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Biological activities of macroalgae in the Moudulung waters: bioactive compounds and antioxidant activity

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Abstract

This study aimed to determine the bioactive compounds and antioxidant activity of macroalgae in Moudolung Waters. The bioactive compounds in seven samples of macroalgae were extracted with methanol. Analysis of macroalgae bioactive compounds was carried out qualitatively by determining the presence of bioactive compounds including alkaloids, flavonoids, saponins, tannins, phenol hydroquinone, and steroids/ terpenoids. antioxidant activity was carried out using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method. Data were analyzed descriptively using Microsoft Excel and all data are expressed as mean. The results showed that these seven macroalgae species have bioactive compounds such as alkaloids, flavonoids, saponins, tannins, phenol hydroquinone, and steroids/terpenoids which function as antioxidants. The highest antioxidant activity was the MA6 (*Turbinaria ornate*) followed by MA1 (*Hormopysa triquetra*), MA4 (*Ulva flexuosa* syn. Enteromorpha flexuosa), MA5 (*Eucheuma spinosum*), MA2 (*Sargassum muticum*), MA3 (*Gracilaria corticate*), and MA7 (*Ulva reticulata*) were 75.25 mg/mL, 90.31 mg/mL, 134.52 mg/mL, 200.22 mg/mL, 216.91 mg/mL, 259.16 mg/mL, and 270.42 mg/mL, respectively. The results suggested that *Turbinaria ornate* and *Hormopysa triquetra* could be used as an important substitute for functional ingredients in foods and pharmaceuticals or nutraceuticals.

1. Introduction

Macroalgae are classified into three main classes, Phaeophyceae (brown algae), Chlorophyceae (green algae), and Rhodophyceae (red algae) (Osório *et al*., 2020). Macroalgae is one of the main components that make up coastal ecosystems that play a role in maintaining the balance of marine ecosystems (Robin *et al*., 2017; Pinteus *et al*., 2018; Van der Loos *et al*., 2019; Meiyasa *et al*., 2020; Tarigan, 2020). Macroalgae have high economic value because they can be applied in aquaculture and biotechnology (Wei *et al*., 2013). Moreover, macroalgae contain about 50% carbohydrates, 1-5% lipids, 10-47% proteins, 8-40% minerals, phenolic compounds about 25%, peptides, enzymes, and vitamins (Beaulieu *et al*., 2015; Biris-Dorhoi *et al*., 2020). This composition varies depending on the species, as well as the season and location of harvest (Suwal *et al*., 2019; Chankaew *et al*., 2021). Macroalgae are also known to have sources of secondary metabolites (Pereira *et al*., 2017). These secondary metabolites include phenolic acids, flavonoids, tannins, saponins, steroids, triterpenoids, alkaloids, and hydroquinones (Akremi *et al*., 2017; Maheswari *et al*., 2018; Pradhan *et al*., 2021; Widowati *et al*., 2021; Kumar, 2021). Sources of these metabolites can act as antioxidants, antimicrobials, anti-inflammatory, antitumor, anticancer, antiviral, and antidiabetic (Maharany *et al*., 2017; Nufus *et al*., 2017; Čagalj *et al*., 2021; Pradhan *et al*., 2021). The antioxidants possessed by these macroalgae play an important role in the food (Pereira *et al*., 2017; Nufus *et al*., 2019), cosmeceutical (Nurjanah *et al*., 2016; Nurjanah *et al*., 2017; Nurjanah, Abdullah, Fachrozan *et al*., 2018; Nurjanah, Aprilia, Fransiskayana *et al*., 2018; Nurjanah, Fauziyah and Abdullah, 2019; Nurjanah *et al*.,

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2019; Sari *et al*., 2019; Dolorosa *et al*., 2020; Nurjanah, Jacoeb, Bestari *et al*., 2020; Nurjanah Suwandi, Anwar *et al*., 2020), nutraceutical (Nurjanah, Abdullah and Diachanty, 2020), and pharmaceutical fields (Lomartire and Gonçalves, 2022). It is known that antioxidant activity is strongly influenced by bioactive components produced by macroalgae (Arguelles, 2021).

