Antioxidant activities of acetone extract of *Sargassum polycystum* from different parts of Thallus

J Santoso1,*, K Khasanah1, K Tarman1 and I K Sumandiarsa2

1Department of Aquatic Products Technology, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University), Jl. Agatis Darmaga Bogor, Bogor 16680, Indonesia
2Study Program of Fisheries Products Technology, Polytechnic of Fisheries Business Expert, Jl. Raya Pasar Minggu, Jakarta 12520, Indonesia

*Corresponding author: jsantoso@apps.ipb.ac.id

**Abstract.** Brown alga *Sargassum polycystum* in Indonesian waters is relatively abundant, and several studies show that brown alga has more antioxidant activity than red and green algae. Analysis of active compounds in the alga is generally carried out as a whole of macroalgae thallus. This research was carried out to study the effect of thallus parts, namely basal, middle, and apical, on the antioxidant activity and fucoxanthin content in acetone extract of *S. polycystum*. The results determined the best part of the thallus as a producer of antioxidant compounds. The extraction was performed by an ultrasonic-assisted method using 90% acetone. The basal part contained the highest fucoxanthin of 1.00±0.025 mg/kg, while the most increased antioxidant activity was in the apical part of 65.78±5.31 µg/mL for the antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, 383.19±28.81 µmol ascorbic acid/g for the antioxidant activity of the *copper reducing antioxidant capacity* (CUPRAC) method, and 964.75±3.91 µmol Fe(II)/g for the antioxidant activity of the *ferric reducing antioxidant power* (FRAP) method. This study shows antioxidant activity had a negative correlation with the fucoxanthin content. It is concluded that fucoxanthin was not the major contributor to an antioxidant activity acetone extract of *S. polycystum* thallus.

**Keywords:** antioxidant; brown alga; fucoxanthin; *Sargassum polycystum*; thallus

1. Introduction

*Sargassum polycystum* is a species of brown alga with a broad, slightly tapered leaf base morphology and has serrations on the leaves. The thallus is rod-shaped and vesicle-shaped, and the primary branches can be growing thickly at the end and produce variety of chemical compounds [1]. Widyartini, Widodo and Susanto [2] reported that *S. polycystum* has a highly polymorphic thallus characteristic among individuals in its population. This seaweed has different thallus shapes and sizes depending on the environmental factors of its habitat and each part of the thallus is predicted to contain different chemicals depending on the growth process.

Green and red algae have lower antioxidant activity compared to brown algae [3]. Total phenols [4, 5], phlorotannins [6], and fucoxanthin [7-9] are antioxidant found in brown algae. The presence of the fucoxanthin pigment gives different colour gradations for each type of macroalgal, which is yellowish-brown to dark brown [10]. Fucoxanthin is one of the carotenoid pigments found in *Sargassum* sp., reducing free radicals [8].

Free radicals can cause oxidative stress that causes several degenerative diseases if the amount exceeds the body’s capacity. In contrast, the internal antioxidants in the body are not sufficient to regulate them. One of the efforts to overcome this is increasing the input of external antioxidants from secondary metabolites of *Sargassum* sp. such as pigment and total phenolic [11]. If a chemical can contribute protons or electrons to diminish free radicals, it is said to have antioxidant activity [12-15]. Extraction is the first step to isolate various types of components contained in macroalgae.
The ultrasonic extraction method or Ultrasound-Assisted Extraction (UAE) effectively extracts active compounds and pigments from solid samples [16]. The ability of the UAE to increase yield reaches 50-500% compared to conventional [17]. It was also discovered that utilizing this procedure increased efficiency by more than 30%, while combining it with other methods such as maceration increased efficiency by 77-93% [18]. The use of selected solvents has substantial yields, such as acetone, which has been widely employed to extract thermolabile chemicals due to its low boiling point [19]. Acetone has been reported to produce high phenol content (79.35 μg/mg) and strong antioxidant activity (23.78 IC50/EC50) [20]. It is supported also by the statement from Luximon-Ramma, Bahoran, and Crozier [21] that the acetone-aquadest mixture was considered a suitable solvent system for antioxidant extraction.

Analysis of antioxidant activity and the content of active compounds were generally carried out as a whole on the thallus of macroalgae. The thallus structure in brown algae consists of three parts, i.e. the blade, holdfast, and stipe. The blade is the leaf part of the thallus, which has a flat shape. Holdfast is the lower part of the thallus, which functions to attach to the substrate. Stipe is a structure that supports the blade [22]. The division of the thallus structure is carried out into the base, middle, and apical parts. The base thallus consists of the holdfast, the central axis, and the lower part of the secondary branch. The middle thallus consists of the middle part of the macroalgae from secondary branches, while the apical part consists of the distal part of the secondary branches [6]. This research used ultra-sound assisted extraction to assess the influence of distinct thallus portions, including the base, middle, and apical, on the antioxidant activity and fucoxanthin concentration of an acetone extract of S. polycystum.

