Antioxidant activity of brown macroalgae _Sargassum_ ethanol extract from Lombok coast, Indonesia

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**Abstract.** The coastal areas of Lombok has very high diversity of macroalgae which are largely unexplored. Among them are the brown macroalgae _Sargassum_ species which are well known for their biological potentials. In this study, we aim to evaluate 4 _Sargassum_ species, _S. cristaefolium_, _S. crassifolium_, _S. polycystum_, and _S. aquifolium_ for their antioxidant potential of _Sargassum_ species found the west coastal Aare of Lombok, Indonesia. To determine the antioxidant capacity of macroalgal extracts, 2,2-diphenyl-1-picrylhydrazyl (DPPH) as well as total phenolic content (TPC) were measured. Extracts of the brown macroalgae are well known to have very active antioxidant activity. Among them _S. cristaefolium_ and _S. crassifolium_ provide stronger antioxidant activity compared to _S. aquifolium_ and _S. polycystum_. Furthermore, correlations were found between TPC macroalgal and their DPPH scavenging activity. This indicates an important role of polyphenols as antioxidants. Overall, brown macroalgae from Lombok coastal area may be a good source of natural bioactive compounds.

**Keywords:** Antioxidant, DPPH, Macroalgae, Polyphenol, _Sargassum_

1. **Introduction**

Oxidative stress and generation of Reactive Oxygen Species (ROS) are involved in pathophysiological process of various diseases such as certain cancers, arthritis, Parkinson’s disease, gastrointestinal diseases, Alzheimer’s disease and aging [1]. Natural antioxidants, compared to those synthetic antioxidants have attracted considerable attention by researchers and consumers since there is concern of synthetic antioxidants consumption due to their instability and possible activity as carcinogens [2]. Macroalgae or commonly known as seaweed is one of the most known sources for functional foods and pharmaceutical constituents with antioxidant activity [3]. As benthic organisms, macroalgae grows by attaching itself to a rocky or reef substrate in the intertidal zone [4]. The intertidal environment which provides unfavourable UV conditions which leads to the formation of free radicals and other strong oxidising agents. Hence, macroalgae is suggested to develops antioxidative defence systems to survive in such conditions. Previous studies have reported macroalgae to be enriched with various antioxidant compounds such as carotenoids, vitamins E and C, fucoxanthin, mycosporine-like amino acids, polysaccharides and polyphenols.
Antioxidative properties of macroalgae extracts have been studied in several geographic regions, but only a few studies have been performed on macroalgae species in tropical regions such as Indonesia. More information on the antioxidant activity of macroalgae inhibiting these tropical regions are needed, as these species are expected to develop a very effective antioxidant defence system due to the strong UV radiation in the tropical environment. Previous studies have demonstrated that UV radiation increases antioxidant defense in macroalgae. In the West Lombok coast there are numerous brown macroalgae Sargassum species exposed to high solar irradiation and they have never been studied for their antioxidant activities. Additionally, brown macroalgae are known to contain several potential antioxidants, such as fucoxanthin, carotenoids, and tocopherols [5].

The present study evaluated the antioxidative potential of 4 commonly found Sargassum species in Batu Layar coast of Lombok island by measuring the antioxidant activity and correlate with total content of phenol and flavonoid compounds in ethanolic extracts. The antioxidant activities were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.

2. Materials and Methods

2.1. Preparation of Sargassum species ethanol extract

All 4 Brown macroalgae Sargassum species (Sargassum crassifolium, Sargassum cristaefolium, Sargassum aquilifolium, Sargassum polycystum) were collected from Batu Layar Coast of Lombok island (8°29′54.251″ S and 116°4′36.664″ E). The macroalgae were identified regarding the algae electronic database. After collection, the samples were directly rinsed with seawater to remove sand debris. Samples were then dried under room temperature conditions for 5 days without direct exposure to sunlight. Macroalgae dry samples were ground and dissolved in absolute ethanol solvent 5x volume of the sample weight (w/v). Mixed solutions were incubated at room temperature for the 48h maceration process followed by stirring every 6 hours. After 24 hours, the mixed solutions were filtered with Whatman grade 1 filter papers. Finally, macroalgae extracts were subjected to the rotary evaporator (Sanjing, China) to evaporate the remaining ethanol solvents. Obtained filtrates were then used as macroalgae ethanol extracts for further experiments.

2.2. Determination of the total phenolic content

The total phenolic contents of macroalgae Sargassum extracts were determined using the Folin and Ciocalteu reagent, following the method described by Singleton and Rossi with slight modifications [6]. Sample and standard readings were made using a UV-Vis Spectrophotometer (Multiskan GO, Thermoscientific) at 765 nm against the reagent blank. The test sample (0.2 mL) was mixed with 0.6 mL of distilled water (dH2O) and 0.2 mL of Folin-Ciocalteu’s phenol reagent (1:1). After 5 min, 1 mL of saturated sodium carbonate solution (8% w/v in dH2O) was added to the mixture and the volume was topped up to 3 mL with dH2O. The reaction was kept in the dark for 30 min and the absorbance of blue color from different samples was measured at 765 nm. The phenolic content was calculated as gallic acid equivalents per gram of dry macroalgae material (mg GAE g\(^{-1}\)) on the basis of a standard curve of gallic acid (5-500 µg/mL, \(Y = 0.0037x + 0.1102; \ R^2 = 0.994\)). All determination were carried out in triplicate.