Several researchers have reported that macroalgae have the potential as a source of antioxidants. As reported by Kokilam *et al*. (2013), the macroalgae such as *Heterocapsa triquetra, Sargassum wightii, Padina tetrastromatica,* and *Chnoospora minima* have antioxidant activity (85.08%, 69.31%, 61.04%, and 46.91%, respectively). In addition, the other species such as *P. tetrastromatica, Gracilaria corticata* and *G. edulis* also have been reported to have antioxidant activity (50%, 23.95%, 20.32%, respectively) and antimicrobial activity against several pathogenic bacteria such as *Vibrio cholerae, Shigella flexneri, Pseudomonas aeruginosa*, and *Bacillus subtilis* (Maheswari *et al*., 2018; Arulkumar *et al*., 2018).

Several macroalgae species were also reported to have antioxidant activity such as *P. tetrastromatica* (25.25 g/mL; Naw *et al*., 2020), *Sargassum ilicifolium* (15.78 g/mL; Arguelles, 2021), *Caloglossa beccarii* DeToni (0.178 mg/mL; Chankaew *et al*., 2021), *Ulva rigida* (2.4 mg/mL; Dimova *et al*., 2021), *Sargassum polycystum* (3.83 mg/mL; Neoh *et al*., 2021), and *Padina gymnospora* (369.99 mg/mL; Rahman *et al*., 2021). In addition, they also have the potential to be antidiabetic, anti-inflammatory, and antimicrobial properties against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Naw *et al*., 2020; Arguelles, 2021; Fuente *et al*., 2021; Pradhan *et al*., 2021).

These studies show that macroalgae have the potential as antioxidants and can be used as functional foods, as in the nutraceutical and pharmaceutical fields. However, the distribution of macroalgae in the waters of East Sumba is very little reported. In a previous study, the macroalgae species in Moudolung waters have been identified with a total of fifteen species (Meiyasa *et al*., 2020). However, information about the bioactive compounds and antioxidant activity of macroalgae scattered in East Sumba, especially Moudolung waters, has not been reported. Therefore, this study aimed to determine the bioactive compounds and antioxidant activity of several macroalgae in Moudolung waters.

2. Materials and methods

2.1 Macroalgae collection

Seven species of macroalgae (*Hormophysa triquetra, Sargasum muticum, Gracilaria corticate*, *Ulva flexuosa* syn. *Enteromorpha flexuosa*, *Eucheuma spinosum*, *Turbinaria ornate*, and *Ulva reticulata*) were collected from Moudolung waters in East Sumba Regency. The seven samples were obtained in August 2020 (in the dry season, with latitude -9.5446387 and longitude 120.2223472,15).

2.2 Identification of macroalgae

Macroalgae identification was carried out based on the type of macroalgae obtained from each station survey. Samples were taken at three stations, namely station I (sand station), station II (seagrass), and station III (rocky/rocky) using a cruising survey with a sampling area of approximately 50×50 m. The sample is then washed, put in clear plastic, stored in a cool box, and brought to the laboratory for identification. Identification using the macroalgae identification book according to the website http://www.algaebase.org (Guiry and Guiry, 2021).

2.3 Preparation of macroalgae extracts

Preparation of macroalgae extracts according to Gazali *et al*. (2018). Macroalgae samples were dried in the sun for 3 days. The dried sample was mashed using a blender until it became a dry simplisia powder. The simplisia was weighed as much as 200 g and put into an Erlenmeyer glass. The samples were extracted with methanol in a ratio of 1:5 (w/v) in a conical flask for 36 hrs. All the supernatants were collected together and then the solvent was removed by a rotary evaporator. The filtrate thus obtained was allowed to concentrate and stored for further studies.