2. Materials and method
2.1. Sample preparation
The raw material used in this study was the brown algae S. polycystum obtained from Tidung Island, Seribu Islands, Jakarta. After removing sand and other impurities, samples were rinsed in clean saltwater and transferred to the laboratory at a low temperature. The samples were cleaned with tap water, rubbed with paper towels, and sliced into three portions of the thallus, namely basal, middle, and apical, before being stored at -20 ºC until extraction or analysis.

2.2. Preparation of acetone extract
Acetone extract from three parts of the thallus was prepared as follows. Each thallus sample (50 g wet sample) was homogenised with 300 mL of acetone using a mixer, continued extraction using ultrasonic (40 kHz) for 60 min at 35 ºC. The extract was then centrifuged for 20 min at 4 ºC at 3580 g. To make a crude extract, the collected supernatants were evaporated in a vacuum rotary evaporator at 30-40 ºC at a pressure of 60–70 cmHg. The crude extracts of acetone were kept at -20 ºC until analysed.

2.3. Analytical methods
2.3.1. Proximate composition and crude fiber. The approved AOAC method [23] was used to calculate moisture, ash, fat, and protein contents. By deducting the percentages of moisture, ash, fat, and protein from 100%, the carbohydrate content was computed. The official AOAC technique [23] was also used to determine crude fibre content, which involved dissolving in sulfuric acid and sodium hydroxide under boiling conditions and weighing the residue after drying.

2.3.2. Phytochemical component and yield. Biochemical testing was conducted by examining the different phytochemical content in aceton extract of seaweed, namely alkaloids, flavonoids, saponins, steroids, and tannins. Phytochemical analysis procedure refers to Harborne [24]. The extract yield before analysis was also determined by comparing the dry weight of the extract after the vacuum evaporation process with the initial weight of samples. The yield was expressed as percentage.

2.3.3. Antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test was carried out according to the method of Blois [12]
with slight modifications. DPPH solution of 0.1 mM was prepared by dissolving 0.004 g of DPPH in 100 mL of ethanol. The blank solution was made by adding 0.5 mL of DPPH solution to 3.5 mL of 90% acetone solvent. Samples of acetone extract with concentrations of 100, 125, 150, 175, and 200 ppm were mixed with 0.5 mL of DPPH solution, homogenized, and incubated at room temperature for 30 min before being detected at a wavelength of 517 nm. Parallel controls (without acetone extract) were also analysed in the same way. The proportion of DPPH radicals was used to calculate scavenging activity. A blank sample is absorbance resulting from the reaction of DPPH and 90% ethanol solutions. A sample is absorbance of sample reaction and DPPH.

\[
\text{Antioxidant Activity (\%)} = \frac{\text{A blank sample - A sample}}{\text{A blank sample}} \times 100\% \tag{1}
\]

2.3.4. Antioxidant activity of the cupric reducing antioxidant capacity (CUPRAC) method. With slight adjustments, the analysis was carried out using the approach proposed by Apak et al. [25]. The acetone extract was dissolved in ethanol, then made CUPRAC reagent, namely 1 mL CuCl$_2$·2H$_2$O 0.01 M; 1 mL ethanolic neocuproin 0.0075 M; and 1 mL of 1 M ammonium acetate buffer pH 7. Absorbance measurements used 0.3 mL of sample, 0.8 mL of 90% acetone, and 3 mL of CUPRAC reagent. The solution was allowed to stand for 30 min after being thoroughly mixed, and the absorbance was measured at a wavelength of 450 nm. The positive control was ascorbic acid, while the blank solution was a combination of solutions lacking acetone extract.

2.3.5. Antioxidant activity of the ferric reducing antioxidant power (FRAP) method. Ferric Reducing Antioxidant Power (FRAP) assay was carried out referring to the method used by Benzie and Strain [13] with modifications. The preparation of the FRAP reagent by mixing 0.3 M acetate buffer pH 3.6; 2,4,6-tripyrydyl-triazine (TPTZ) 0.01 M in 0.04 M HCl; and FeCl$_3$·6H$_2$O 0.02 M. The solution was prepared fresh with a ratio of 10:1:1 acetate buffer, TPTZ, and FeCl$_3$·6H$_2$O. The absorbance measurement used 0.3 mL of acetone extract sample that had been dissolved in ethanol, 0.8 mL of 90% acetone, and 3 mL of FRAP reagent. The sample mixture with FRAP reagent was homogenised and then incubated at 37 °C for 30 min. The absorbance was measured at a wavelength of 593-595 nm. The standard used is FeSO$_4$·7H$_2$O which is prepared by dissolving FeSO$_4$·7H$_2$O with distilled water.