2.3. Determination of the total flavonoid content

The total flavonoid content of macroalgae Sargassum extracts were determined with the aluminium chloride colorimetric method [7]. The standard quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 mL methanol, then the standard solutions of quercetin were prepared by serial dilutions with methanol (5 – 500 µg/mL). An amount of 0.6 mL diluted standard quercetin solutions or extracts was seperately mixed with 0.6 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 420 nm wavelength with UV-Vis Spectrophotometer (Multiskan GO, Thermoscientific). The concentration of total flavonoid content in the tested samples was calculated from the calibration plot (\(Y = 0.0031x +\))
0.1388; \( R^2 = 0.9825 \) and expressed as quercetin equivalent per gram of dried macroalgae material (mg QE g\(^{-1}\)). All the determinations were carried out in triplicate.

2.4. DPPH free radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined according to the method of Zhang et al., (2007) with slight modifications [8]. Briefly, 100 µL of each \textit{Sargassum} extract at various dilutions, were mixed with 100 µL of 200 µM DPPH solution (Sigma Aldrich). The mixture was vortexed for 30 min in dark and then, the absorbance was measured at 517 nm in an automated microplate reader (Multiskan GO, Thermoscientific). The antioxidant capacity was calculated using the following equation:

\[
\text{% Inhibition} = \left( \frac{A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}})}{A_{\text{control}}} \right) \times 100
\]

Where the \( A_{\text{control}} \) is the absorbance of the control (DPPH without sample), the \( A_{\text{sample}} \) is the absorbance of the test sample (the sample test and DPPH solution), and the \( A_{\text{blank}} \) is the absorbance of the sample blank (Sample without the DPPH solution). The half-maximal inhibitory concentration (IC\(_{50}\)) was calculated by linear regression analysis and expression as mean of three determinations. Gallic acid was used as positive control.

2.5. Statistical Analysis

Statistical analyses were conducted using GraphPad with a two-tailed unpaired Student’s t-test and one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test. The data are presented as means ± standard deviation (SD). Differences among comparisons were considered statistically significant for \( p \)-values less than 0.05.

3. Results and Discussion

3.1. Antioxidant activity of \textit{Sargassum} species

Results for the DPPH assay, calculated as IC\(_{50}\) (µg/mL), are shown in Table 1. The values between species were not significant. However among all species, the lowest IC\(_{50}\) value, which means the highest radical scavenging activity, was observed in both \textit{S. cristaefolium} and \textit{S. crassifolium}. Overall the antioxidant activity of \textit{Sargassum} species tested from strongest to weakest based on IC\(_{50}\) value is \textit{S.cristaeofolium}, \textit{S. crassifolium}, \textit{S. polycystum}, and \textit{S. aquifolium}. Brown macroalgae \textit{Sargassum} are well known to exhibit strong antioxidant activity due to the high content of phlorotannins [9]. Similar total phenolic content and DPPH antioxidant activity was observed in \textit{Sargassum horneri} ethanol extract [10].

| Sample                | Total Phenol Content (GAE/g) | DPPH IC\(_{50}\) (µg/mL) |
|-----------------------|----------------------------|--------------------------|
| \textit{Sargassum cristaefolium} | 66.13 ± 5.62               | 737.30 ± 23.46           |
| \textit{Sargassum aquifolium}    | 39.83 ± 2.10               | 828.23 ± 27.24           |
| \textit{Sargassum polycystum}    | 38.93 ± 6.03               | 804.30 ± 30.82           |
| \textit{Sargassum crassifolium}  | 52.90 ± 2.87               | 767 ± 40.35              |
| Gallic acid            | 16.56 ± 2.67               | 16.56 ± 2.67             |

3.2. Correlations between the antioxidant activity and total phenol content

The correlation of total phenolic content with DPPH is shown in Table 2. A significant correlation was observed. Previous reports have also suggested that there is a significant correlation between total phenolic content and antioxidant activity of plant extracts. The high correlation confirms the role of phenolic compounds as the main contributor to the antioxidant activities of the \textit{Sargassum} species ethanol extracts. The genus \textit{Sargassum} is known to produce interesting phenolic compounds of high molecular weight, 5000 and 14,000 DA which demonstrated biological activities such as antioxidant, antiproliferative, antiangiogenesis, and antifouling [11].
### Table 2. Correlation between total phenolic content and DPPH radical scavenging activity of *Sargassum* ethanol extracts.

| Assay                                      | Correlation R² phenolics |
|--------------------------------------------|--------------------------|
| IC₅₀ of DPPH radical scavenging activity   | 0.9002**                 |

### 4. Conclusion

In conclusion, the ethanolic extracts of four different *Sargassum* species which contains large amount of phenolic compounds, exhibit high antioxidant activity. Among all species, *Sargassum cristaefolium* and *Sargassum crassifolium* show stronger antioxidant activity compared to *Sargassum polycystum* and *Sargassum aquifolium*. There are also limitations to this study such as other extraction methods should be evaluated to possibly increase its antioxidant activity. Furthermore, other antioxidant assays should also be used to compare the antioxidant activities between the *Sargassum* species. Nevertheless, brown macroalgae *Sargassum* species show promising antioxidant activity in correlation with its high amount of phenolic content.

### Acknowledgments

We thank the Indonesian Ministry of Research, Technology, and Higher Education for funding this research through the International Collaborative Research Grant scheme (LN18/2020).

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