2.4 Analysis of bioactive compounds

The phytochemical screening of different macroalgae extracts was assessed by the standard method as described by Harborne (1998), to identify the major natural chemical groups such as alkaloids, flavonoids, saponin, tannin, phenol hydroquinone, and steroid/terpenoid.

2.5 Antioxidant assays

The antioxidant activity of each seaweed extract and fraction were determined by using DPPH radical scavenging assay according to the method of Gazali *et al*. (2018). The crude seaweed extract samples were dissolved in methanol with concentrations of 10, 20, 30, 40, 50 ppm, respectively. Ascorbic acid was used as a positive control with concentrations of 1, 2, 3, 4, and 5 ppm. Macroalgae extract samples were absorbed in a wavelength of 517 nm using a UV-Visible spectrophotometer. The percentage of free radical activity inhibitor was obtained from the absorbance

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value of the sample. The regression equation was obtained from the relationship between the sample concentration and the percentage of inhibition of free radical activity. The antioxidant capacity of the sample to scavenge 50% of DPPH radical activity is expressed as IC_{50} value. This value was calculated using a linear regression equation. The IC_{50} value is obtained by entering y = 50 and the known values of A and B. Meanwhile, the antioxidant activity of each sample is expressed as the percentage of free radical inhibition calculated by the equation:

$$(\%)\ \text{Inhibition} = \left[1 - \frac{A_{\text{Sample}} - A_{\text{Blannk}}}{A_{\text{Control}} - A_{\text{Blank}}}\right] \times 100$$

Since the percentage of radical inhibition is dependent on the DPPH concentration and may vary among studies, it is necessary to express the antioxidant capacity in the form of the Antioxidant Activity Index (AAI). The AAI is obtained by the following equation:

$$\text{AAI} = \frac{\text{final concentration of DPPH } (\mu g/mL)}{IC_{50}\ (\mu g/mL)}$$

2.6 Data analysis

Data were analyzed descriptively using Microsoft Excel and all data are expressed as mean.

3. Results and discussion

3.1 Distribution of macroalgae in Moudolung waters, East Sumba Regency

Moudolung waters are located in East Sumba Regency, East Nusa Tenggara. This aquatic territory is known as one of the locations with a high distribution of macroalgae. The previous study revealed there are fifteen species of macroalgae found in this area. These macroalgae species are mostly found in dead coral substrates. The high abundance of macroalgae in this area correlates to the quality of the water in this territory. The physicochemical properties of the water in Moudolung area have been observed in our previous study. The water has an average temperature of around 29°C with the dissolved oxygen (DO) range between 7.7 -8.1 mg/L, and the pH between 8.30-8.40 (Meiyasa *et al*., 2020). According to Standar Nasional Indonesia (SNI) good quality water should have a temperature range of 25-30°C, pH 6.8-8.2, and DO > 3.0 (SNI 7904:2013). Therefore, the Moudolung water quality can be categorized as good. Usually, local people use these macroalgae as vegetables, but the information regarding the bioprospection of macroalgae in Moudolung waters has never been reported.

There are fifteen species of macroalgae from Moudolung waters have been successfully identified. The seven species are classified as brown algae i.e., *Hormophysa triquetra, Sargasssum muticum, Turbinaria ortic (Turner) J. Agardh, Sargassum plagyophyllum, Sargassum polycystum, Dictyota pinnatifida*, and *Padina australis*. The five species belong to the red algae i.e. *Gracilaria orticate, Eucheuma spinosum, Gracilaria corticate, C. Agaradh, Achanthopora spicifera*, and *Achanthopora muscoides*. Meanwhile, the last three species are classified as green algae i.e. *Ulva flexuosa, Ulva reticulate*, and *Ulva compressa* L. (Table 1).

The fifteen species were re-selected based on the abundance of macroalgae populations (Meiyasa *et al*., 2020) and then tested for their bioactive compounds and antioxidant activity. These species are *Hormopysa triquetra* (Figure 1), *Sargasum muticum* (Figure 2), *Gracilaria corticate* (Figure 3), *Ulva flexuosa* syn. *Enteromorpha flexuosa* (Figure 4), *Eucheuma spinosum* (Figure 5), *Turbinaria ornate* (Figure 6), *Ulva reticulate* (Figure 7).