2.3.6. Fucoxanthin analysis. A High-Performance Liquid Chromatography Instrument (Shimadzu LC-10AD) with a photodiode array detector and a Shim-Pack VP-ODS C18 2x150 mm column was used to analyze fucoxanthin. The mobile phase utilized was a gradient of distilled water-acetonitrile for 40 minutes at a flow rate of 0.2 mL/min. Each thallus (0.05 g) acetone extract was dissolved in 5 mL of ethanol to obtain a concentration of 10,000 ppm. The acetone extract sample was then filtered using Whatman 42 filter paper, injected into the instrument using an autosampler of 10 µL each. The standard curve of fucoxanthin was prepared by dissolving 1 mg of standard fucoxanthin with 1 mL of ethanol. Dilutions were made with concentrations of 20, 40, 60, 80, and 100 ppm. The concentration series were then injected into the instrument at 10 µL each. Using the linear regression equation of the fucoxanthin standard, the concentration of fucoxanthin in each acetone extract was estimated based on the peak area. The method that has been described refers to the previous research conducted by Nursid et al. [27] and Nursid and Noviendri [28].

2.4. Statistical analysis
The outcomes are presented as a mean with a standard deviation. To analyze the data among multiple treatments, a complete random design with a single component, namely acetone extract of section of the thallus (basal, middle, apical), was used. The data was analyzed using analysis of variance at a confidence level of 95%, and continued with the Duncan test.
3. **Results**

3.1. **Proximate composition and crude fibre**

Proximate composition and crude fibre content of three-part of the thallus, including the whole of the thallus, is presented in table 1. Apical and basal contained the highest moisture and ash, respectively and were significantly different compared to other parts. In the case of protein content, both basal and apical parts had higher content than the middle part, while the fat content had no significant difference. The basal and middle part of the thallus had higher carbohydrate content, and for crude fibre, the higher content was found in the basal.

| Parameter          | Basal     | Middle    | Apical    | Whole     |
|--------------------|-----------|-----------|-----------|-----------|
| Moisture (%)       | 83.26±0.01a | 83.96±0.01b | 85.14±0.01c | 89.28±0.01 |
| Ash (%)            | 5.27±0.06b | 5.00±0.08a | 4.81±0.06a  | 3.42±0.05  |
| Protein (%)        | 1.51±0.02b | 1.42±0.00a | 1.50±0.01b  | 1.00±0.01  |
| Fat (%)            | 0.79±0.01a | 0.80±0.01a | 0.81±0.01a  | 0.20±0.00  |
| Carbohydrate by-difference (%) | 7.71±0.04b | 7.56±0.14b | 6.35±0.04a  | 4.73±0.01  |
| Crude fibre (%)    | 1.47±0.07b | 1.26±0.07a | 1.41±0.02ab | 1.39±0.06  |

Different superscript letters (a, b, c) in each treatment of the thallus section show significant differences (p<0.05).

3.2. **Yield crude extract of acetone**

Yield crude extract of acetone was significantly affected by part of the thallus, as shown in table 2. The maximum yield was found in the apical thallus of *S. polycystum*, followed by the middle, and the lowest was found at the basal, with each value being statistically different. It suggests that how other sections of the thallus are treated throughout the extraction process has an impact on the output of crude acetone extract.

| Thallus parts | Yield (%) |
|---------------|-----------|
| Basal         | 0.41±0.04a|
| Middle        | 1.78±0.05b|
| Apical        | 3.33±0.07c|

Different superscript letters (a, b, c) in each treatment of the thallus section show significant differences (p<0.05).

3.3. **Phytochemical components of crude extract of acetone**

The components of the active compound in the thallus of the brown alga *S. polycystum* can be detected through qualitative phytochemical testing. Phytochemical tests carried out included alkaloids, steroids, saponins, phenols, tannins, and flavonoids. As shown in table 3, all parts of each thallus contained all phytochemical components tested, except saponins and tannins.

| Parameter                          | Basal     | Middle    | Apical    |
|------------------------------------|-----------|-----------|-----------|
| 2,2-diphenyl-1-picrylhydrazyl (DPPH) | 75.2±0.04b | 76.0±0.05a | 74.8±0.03b |
| Cupric reducing antioxidant capacity (CUPRAC) | 82.0±0.06a | 83.0±0.04b | 81.0±0.02c |
| Ferric reducing antioxidant power (FRAP) | 92.0±0.07c | 93.0±0.05b | 91.0±0.03a |

Different superscript letters (a, b, c) in each treatment of the thallus section show significant differences (p<0.05).

