. minor* from Teluk Sepang Beach.

### 3.2 Nutritional composition of *P. minor*

Based on this research, the composition of *P. minor* from the highest to the smallest was moisture content, carbohydrate content, ash content, crude fiber content, protein content, and lipid content, respectively (Table 2.).

Table 2. Nutritional composition of *P. minor*.

Chemical composition	Content
Moisture (% , wet basis)	68.00 $\pm$ 0.00
Ash (% , dry basis)	42.97 $\pm$ 0.16
Crude Protein (% , dry basis)	1.95 $\pm$ 0.15
Crude Lipid (% , dry basis)	1.79 $\pm$ 0.72
Crude Fiber (% , dry basis)	6.01 $\pm$ 0.27
Carbohydrate (% , dry basis)	49.27 $\pm$ 0.24

\*Values are expressed as mean ± SD (n = 2)

### 3.3 Extract yield of *P. minor*

The extraction process is used to obtain active compounds from raw materials using specific solvents. To determine the effectiveness of the solvent used, it is necessary to measure the extract yield. In this study, the yield of *P. minor* extract was 0.91±0.04%.

### 3.4 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

Antioxidants are substances that have the ability to neutralize the harmful effects of free radicals. The antioxidant activity of *P. minor* ethanol extract was assessed by DPPH tests. Antioxidant activity is measured by the IC<sub>50</sub> value (inhibition concentration of 50%), which is the concentration of samples that can inhibit 50% free radicals. The IC<sub>50</sub> value of fresh *P. minor* extract was 11.26 ppm exhibited very strong antioxidant, calculated using the regression equation  $y = 0.1881x + 47.882$  (Fig. 3.). Meanwhile, ascorbic acid as a control positive has an IC<sub>50</sub> value of 3.27 ppm.

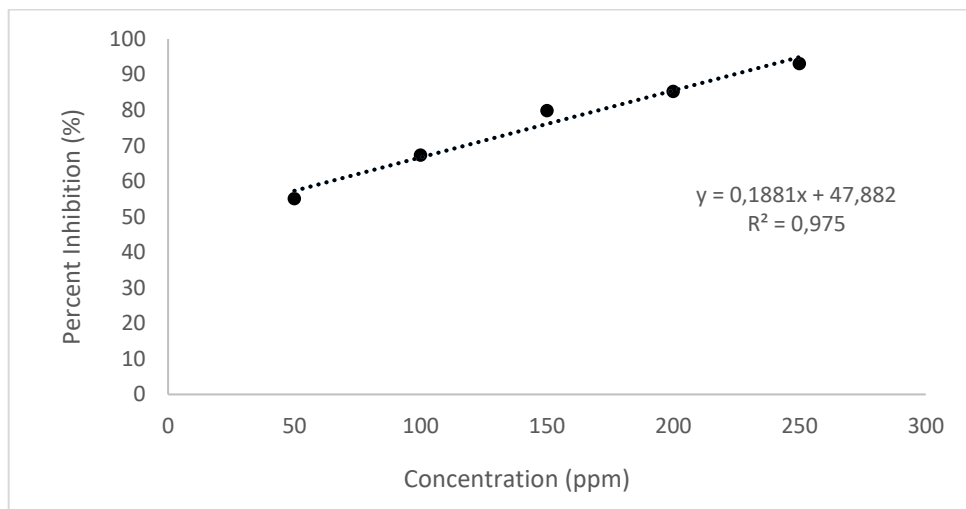


Fig. 3. The sample concentration and percent inhibition correlation.

## 4 Discussions

### 4.1 Morphological of *P. minor*

*P. minor* had yellowish brown to light brown or slightly whitish thallii due to light calcification. *P. minor* is characterized by its 2-layered thallus, with hairs restricted to the ventral surface. This species widely distributed in intertidal to subtidal zones, grows in a solid substrate including rocky or sandy bottom [4] and in many tropical areas, including Indonesia.

## 4.2 Nutritional composition of *P. minor*

The results showed that *P. minor* contained high amounts of water ( $68 \pm 0.00$  % wb). Vijay *et al.* [5] reported that seaweed contain 80-90% of water. Marine algae's polysaccharides increase water holding capacity, leading to increased initial moisture content. Hydrogen bonds between polysaccharides form extrinsic moisture, while tight junction proteins regulate intrinsic moisture.

*P. minor* also contained high carbohydrates, which was  $49.27 \pm 0.24$  %. Meanwhile, [6] stated that carbohydrate content of *P. minor* from Puntondo Coast, Takalar, Indonesia, which was 48.06%. This variation can be influenced by environmental factors such as seasonal differences and light intensity. Seaweed carbohydrate levels are generally higher in the dry season because increased sunlight intensity accelerates photosynthesis, causing the algae to form and store more sugars and polysaccharides [7]. Kamal *et al.* [8] reported that the carbohydrate content of all macroalga studied in Egyptian Red Sea Shore peaked in summer compared to winter. High water temperatures indicate increased sunlight intensity, which also increases the rate of photosynthesis.