3.2 Bioactive compounds of macroalgae from Moudolung waters

Bioactive components are non-nutritive chemical compounds from plants that have a protective or preventive effect against disease. El-Din and El-Ahwany (2016); and Cyril *et al*. (2017) also explained that this bioactive compound has a broad spectrum of functional bioactivity, including antioxidant, antibacterial, antiviral, antifungal, antifouling, and anti-inflammatory. In this study, the seven species of macroalgae from Moudolung waters were tested qualitatively through phytochemical screening to detect the presence of the bioactive components they contain.

Table 1. Classification of distribution of macroalgae in Moudolung waters, East Sumba Regency

Brown algae (Phaeophyta)	Red algae (Rhodophyta)	Green algae (Chlorophyta)
1. *Hormophysa triquetra*	1. *Gracilaria corticate*	1. *Ulva flexuosa*
2. *Sargasssum muticum*	2. *Eucheuma spinosum*	2. *Ulva reticulate*
3. *Turbinaria ornata (Turner) J. Agardh*	3. *Gracilaria salicornia C. Agaradh*	3. *Ulva compressa L.*
4. *Sargasum plagyophyllum*	4. *Achanthopora spicifera*	
5. *Sargassum polycystum*	5. *Achanthopora muscoides*	
6. *Dictyota pinnatifida*		
7. *Padina australis*		

Source: Meiyasa *et al*. (2020)

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Figure 1. *H. triquetra*

Figure 2. *S. muticum*

Figure 3. *G. corticate*

Figure 4. *U. flexuosa*

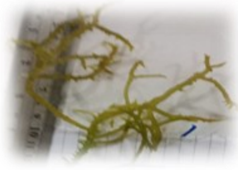

Figure 5. *E. spinosum*

Figure 6. *T. ornate*

Figure 7. *U. reticulate*

The results showed that the macroalgae obtained from Moudolung waters have different content of bioactive compounds. Based on this study, all Moudolung macroalgae samples contain bioactive compounds such as alkaloid, saponin, and steroid or terpenoid. Moreover, the phenol hydroquinone was detected in all samples except for the MA1 sample. Meanwhile, flavonoid was only detected in MA4 and MA7 samples. Then, tannin was only detected in MA1, MA4, and MA5 samples (Table 2). This data suggests that the three groups of bioactive compounds (i.e. alkaloid, saponin, and steroid or terpenoid) are the major compounds that might have an important role in the biological activity of macroalgae in this study. It was further reported that the MA4 sample identified as *Enteromorpha flexuosa* was the only extract that showed positive results for all test parameters (Table 2). The *E. flexuosa* (MA4 sample) also contain the highest flavonoids, qualitatively. On the other hand, the *Turbinaria ornate* (MA6 sample) contains the highest saponins, phenolic hydroquinone, and steroids or terpenoids, respectively (Table 2). The bioactive compound of macroalgae from several Indonesian aquatic territories has been studied and reported. For example, *Ulva lactuca*, *Gracilaria* spp., and *Padina* sp. obtained from the waters of Sanur (Bali) have bioactive compounds such as phenols, flavonoids, and alkaloids (Julyasih and Wisiyanti, 2020). Hudaifah (2020) reported that macroalgae obtained from Kencono Source waters (East Java) with *Halimeda opuntia* species have bioactive compounds such as alkaloid, flavonoid, and triterpenoid, while *Ulva lactuca* has bioactive compounds such as alkaloid, flavonoid, triterpenoid, saponin, and tannin. In addition, Edison (2020) reported that macroalgae species *S. plagyophyllum* cultivated in the waters of Panjang (Yogyakarta) had bioactive compounds such as flavonoid, steroid/triterpenoid, saponin, alkaloid, and phenolic. Furthermore, Dolorosa *et al*. (2017) reported that samples of macroalgae obtained from Serang Waters (Banten) with *S. plagyophyllum* species had bioactive compounds such as alkaloid, steroid, flavonoid, saponin, and tannin. Meanwhile, *E. cottonii* has alkaloid and terpenoid bioactive compounds.