3.4. **Antioxidant activities of crude extract of acetone**

To evaluate the antioxidant activities of each part of thallus extract, we performed three analysis methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH), cupric reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP). The part of thallus significantly affected the antioxidant activity, measured by the DPPH method (p<0.05). The apical thallus had the highest antioxidant activity, followed by the middle thallus and the basal thallus, with each part of the thallus having significantly different values (figure 1). The apical thallus, which was included in the strong category, had the highest antioxidant activity in the DPPH method, with an IC_{50} value of 65.785.31 g/mL.

Table 1. Yield of acetone extract of thallus parts variation.

Table 2. Proximate composition and crude fiber of each parts of thallus and whole thallus.
The antioxidant capacity measured by the CUPRAC method, like the antioxidant activity measured by the DPPH method, was significantly affected by the part of the thallus employed (p < 0.05). The apical thallus had the highest antioxidant capacity value and significantly differed from the middle thallus, and the basal thallus with values were 383.19, 346.66 and 352.94 µmol ascorbic acid/g, respectively (figure 2). Similar results were also obtained from the evaluation of antioxidants using the FRAP method. As shown in figure 3, the highest value of FRAP was obtained from the apical part of the thallus (964.75 µmol Fe²⁺/g) followed by the middle part (941.25 µmol Fe²⁺/g) and the basal part (891.07 µmol Fe²⁺/g) which each value was significantly different (p <0.05).

3.5. Fucoxanthin content
The fucoxanthin content in the crude extract of acetone is also significantly affected by the part of thallus used (figure 4). In contrast to the antioxidant activities described previously, the highest fucoxanthin content was found in the basal part (1.00 mg/kg). The middle and apical contained fucoxanthin of 0.83 and 0.73 mg/kg, respectively. Each part has a statistically significant difference in fucoxanthin content (p <0.05).

| Parameter   | Sargassum polycystum |        |        |
|-------------|-----------------------|--------|--------|
|             | Apical                | Middle | Basal  |
| Alkaloids   |                       |        |        |
| Dragendorff | +                     | +      | +      |
| Meyer       | +                     | +      | +      |
| Wagner      | +                     | +      | +      |
| Steroids    | +                     | +      | +      |
| Saponins    | -                     | -      | -      |
| Phenol      | +                     | +      | +      |
| Tannins     | -                     | -      | -      |
| Flavonoids  | +                     | +      | +      |

+ = detected; - = not detected

| Table 3. Phytochemical components of different parts from S. polycystum thallus. | Sargassum polycystum | Sargassum polycystum¹ |
|-------------------------------|----------------------|-----------------------|
| Parameter   | Apical | Middle | Basal | | Apical | Middle | Basal |
| Alkaloids   |        |        |       | |        |        |       |
| Dragendorff | +      | +      | +     | | +      | +      | +     |
| Meyer       | +      | +      | +     | | +      | +      | +     |
| Wagner      | +      | +      | +     | | +      | +      | +     |
| Steroids    | +      | +      | +     | | +      | +      | +     |
| Saponins    | -      | -      | -     | | -      | -      | -     |
| Phenol      | +      | +      | +     | | +      | +      | +     |
| Tannins     | -      | -      | -     | | -      | -      | -     |
| Flavonoids  | +      | +      | +     | | +      | +      | +     |

¹ whole thallus Diachanty, Nurjanah, and Abdullah [26]
4. Discussion

The whole thallus has a different chemical composition value from other thallus parts. It may be due to the different reproductive phases between the parts of the thallus. Macroalgae in the generative phase tend to have more primary metabolites because they do not have difficulty growing in comfortable environmental conditions [29]. Macroalgae with a layered structure of laminar have lower moisture content than seaweed with a tubular [30]. The highest content of ash was found in the basal thallus. Kumar, Sahoo and Levine [31] reported that different parts of the thallus in S. wightii had other ash content. The primary axis thallus and old leaves have higher ash content than young leaves. It is due to the higher mineral accumulation in the older thallus because of direct contact with the rock and substrate. Kumar, Sahoo and Levine [31] reported that the highest protein and fat content of S. wightii were found in young leaves, with values were 12.7±0.96% and 3.5±0.53% dry weight, respectively. This condition is related to a higher rate of cell division in part of young thallus. Kumar, Sahoo and Levine [31] reported that different parts of the thallus in S. wightii had different ash content. In comparison, S. wightii had the maximum protein content in late winter (January-March), when the young thallus was in the developing phase. S. polycystum thallus has lower fat content than other chemical compositions. Ortiz et al. [32] reported that Ulva lactuca and Durvillaea antarctica have lower fat content than other chemical compositions.

