Evaluation antioxidant capacity and proximate composition in brown seaweed *S. crassifolium* found in Lombok coast, Indonesia

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Abstract. Seaweed is one of the marine-biota that is widely known for its uses, one of them is in the food functional. Functional food is a program that promotes a healthy diet so that it can prevent a disease when consuming food. With functional food, daily eating patterns can be maintained and also the content of the food consumed daily has a good effect on the body. It is known that in seaweed, especially brown algae (*S. crassifolium*), it contains antioxidants and other compounds that are good for the health. In this study, phytochemical content and proximate composition was evaluated in *S. crassifolium*. Including total flavonoid content and total phenolic content, antioxidant capacity both of DPPH and ABTS assay used three type of solvents such as Ethanol, n-Hexana, and ethyl acetate for comparable the best solvents. Also, proximate analysis to determine the content of moisture content, crude fat content, crude protein content, and carbohydrates content. The results showed that n-Hexana and ethyl acetate solvents were significantly than ethanol. Which mean both of them had highest values of total flavonoid content (TFC), total phenolic content (TPC), and antioxidant activity of DPPH radical scavenging activity and ABTS radical scavenging activity. The results of chemical composition in proximate compounds showed that *S. crassifolium* was suitable to be candidate of functional food.

1. Introduction

Indonesia is an archipelagic country with a tropical climate which has an area of 6,400,000 km² of water and a coastline of 110,000 km. This region makes Indonesia a very high producer of natural seaweed resources [1]. Seaweed is a marine biota that can be found in shallow waters that have a substrate to be used as a medium for seaweed to attach. Seaweed or macro algae are divided into 3 groups, namely *Phaeophyta* (brown algae), *Rhodophyta* (red algae), and *Chlorophyta* (green algae) [2]. In the coastal areas in Lombok, West Nusa Tenggara, which are in the Wallacea area, this causes the high and unique marine life of NTB, including seaweed resources. The results of the exploration and identification conducted by Sunarpi *et al.*[3] showed that there are about 88 species of macroalgae in the sea waters of West Nusa Tenggara, most of which have not been exploited. It is also known that there are five types of brown algae (*Phaeophyta*), one of them is *S. crassifolium*. 

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2. Material and Methods

2.1 Sample collection and preparation

The brown macroalgae *Sargassum crassifolium* was collected from batu layar coastal, West Lombok, West Nusa Tenggara, Indonesia (8°31’03.1"S 116°03’40.8”E). Macroalgae was taken on August 2021 in intertidal zone coastal. Sample that have been collected are brought to Bioscience and Biotechnology Research Center, University of Mataram for preparation. The samples were washed with flow water and dried at room temperature (±27°C) for 3-5 days. After that, dried sample was cut using scissors into small cutting and then blended. Then, the powder of *S. crassifolium* was extracted with 3 kinds of solvents which were Ethanol, n-Hexana, and Ethyl Acetate. After extracting, samples were evaporated to remove the solvents in each sample to get crude extracts. Stock solution was made by dissolve the crude extracts with DMSO to gain 1% concentration.

2.2 Total Flavonoid Content

Total flavonoid content measured by Baek *et al.* [10] that have been modified. 25 µL samples was mixed with 125 µL distilled water or ethanol to 96 well plate. 5% of sodium nitrite (NaNO2) was added to this mixture. In the other well, 25 µL samples was mixed with 197 µL ethanol was added. After 5 minutes, incubation, 10% aluminium chloride (AlCl3) was added. After another 50 minutes incubation, 1 M natrium hydroxide (NaOH) was added into well. Then, the absorbance was measured at 510 nm using UV-spectrofotometer. Quercetein was dissolved in methanol and then diluted at range 25-150 mg/mL as standars.

2.3 Total phenolic Content

Total phenolic content was measured modified as described by Baek *et al.* [10] with Follin Ciocalteu (FC) reagent. 20 µL samples was mixed with Follin-Ciocalteu reagent added into 96 well plate. In the other well, 20 µL samples and 140 µL ethanol was added. 40 µL natrium bicarbonate (Na2CO3) was added after 60 minutes incubation and the absorbance was measured using UV-spectrofotometer at 765 nm wavelength. Total phenolic content in *S. crassifolium* was expressed as gallic acid equivalents (GAE)/gr.
2.4 ABTS Radical Scavenging Activity
ABTS radical scavenging activity assay was measured modified from Youn et al. [11]. 100 μL of samples was mixed with ABTS reagent added into 96 well plate. 100 μL of samples mixed with 100 μL ethanol into other well. After 3 minutes later, the absorbance was measured at 734 nm wavelength. ABTS radical scavenging activity was expressed with mg vitamin C equivalent (VCEAC)/100 gr.

2.5 DPPH Radical Scavenging Activity
DPPH radical scavenging activity assay was carried out according Youn et al. [11] that have been modified. 100 μL samples was mixed with DPPH reagent into 96 well plate. In the other well, 100 μL of samples and 100 μL ethanol was added. After 30 minutes incubation, the absorbance was measured at 517 nm. Vitamin C equivalent was expressed of DPPH radical scavenging activity.

2.6 Chemical Composition
Chemical composition includes moisture content, crude fat content, crude protein content, and carbohydrate content are determined based on proximate analysis which refers to AOAC (2010) method [12].

2.7 Statistical Analysis
Statistical analysis was performed using GraphPad Prism 9. with a two-tailed unpaired Student t-test and one-way analysis of variance (ANOVA) and continued with the Tukey-Kramer test. The difference between the comparisons is statistically considered significant if the p-value is less than 0.05 (p<0.05).