In alignment with this study's result, the variation in the number of bioactive components among macroalgae genera or species has been reported. There are some reports of the bioactivity of the *Enteromorpha* sp. extract. On *in vitro* tests, Narasimhan *et al*. (2013) discovered the methanolic extracts of seaweeds *Enteromorpha antenna*, *Enteromorpha linza*, and *Gracilaria corticata* possess high total phenolic content and show good free radical scavenging activity against nitric oxide, hydrogen peroxide, hydroxyl radicals, free radical scavenging (DPPH). They also possess FRAP (ferric reducing ability plasma) ability and reducing power. In another Enteromorpha species, for example,

Table 2. Qualitative data on the bioactive components of the seven macroalgae species from Moudolung waters

Bioactive compound	Samples						
	MA1	MA2	MA3	MA4	MA5	MA6	MA7
Alkaloids	++	++	++	+++	++	++	++
Flavonoids	-	-	-	++	-	-	+
Saponins	+	+	++	+	+	++	++
Tannins	+	-	-	+	+	-	-
Phenol Hydroquinone	-	++	+	+	+	++	+
Steroid or Terpenoids	+	+	+	+	+	++	+

MA1: Extract *Hormopysa triquetra*, MA2: *Sargasum muticum*, MA3: *Gracilaria corticate*, MA4: *Ulva flexuosa* syn. *Enteromorpha flexuosa*, MA5: *Eucheuma spinosum*, MA6: *Turbinaria ornate*, MA7: *Ulva reticulata*, (-): no detected, (+): weak, (++): moderate, (+++): strong

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Enteromorpha prolifera the main antioxidant compound was the chlorophyll compound, pheophorbide a, rather than phenolic compounds (Cho *et al*., 2011). This suggests that the variation of the antioxidant properties of macroalgae is determined by their phytochemical contents.

The phytochemical analysis content of several macroalgae including *Enteromorpha flexuosa* also has been done in another research. Research by Abirami and Kowsalya (2012) showed that carbohydrates, protein, gums and mucilage, phenols, and starch are present in several underexploited macroalgae i.e., *Acanthophora spicifera*, *Gracilari edulis*, *Padina gymnospora*, *Ulva fasciata* and *Enteromorpha flexuosa*. Meanwhile the alkaloids were absent in all the selected seaweeds, phenols were found to be plentiful, and quinones were absent in the brown seaweed like *P. gymnospora* and green seaweed such as *U. fasciata, E. flexuosa* (Abirami and Kowsalya, 2012).

Pramitha and Sree Kumari (2016) reported that the presence of alkaloids, phenolic, and flavonoid compounds and tannins in macroalgae could act as antioxidants, antimicrobials, anti-inflammatory, and anticarcinogenic. More specifically, flavonoids have been known to be effective scavengers for oxidative compounds, such as singlet oxygen and free radicals, which are associated with disease-causing agents. Meanwhile, alkaloids are known to have a broad spectrum of pharmacologic activity, including anticancer and antibacterial agents. Another bioactive compound class, Saponins, also have various biological activities, including antioxidants, antimicrobials, anticancer, and lowering cholesterol levels (Güçlü-Üstündağ and Mazza, 2007). In plants, the Saponins act as protective agents against pathogen invasion, which effectively prevents several diseases. Therefore, these compounds have a healing role and may act as a natural medicine (Nithya *et al*., 2016).