Furthermore, a study by El-Manawy *et al* [7] stated that seaweed polysaccharide content varies depending on the reproductive stage; the more mature the seaweed, the higher the polysaccharide content. During the juvenile phase, a large amount of energy is allocated to the synthesis of structural and reserve polysaccharides. Because the algae retain a lot of growth resources during this period, polysaccharide synthesis is decreased. Some of the stored polysaccharides are released during the vegetative phase to supply the energy required for the development of mature reproductive structures and spores. During this stage, yields are comparatively stable and start to rise as the algae mature. When algae reach the reproductive phase, some of the accumulated polysaccharides are mobilized to support the development of reproductive structures and spore formation. Because reproduction uses a lot of energy, polysaccharide stores are used up at this stage. As a result, polysaccharide levels rise as algae grow.

Carbohydrates in seaweed have health benefits, including antioxidants, anticoagulants, anti-inflammatory, anti-arthritis, immunomodulatory, and immunostimulant properties. Carbohydrates in brown algae are found in the form of fucoids, laminarin, cellulose, and alginate. One component of carbohydrates is crude fiber. The crude fiber of *P. minor* was  $6.01 \pm 0.27$ %. Seaweed contains low crude fiber, less than 10% [5]. The variation in crude fiber content could be related to variability in species, photosynthetic activity, growth stage, and seasonality, which are caused by environmental changes that affect photosynthesis and nutrient absorption [6]. The crude fiber content in seaweed is very good for digestive health, can prevent colon cancer, and is good for diet.

Seaweed has a greater ash concentration than terrestrial plants or animal products. Seaweed grows in a seawater environment rich in dissolved minerals, causing the algal cells to continuously absorb inorganic ions across the entire thallus surface, not just through the roots, resulting in more effective nutrient uptake. As a result, seaweed has a high ash content [5]. Brown seaweed generally has a higher ash content compared to red and green seaweed due to its alginate and sulfated polysaccharide content, which is rich in polygalacturonic acid units that act as anionic carboxylic binding sites that can interact with metallic cations.

In this study, the ash content of *P. minor* was  $42.97 \pm 0.16$ %, higher than in the research of Khadijah *et al* [6] from Puntondo coast, Takalar, Indonesia, and Salosso *et al* [9] from Kelapa Lima coastal waters, Kupang Bay, Indonesia which was 38.02% and 34.58%, respectively. The ash content of seaweed varies according to several factors, including age, species, geographical conditions, physiology, and mineralization methods [6]. Moreover, salinity is also suspected to be a factor influencing seaweed ash content. Excessive salinity levels can cause cells water content to rapidly drop, which raises the salt concentration inside

the cells. During the study, Teluk Sepang Beach had a relatively high salinity of 34.67 ppt. This condition is thought to have contributed to the high ash content of *P. minor*.

Seaweed ash content is also influenced by the mineral content of the water. Mineral content differences between locations can result in varied seaweed ash content [6]. Unfortunately, this study did not measure ash or mineral content at Teluk Sepang Beach, so we cannot confirm whether the ash content of *P. minor* is influenced by the mineral amount of its environment. Besides that residual salt adhering to the seaweed during sample also influences ash content. Improper seaweed washing can leave salt residue on the surface of the thallus, resulting in a high ash content.

Protein and lipid content in *P. minor* was  $1.95 \pm 0.55\%$  and  $1.79 \pm 0.72\%$ , respectively. Seaweed has low protein and lipid content, namely 1-44% and 1-6% [5], consecutively. The protein content of *P. minor* in this study were lower compared to the study by Khadijah et al [6] on *Padina* sp. from Puntondo Coast, Takalar, Indonesia, which reported a protein content of 12.33%, but almost have the same lipid content (1.60%).

Differences in seaweed protein content are influenced by variations in species, habitat, season, and environmental conditions such as water temperature, salinity, light intensity, current strength, and nutrient supply [5]. According to Kamal et al [8], the protein content of seaweed is generally higher during winter and spring compared to summer, when rapid growth occurs due to increased photosynthetic rates. This statement is consistent with the present study, where protein levels were low because the sample was collected during the dry season. Furthermore, El Manawy et al [7] added that the protein content have a positive correlation with nitrate and phosphate levels in the water. According to Darmawan et al [10], the optimal nitrate : phosphate ratio for seaweed growth is 30 : 1. Meanwhile, the research location has a low nitrate : phosphate ratio of 12 : 1, which impacts the low protein content of *P. minor*.