The highest crude fibre and carbohydrate content of this macroalga was found in the basal thallus. Hidayat et al. [33] reported that crude fibre content in Sargassum sp. was 0.43% wet weight. This result was lower than the crude fibre content of S. polycystum thallus in our research. Kumar, Sahoo and Levine [31] reported that the highest total carbohydrate from the thallus of S. wightii was found on the central axis, followed by old and young leaves. Differences in the results of carbohydrate content can be caused by differences in the methods used in determining carbohydrate levels; this study uses the by difference method.

The use of pretreatment such as dimethyl sulfoxide (DMSO) as immersion pre-treatment before extraction has proven to produce a high yield extract. Seely, Duncan, and Vidaver [34] stated that the use of pre-treatment in the form of immersion in DMSO could damage the cell membrane of the sample;
as a consequence, many active compounds came out and resulted in a high yield. Mahindrakar and Rathod [18] reported that the extraction yield increased significantly related to the increasing ratio of material and solvent, from ratio 1:5 to 1:15.

Harborne [24] stated that solvent polarity also influences the yield of extracts. The component will be extracted well when it has the same polarity as the solvent. Hemlatha et al. [35] reported that the extraction of S. polycystum using the maceration method and methanol solvent resulted in a crude extract yield of 4.62%. Extraction of Sargassum sp. using the maceration method with methanol and ethanol solvents resulted in 2.69% and 0.54% of yield, respectively [10, 11]. Moreover, Gazali, Nurjanah and Zamani [35] reported that extraction of Sargassum sp. through maceration using ethanol, ethyl acetate, and n-hexane as solvents produced yields of 0.565, 0.420, and 0.265%, respectively.

Macroalgae like plants can produce active compounds that play an essential role in the health aspect. The apical, middle and basal parts of S. polycystum thallus contained alkaloids, steroids, phenols, and flavonoids. Diachanty, Nurjanah and Abdullah [36] reported that ethanol extract of S. polycystum (whole thallus) had steroids, triterpenoids, and flavonoids. Moreover, Gazali, Nurjanah and Zamani [35] reported that ethanol and n-hexane extracts of Sargassum sp. showed positive results for alkaloid compounds in Meyer's reagent and triterpenoids. In contrast, ethyl acetate extract was positive for phenol. Phenol was detected in the ethyl acetate extract of S. polycystum as reported by Sami et al [37]. Dia, Nurjanah and Jacob [38] stated that steroids were more detectable in ethanol extracts than ethyl acetate extracts and n-hexane extracts in the extract of Bruguiera gymnorrhiza leave.

The DPPH method for determining antioxidant activity involves employing DPPH free radicals to capture hydrogen generated from antioxidants in brown seaweed extracts. Antioxidants will donate protons or hydrogen to DPPH, which will break free radical chains to form non-radical compounds. The categories of antioxidant power include very strong (IC₅₀ < 50 ppm), strong (IC₅₀ = 50-100 ppm), moderate (IC₅₀ = 100-150 ppm), and weak (IC₅₀ = 150-200 ppm). The IC₅₀ value indicates the concentration of the sample required to inactivate free radicals by 50% [14]. The lower IC₅₀ value has the stronger sample extract's ability to reduce free radicals.

Diphenylpicrylhydrazyl (DPPH) is a stable free radical based on electron delocalization between molecules as a whole, which prevents the molecules from merging. It is the delocalisation of electrons that gives rise to a dark purple colour. When a DPPH solution is combined with an extract containing antioxidants that may donate hydrogen atoms, diphenylpicrylhydrazine is produced, which is not a radical. The purple tint is fading, indicating that it is turning yellow [14]. The donor molecule that has donated its electron becomes a radical and reacts with another molecule of the same type produced by a parallel reaction. The DPPH technique produces a dark purple tint and a significant absorption at a wavelength of 517 nm [12].

CUPRAC is widely used in measuring antioxidant capacity because it is selective, the reagent is more stable, and can be used to measure antioxidants simultaneously. The CUPRAC method uses a chromogenic reagent in the form of CAS (neucuproin) copper (II) (Cu(Nc)₂⁺)[15]. The mechanism of the CUPRAC method is the reduction of Cu(II) to Cu(I). The Cu(I)-neucuproin complex gives a yellow colour and is absorbed in a wavelength of 450 nm. The copper(II) bis(neucuproin) chromogenic reagent in the CUPRAC assay works at pH 7 [15]. The yellow colour's intensity depends on the amount of Cu(II) reduction to Cu(I). If a material reduces Cu(II) to Cu(I), at the same time oxidation occurs, the material can act as an antioxidant [15, 25]. Furthermore, the FRAP approach can be utilized to determine antioxidant capability in everything from crude extracts to purified molecules. The FRAP method's antioxidant capacity is determined by measuring an extract's ability to donate electrons to decrease Fe³⁺ in the Fe³⁺-TPTZ complex to Fe²⁺-TPTZ [39].