3.3 Antioxidant activity and antioxidant activity index macroalgae methanol extract from Moudolung waters

The antioxidant potential and bioactivity of the seven methanol extracts of macroalgae species from Moudolung waters were evaluated using the DPPH radical scavenging method, which then represented the value of IC_{50} and antioxidant activity index (AAI) is a method to standardize antioxidant test results based on the DPPH method. According to Scherer and Godoy (2009), poor antioxidant activity when AAI < 0.5 moderate antioxidant activity when AAI is between 0.5 and 1.0, strong antioxidant activity when AAI is between 1.0 and 2.0, and very strong when AAI > 2.0. Most of the studies related to antioxidants express their results in IC_{50} values, which were defined as the concentration of extract needed to reduce as much as 50% of the initial concentration of DPPH radical compounds (Firdaus, 2013). The result of this study is shown in Table 3.

Based on the DPPH radical scavenging test, seven species of macroalgae from Moudolung waters had an average IC_{50} value of 75.25–270.42 mg/mL, where the IC_{50} values were from the lowest to the highest were in samples MA6, MA1, MA4, MA5, MA2, MA3, and MA7, respectively (Table 3). This study indicates that the *Turbinaria ornate* (MA6) sample has the strongest antioxidant activity compared to the other six samples because, at a lower concentration, the MA6 extract has a higher ability to reduce radical agents than other types of macroalgae. This is also supported by data on bioactive components (Table 2) which shows that the *Turbinaria ornate* (MA6) sample contains the most saponins, phenolic hydroquinone, and steroids or terpenoids compared to the other six extracts and that the MA6 extract has better radical scavenging ability than the other six samples. According to our experiments, the AAI value of the standard ascorbic acid is still higher than Moudulung macroalgae sample (Table 3). These results suggest that the Moudulung macroalgae sample is less effective in scavenging DPPH radicals compared to a commercial antioxidant.

Table 3. IC_{50} value of methanol extract of seven macroalgae species from Moudolung waters

Samples	IC_{50} value (mg/mL)	AAI
MA1	90.31±1.71	0.55
MA2	216.91±28.79	0.23
MA3	259.16±14.29	0.19
MA4	134.52±3.28	0.37
MA5	200.22±28.78	0.25
MA6	75.25±1.95	0.66
MA7	270.42±35.20	0.18
Ascorbic Acid Standard	8.56±1.08	

MA1: Extract *Hormopysa triquetra*, MA2: *Sargasum muticum*, MA3: *Gracilaria corticate*, MA4: *Ulva flexuosa* syn. *Enteromorpha flexuosa*, MA5: *Eucheuma spinosum*, MA6: *Turbinaria ornate*, MA7: *Ulva reticulata*

The high antioxidant activity in the sample of macroalgae extract is thought to be due to the presence of phenolic components contained. The high scavenging ability is related to the hydroxyl group present in phenolic compounds (Gazali *et al*., 2018). Moreover, the lower IC_{50} value indicated the more effective the antioxidant in reducing DPPH radical compounds through the hydrogen atom donor (H) mechanism (Sarini *et al*., 2014; Zulfafamy *et al.,* 2018). Molyneux (2004) reported that based on the IC_{50} value, the activity of extracts of natural ingredients was categorized as follows: active (≤20 mg/mL); moderate (>20-100 mg/

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mL), weak (>100-1000 mg/mL); and inactive (>1000 mg/mL). Thus, the antioxidant activity of macroalgae extract samples in this study could be classified at moderate levels (*Turbinaria ornate* and *Hormopysa triquetra*) and weak (*Sargasum muticum, Gracilaria corticata, Ulva flexuosa* syn. *Enteromorpha flexuosa, Eucheuma spinosum*, and *Ulva reticulata*).

These results are supported by the previous study done by Deepak *et al*. (2017). The study of Deepak *et al*. (2017) on hexane and aqueous extract of *T. ornate* revealed the DPPH radical scavenging with IC_{50} values (mg/mL) of the hexane-extract, aqueous-extract and ascorbic acid were: 45.38, 116.50, and 21.31, respectively. Further, Deepak *et al*. (2017) work also revealed the FRAP ability and antiproliferative activity from both hexane and aqueous extracts of *T. ornate.* The phytochemicals screening done by Deepak *et al*. (2017) on *T. ornate* extract detected the main components including flavonoid, saponin, alkaloid, and fixed oil and fat which are comparable to our results.