Variations in lipid content are influenced by several factors, including species, season, and environmental conditions such as salinity and water temperature. According to Xiong et al [11], high salinity leads to ion stress, osmotic stress, and secondary stress. Ion stress results in Na<sup>+</sup> poisoning. Excessive Na ions on the thallus surface can inhibit K<sup>+</sup> absorption from the environment. Osmotic stress can disrupt water and nutrient absorption because of high environmental osmotic pressure. Ion stress and osmotic stress due to high salinity will cause secondary stress, specifically damage to cell structures and macromolecules such as lipids. Salinity at the research location was relatively high, resulting in low lipid content.

Higher temperatures generally result in lower lipid content. Vijay et al. [5] added that lipid content in tropical seaweed is lower than in temperate seaweed. Under stress conditions due to high temperatures, seaweed adjust their lipid metabolic pathways to synthesize and accumulate neutral lipids in the form of triacylglycerols. It provides a carbon and energy storage function in cells, allowing algae to tolerate high temperature. The combination of these various factors can explain the difference in lipid content of *P. minor* compared to previous studies.

Although seaweed contains relatively low levels of protein and lipid, the amino acids and fatty acids present contribute positively to human health. According to Salosso et al [9], *P. australis* is rich in aspartic and glutamic acid, which can act as an immune system, maintaining digestion and generate neurotransmission in the nerves of the brain and muscles. Proteins derived from macroalgae are also important as a source of peptides and amino acid extracts, especially after enzymatic digestion, which enhances their solubility in water, allowing them to be utilized in numerous industries [8]. Low lipid content provides health benefits for people with obesity. However, seaweed has an essential amino acid that is quite complete and rich in unsaturated fatty acids that are good for brain intelligence, such as EPA and DHA. Corsetto et al. [1] stated that seaweed fatty acids perform important functions in nutrition and cell membrane development, including essential-linolenic fatty acids that

cannot be produced by mammals, whereas terrestrial plants can only produce it in small quantities. Based on this, seaweed has the potential to be an alternative supplement for unsaturated fatty acids, essential amino acid, and natural flavourings.

### 4.3 Extract yield and of *P. minor*

The yield obtained in this study was lower than that reported by Afrin et al [12] for *P. tetrastromatica* from Saint Martin's Island, Bangladesh, which was 14.26±0.85%. Herliany et al [2] suggests that variations in yield may result from differences in seaweed species, specifically due to the polarity of active compounds between species. Typically, extracts are most efficient when the solvent and active compound have similar polarity. When a seaweed species contains a higher concentration of polar active compounds, extraction with a polar solvent typically results in higher yield. Moreover, extraction yield may vary depending on the extraction method, environmental conditions, and season [2, 13].

### 4.4 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

There are different IC<sub>50</sub> value of same genus from several coastal waters. Gazali *et al.* [14] reported that fresh *P. australis* from West Aceh, Indonesia, had an IC<sub>50</sub> value of 265 µg/ml, while [14] found that dried *Padina* sp. from Selayar Island, South Sulawesi, had an IC<sub>50</sub> value of 116.83 ppm. Various factors can influence antioxidant activity, including environmental conditions, seasons, harvest age, species, drying processes, and extraction procedures. According to Herliany *et al* [2], season influences the condition of the aquatic environment, which can impact the content of seaweed. The bioactive content in seaweed is influenced by seasonal variables that cause environmental changes, such as direct exposure to sunlight, nutrients, salinity, pH, and water temperature. The extraction method affects the antioxidant properties of seaweed because the suitable solvent allows for the optimal extraction of components from plants [13]. The polarity of seaweed's compounds will determine the appropriate solvent for extraction. Polar compounds dissolve in polar solvents (methanol, ethanol, water, and butanol), while non-polar compounds dissolve in non-polar solvents (n-hexane, ether, and chloroform).

This study found that *P. minor* from Bengkulu has strong antioxidant activity, similar to ascorbic acid. Because of this, *P. minor* has potential as an antioxidant supplement. Antioxidants are essential for counteracting free radicals, which contribute to degenerative diseases. Antioxidant needs can be met by consuming external sources. Further research should examine how processing methods affect *P. minor*'s antioxidant activity to identify optimal methods that preserve its efficacy.

## Conclusion

*P. minor* has high nutritional value, making it a potential functional food. The nutritional composition from the highest to the lowest is water, carbohydrates, ash, crude fiber, protein and lipid, respectively. The high antioxidant activity of *P. minor* suggests its potential application in the development of functional foods or nutraceuticals; however, further purification and compound identification are required.

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