Our findings were consistent with a recent study conducted by Abdala-Diaz et al. [4], who discovered that the greatest antioxidant activity was observed in the apical thallus of Cystoseira tamariscifolia. These results are suspected in the apical thallus containing many active compounds that can reduce free radicals [40]. On the other hand, Sami et al. [37] reported that multilevel extraction of S. polycystum (whole thallus) with methanol, ethyl acetate, and n-hexane by maceration produced weak antioxidant activity. According to the report, the values of IC₅₀ were 491.02, 411.80 and 502.70 µg/ml, respectively.
Furthermore, Diachanty, Nurjanah, and Abdullah [36] found that an ethanol extract of *Sargassum polycystum* (whole thallus) showed IC50, CUPRAC, and FRAP antioxidant capabilities of 3.4 mg/L, 201 mol troloks/g, and 105.357 mol troloks/g, respectively. The difference in results can be influenced by the habitat of the sample, type and concentration of solvent, extraction method, temperature, and extraction time. Wati, Suhendra and Wartini [41] reported that differences in solvent concentration and extraction temperature have different antioxidant activities, where antioxidant activity decreases at high solvent concentrations and temperatures. The high antioxidant capacity in the extract can be influenced by the content of phenolic compounds, polyphenols, vitamin C, vitamin E or other compounds that can reduce free radicals [15, 25].

Fucoxanthin is a carotenoid with an uncommon allenic link and oxygenic functional groups in its molecule such as epoxy, hydroxyl, carbonyl, and carboxyl moieties that contribute to its distinctive structure. Carotenoids' reactions with radicals are influenced not only by the carotenoids, but also by the radicals' type [42, 43]. Although the basal gets the least amount of sunlight, the basal thallus has a higher fucoxanthin content than other thallus parts. It is related to the complementary chromatic adaptation of macroalgae, modifying their pigmentation to compensate for low light availability to maximise their absorption. *Ulva lactuca, Codium fragile, Porphyra umbilicalis,* and *Chondrus crispus* found in a protected (shady) position with deeper depths had higher pigment concentrations than direct sunlight [44]. Spatial and temporal characteristics of seaweed habitat also influence the quantity of fucoxanthin content in *S. polycystum* that grow in western Indonesian waters [45].

Sudhakar, Ananthalakshmi and Nair [9] reported the extraction of fucoxanthin *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. with 90% acetone solvent containing fucoxanthin 0.09±0.006 to 0.38±0.046 mg/g dry weight. The fucoxanthin content of *Sargassum polycystum* extract with 90% acetone as solvent ranged from 0.59±0.01-0.71±0.01 g/g [41]. These results were different from the results of our study, which was extracted from different types of thallus. The low content of fucoxanthin is caused by other compounds that are not fucoxanthin which is more extracted from the thallus of *S. polycystum* than fucoxanthin. Gross [46] stated that fucoxanthin had conjugated double bonds in its structure to be easily damaged by oxidative degradation such as temperature, oxygen, enzymes, and light.

We also calculated the correlation value between antioxidant activity and fucoxanthin content to see if there was a link (r). Using the DPPH, CUPRAC, and FRAP techniques, the correlation values between fucoxanthin content and antioxidant activities were -0.997, -0.954, and -0.999, respectively. The highest antioxidant activity was found in the apical thallus, while the highest fucoxanthin was found in the basal thallus. These results indicate that fucoxanthin only contributes 1% to the antioxidant activity of *S. polycystum* thallus acetone extract, meaning 99% is determined by other unknown compounds but has high antioxidant activity. The antioxidant activity of the acetone extract of the thallus of *S. polycystum* was not correlated with fucoxanthin because the samples tested were still crude extracts that still contained other chemical compounds, including phenolic, chlorophyll, and others. Carotenoids were not the key contributors to guava's antioxidant activity, according to Thaipong et al. [47]. Total phenols [4, 5] and phlorotannins [6] are two other antioxidant molecules. The phytochemical profile of *S. polycystum* thallus acetone extract revealed positive results for alkaloids, steroids, phenols, and flavonoids, all of which are predicted to have antioxidant properties.

5. Conclusions
The antioxidant activity and fucoxanthin content of crude acetone extracts of *S. polycystum* in different sections of the thallus (basal, middle, and apical) were significantly different. The apical thallus had the most antioxidant activity, while the basal section of the thallus had the highest fucoxanthin content. The antioxidant activity of *S. polycystum* acetone extract displayed a negative association with fucoxanthin levels, indicating that fucoxanthin was not the main contributor to the antioxidant activity.
References

[1] Pansing J, Gerung G S, Sondak C F A, Wagey B T, Ompi M and Kondoy K I F 2017 Sargassum sp. morphology in Raja Ampat archipelago, West Papua Jurnal Pesisir dan Laut Tropis. 1 3-17.