3. Results and Discussion
3.1 Collected samples.
The sampling site is located at Batu Layar Coastal, west Lombok (8°31’03.1”S 116°03’40.8”E). Batu Layar is one of coastal in Lombok that have abundant of macroalgae especially Phaeophyta (brown algae). S. crassifolium (Fig. 1). It mainly has a cylindrical thallus. The branches are luxuriant, the leaves broadened, elongated or like a sword. Mostly, having solitary air bubbles. the color of thallus is brown. Special features belonging to S. crassifolium included thallus flattened, smooth, a rough main shaft, and a holdfast (the area used for sticking) asa disk. Alternating across on a regular basis. The oval leaf in length and thallus 13.5 to 14 cm [13].

![Fig 1. Morphology of S. crassifolium.](image-url)
3.2. Total flavonoid and phenolic content.

Total flavonoid content (TFC) of the extracts were quantification sample values of standard curve = -0.0013x + 0.8277 and expressed in mg Quercetin Equivalent per gram of extract. Total phenolic content (TFC) was y = 0.005x + 0.0208 standard curve and expressed in mg Gallic Acid Equivalent (GAE) per gram of extract.

![Figure 2. Phytochemical constituent analyses of S. crassifolium extracts (a) total flavonoid content (TFC) (b) total phenolic content (TPC). Different letters indicate significant difference between groups (p<0.05).](image)

Results for total flavonoid content and total phenolic content shown in (Fig. 2). Flavonoids are secondary metabolites belonging to a large group of hydroxylated polyphenolic compounds[14]. The effect of n-Hexana and ethyl acetate solvents on total flavonoid content (TFC) were not significant (p>0.05) as on total phenolic content. While ethanol solvent showed significantly with other solvents (p > 0.05). Both of n-Hexana (79.6 mg QE/100 gr) and Ethyl acetate (67.7 mg QE/100 gr) were higher than Ethanol (49.07 mg QE/100 gr). As well as total phenolic content on n-Hexana (79.4 mg GAE/100 gr) and ethyl acetate (82.6 mg GAE/100 gr) were highest values than ethanol solvent (16.7 mg GAE/100 gr). Which mean the highest flavonoid and phenolic compound were observed in both n-Hexana and ethyl acetate extracts. This was accordance with Hidayati et al.[15] which mention that n-Hexana and ethyl acetate extracts have highest total phenolic content than ethanol extract. The high content of active compounds in this Sargassum extracts is considered to have soluble biopolyphenol compounds such as flavanols and tannins[15].

3.3 Antioxidant Activity.

Results for antioxidant activity between ABTS radical scavenging activity and DPPH radical scavenging shown in (Fig.3). Based on results, ethanol solvent showed that have significantly (p > 0.05) with n-Hexana and ethyl acetate solvents. While n-Hexana and ethyl acetate showed that not significantly (p < 0.05) each other. Same as TFC and TPC, results of ABTS radical scavenging activity and DPPH radical scavenging activity have high values on n-Hexana and ethyl acetate solvents. Accoding Prasedya et al. Among all antioxidant test, S. cristaefolium extract showed the strongest activity against ABTS radicals. ABTS test is considered more sensitive in identifying antioxidant activity because of the faster reaction kinetics, and the
response higher antioxidant capacity than other radicals. Overall, extracts with smaller particle sizes had more potential to react with free radicals and stop them from becoming stable non-reactive forms [16].

![Fig 3. Radical scavenging activity of S. crassifolium analysed by (a). ABTS and (b), DPPH assays. Different letters indicate significant difference between groups (p<0.05).](image)

3.3. Chemical Compositions.

Proximate compound was shown in (Table. 1). Moisture content S. crassifolium obtained by 87.2114%, this result has similarities with Dewinta et al.[17] regarding the water content in S. crassifolium, which is 88.56%. Conducted by Novianti & Arisandi [18], the water content of S. crassifolium is around 10%. This difference in water content is strongly influenced by the temperature and humidity of the environmental conditions of storage of the material. This water content can affect the final yield of S. crassifolium simplicia. In addition, differences in water content in Sargassum are also influenced by harvest age, species, and environment [19]. Although it has high water content, Sargassum is not a perishable material, Sargassum remains fresh and has a distinctive aroma after a long process. reduced water content in a food ingredient can increase the concentration of fat, ash, protein, carbohydrates, and minerals [17]. Normally, seaweed has low fat content and is not considered a source of fat. Yanthong [17] reported that Sargassum has lipid content ranged from 2.02 to 2.62% dry weight. While in this study, the fat content obtained in S. crassifolium is around 1.0231% which is a low value, Sargassum is can be considered an ingredient that contains low calories and has many health benefits. Based on (Table. 1) crude protein content around 2.7%. The lowest protein value in brown algae was recorded at about 5-11% in dry weight. the lack of protein contained in Sargassum can be caused by the season period when the sample was collected. The highest value of protein in sargassum was found in winter and spring, while in summer the protein content was recorded to be low[20]. Carbohydrate content in S. crassifolium is 4.03%, this value higher than [17] that had 3.79% carbohydrate content. The high content of chemical compounds in brown algae includes alginate, this compound is known to be used as functional food[21].
Table 1. Proximate Compound of *S. crassifolium*.

| Parameters          | Average (%) |
|---------------------|-------------|
| Moisture content    | 87.2114     |
| Crude fat content   | 1.0231      |
| Crude protein content| 2.7107     |
| Carbohydrate content| 4.030       |

4. Conclusion
In conclusion, *S. crassifolium* contains high values of flavonoids and phenols. This potentially correlates to its antioxidant capacity. In addition, the proximate composition in *S. crassifolium* could be considered for further development as functional food.

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