[2] Widyartini D S, Widodo P and Susanto A B 2017 Thallus variation of Sargassum polycystum from Central Java, Indonesia Biodiversitas 18 1004-1011.

[3] Kelman D, Posner E K, McDermid K J, Tabandera N K, Wright P R and Wright A D 2012 Antioxidant activity of Hawaiian marine algae Marine Drugs 10 403-416.

[4] Abdala-Diaz R T, Cabello-Pasini A, Marquez-Garrido E and Figueroa F L 2014 Intra-thallus variation of phenolic compounds, antioxidant activity, and phenolsulphatase activity in Cystoseira tamariscifolia (Phaeophyceae) from southern Spain Ciencias Marinas 40 1-10.

[5] Kang K, Park Y, Hwang H J, Kim S H, Lee J G and Shin H C 2003 Antioxidative properties of brown algae polyphenolics and their perspectives as chemopreventive agent against vascular risk factors Archives of Pharmacal Research 26 286-293.

[6] Connan S, Delisie F, Deslandes E and Gall E A 2006 Intra-thallus phlorotannin content and antioxidant activity phaeophyceae of temperate waters Botanica Marina 49 39-46.

[7] Takashima M, Shichiri M, Hagihara Y, Yoshida Y and Niki E 2012 Capacity of fucoxanthin for scavenging peroxy radicals and inhibition of lipid peroxidation in model systems Free Radical Research 46 1406-1412.

[8] Nursid M, Wikanta T and Susilowati R 2013 Antioxidant activity, cytotoxicity, and fucoxanthin content of brown seaweed extract from Binuangean Beach, Banten Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 8 73-84.

[9] Sudhakar M P, Ananthalakshmi J S and Nair B B 2013 Extraction, purification and study on antioxidant properties of fucoxanthin from brown seaweeds Journal of Chemical and Pharmaceutical Research 5 169-175.

[10] Renhoran M, Noviendri D, Setyaningsih I and Uju 2017 Extraction and purification of fucoxanthin from Sargassum sp. as an anti-acne Jurnal Pengolahan Hasil Perikanan Indonesia 20 370-379.

[11] Sedjati S, Supriyantini E, Ridlo A, Soenardjo N and Santi V Y 2018 Pigment content, total phenolic, and antioxidant activity of Sargassum sp Jurnal Kelautan Tropis 21 137-144.

[12] Blois M S 1958 Antioxidant determinations by the use of a stable free radical Nature 181 1199-1200.

[13] Benzie I F F and Strain J J 1996 The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay Analytical Biochemistry 239 70-76.

[14] Molyneux P 2004 The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity Songklanakarin Journal Science Technology 26 211-219.

[15] Apak R, Guclu K, Ozurek M and Karademir SE 2004 Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method Journal of Agricultural and Food Chemistry 52 7970-7981.

[16] Priego-Capote F and de Castro M D L 2007 Ultrasound in analytical chemistry Analytical and Bioanalytical Chemistry 387 247-257.

[17] Michalak I and Chojnacka K 2014 Algal extracts: Technology and advances Engineering in Life Sciences 14 581–591.

[18] Mittal R, Tavanandi H A, Mantri V A and Raghavaraao K S M S 2017 Ultrasound assisted methods for enhanced extraction of phycobiliproteins from marine macro-algae, Gelidium pusillum (Rhodophyta) Ultrasonics Sonochemistry 38 92–103.

[19] Rowe RC, Sheskey PJ and Quinn ME 2009 Handbook of Pharmaceutical Excipients: Sixth Edition (London: Pharmaceutical Press).

[20] Alagesan V, Ramalingam S, Kim M, Venugopal S 2019 Antioxidant activity guided isolation of a coumarin compound from Ipomoea pescaprea (Convolvulaceae) leaves acetone extract and
its biological and molecular docking studies European Journal of Integrative Medicine 32 1-55.
[21] Luximon-Ramma R, Bahorun T and Crozier A 2003 Antioxidant actions and phenolic and vitamin C contents of common Mauritius exotic fruits Journal Science Food Agriculture 83 496-502.
[22] Castro P and Huber M E 2003 Marine Biology: Fourth Edition (New York: MacGraw-Hill Companies).
[23] [AOAC] Association of Official Analitycal Chemist 2005 Official Method of Analysis of the Association of Official Analitycal of Chemist (Virginia: Published by The Association of Analytical Chemist, Inc).
[24] Harborne JB 1987 Phytochemical Methods A Guide to Modern Technique of Plant Analysis. Third Edition (London: Chapman and Hall)
[25] Apak R, Guclu K, Özyürek M, Ozyurek M, Bektaşoğlu B and Celik S E 2008 Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay Microchimica Acta 160 413-419.
[26] Diachanty S, Nurjanah and Abdullah A 2017 Antioxidant activity of various types of brown seaweed from the waters of the Thousand Islands Jurnal Pengolahan Hasil Perikanan Indonesia 20 305-318
[27] Nursid M, Noviendri D, Rahayu L and Novelita V 2016 Isolation of fucoxanthin from brown seaweed Padina australis and its cytotoxicity against MCF7 cells and Vero cells Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 11 83-90.
[28] Nursid M and Noviendri D 2017 Fucoxanthin and total phenolic content in sun-dried Padina australis brown seaweed Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 12 117-124.
[29] Wiencke C and Bischof K 2012 Seaweed Biology: Novel Insights into Ecophysiology, Ecology, and Utilisation (London: Springer Science).
[30] Garcia J S, Palacios V and Roldan A 2016 Nutritional potential of four seaweed species collected in the Barbate Estuary (Gulf of Cadiz, Spain) Journal of Nutrition and Food Science 6 1-7.
[31] Kumar S, Sahoo D and Levine I 2015 Assessment of nutritional value in a brown seaweed Sargassum wightii and their seasonal variation Algal Research 9 117-125.
[32] Ortiz J, Romero N, Robert P, Araya J, Lopez-Hernandez J, Bozzo C, Navarrete E, Osorio A and Rios A 2006 Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulva lactuca and Durvillaea antarctica Food Chemistry 99 1-7.
[33] Hidayat T, Nurjanah, Nurilmala M and Anwar E 2018 Characterization of tropical seaweed from the Thousand Islands as a source of cosmetic raw materials Creative Research Journal 4 49-62.
[34] Seely G R, Duncan M J and Vidaver W E 1972 Preparative and analytical extraction of brown algae with dimethyl sulfoxide Marine Biology 12 184-188.
[35] Hemlatha N, Pee P P, Kee S H Y, Ow J T, Yan S W, Chew L Y and Kong K W 2017 Malaysian brown seaweeds Sargassum siliculosum and Sargassum polycystum: low density lipoprotein (LDL) oxidation, angiotensin converting enzyme (ACE), α-amylase, and α-glucosidase inhibition activities Food Research International 99 950-958
[36] Gazali M, Nurjanah and Zamani N P 2018 Exploration of bioactive compounds of brown algae Sargassum sp. Agardh as an antioxidant from the west coast of Aceh Jurnal Pengolahan Hasil Perikanan Indonesia 21 167-178.
[37] Sami F J, Soekamto N H, Firdaus and Latip J 2019 Antioxidant activity test of extracts of brown algae Sargassum polycystum and Turbinaria deccurens from Dutungan Island, South Sulawesi against DPPH radicals Jurnal Kimia Riset 4 1-6.
[38] Dia S P S, Nurjanah and Jacob M A 2015 Chemical composition and antioxidant activity of roots, bark and leaves of lindur Jurnal Pengolahan Hasil Perikanan Indonesia 18 205-219.
[39] Katalinic V, Milos M, Kulisic T and Jukic M 2006 Screening of 70 medicinal plant extract for antioxidant capacity and total phenolics Food Chemistry 94 550-557.
[40] Gomez I, Espanol S, Veliz K and Huovinen P 2016 Spatial distribution of phlorotannins and its relationship with photosynthetic UV tolerance and allocation of storage carbohydrates in blades of the kelp Lessonia spicata Marine Biology 163 1-14.
[41] Wati N K E, Suhendra L and Wartini N M 2020 Characteristics of fucoxanthin content and antioxidant activity of brown algae extract (Sargassum polycystum) in the treatment of acetone solvent concentration and maceration temperature Jurnal Rekayasa dan Manajemen Agroindustri 8 80-90.
[42] Peng J, Yuan J-P, Wu C-F and Wang J-H 2011 Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health Marine Drugs 9 1806–1828.
[43] Sachindra N M, Sato E, Maeda H, Hosokawa M, Niwano Y, Kohno M and Miyashita K 2007 Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites Journal of Agricultural and Food Chemistry 55 8516-8522.
[44] Ramus J, Beale S I, Mauzerall D and Howard K L 1976 Changes in photosynthetic pigment concentration in seaweeds as a function of water depth Marine Biology 37 223-229.
[45] Sumandiarsa I K, Bengen D G, Santoso J and Januar H I 2021 The Impact of spatio-temporal variation on seawater quality and its effect on the domination of Sargassum polycystum on small islands in western Indonesian waters Environment Asia 14 80-92.
[46] Gross J 1991 Pigments in Vegetable, Chlorophylls, and Carotenoids (New York: Van Nostrand Reinhold).
[47] Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L and Byrne D H 2006 Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts Journal of Food Composition and Analysis 19 669-675.