Scherer and Godoy (2009) reported that in determining the IC_{50} value if different DPPH concentrations were used, it could give different results even though the samples tested were the same. Therefore, in this study, it is necessary to calculate the AAI, which will relate the value of the DPPH concentration used in the test and the resulting IC_{50} value. This aims to obtain consistent data on each test sample and minimize variables that might affect the results (such as different extraction methods and sampling locations). The results showed that the seven samples of macroalgae methanol extract from Moudolung waters had relatively different AAI values between samples. The AAI values obtained in this study ranged from 0.18–0.66 (Table 3). The strongest antioxidant activity was indicated by the highest AAI value, which was found in the MA6/*Turbinaria ornate* sample.

Scherer and Godoy (2009) explained that based on the AAI value, the antioxidant activity of an extract/ compound could be categorized into several levels, namely very strong (> 2.0); strong (1.0–2.0); moderate (0.5–1.0); and weak (<0.5). The samples of the macroalgae methanol extract were classified as moderate antioxidants, and others were classified as weak based on the research data. Some of them classified as moderate are *Turbinaria ornate* and *Hormopysa triquetra*, while the other five, *Sargasum muticum, Gracilacorticate, Ulva flexuosa syn. Enteromorpha flexuosa, Eucheuma spinosum*, and *Ulva reticulata* are classified as weak.

Determination of antioxidant bioactivity of macroalgae species from other water areas using the DPPH radical scavenging assay has been reported. Gazali *et al*. (2018) reported that the n-hexane fraction of *Sargassum* sp. found in the waters of Lhok Bubon, West Aceh, has an IC_{50} of 148.16 mg/mL. However, Firdaus (2013) reported that the methanol fraction of *Sargassum aquifolium* obtained from the waters of Talango Island Sumenep had an IC_{50} was 66.16 mg/mL. Another study reported that the methanol fraction of *Turbinaria conoides* obtained at waters of Pasauran and *E. cottonii* from Lontar Serang had an IC_{50} were 15.15 mg/mL and 23.15 mg/mL, respectively (Yanuarti *et al*., 2017). In addition, Diachanty *et al*. (2017) reported that the ethanol fractions of *Sargassum polycystum, Padina minor*, and *Turbinaria conoides* obtained from the waters of the Thousand Islands had an IC_{50} were 3.4 mg/mL, 1.9 mg/mL, and 9.6 mg/mL, respectively. According to Mellouk *et al*. (2017) and Øverland *et al*. (2019), the variation in the bioactive components of macroalgae is naturally influenced by the type of macroalgae, harvest time, growing habitat, and environmental conditions (fluctuations in environmental changes, nutrient content, intensity of light/ultraviolet radiation, temperature, osmotic pressure, salinity, desiccation, and contamination of pathogens). On the other hand, bioactivity testing methods and extraction variables (type and volume of solvent, technique, temperature, and extraction time used) will also provide information on the performance of different activities (Pedro *et al*., 2016; Zulfafamy *et al*., 2018).

Based on the research, the seven methanol fractions of macroalgae from Moudolung waters contain several potentially bioactive compounds that play protective agents against free radical compounds through the radical scavenging mechanism, especially in the MA6 (*Turbinaria ornate*) and MA1 (*Hormopysa triquetra*) samples which have more robust anti-radical activity than other types of macroalgae. This indicates that the macroalgae found in the Moudolung waters are very prospective to be developed in the food industry (food ingredients, processed food, and functional food) and non-food (pharmaceuticals and cosmetics).

4. Conclusion

The bioactive compounds through phytochemical screening showed that alkaloids, saponins, steroids, or terpenoids were the main compounds found in all test samples. However, the MA6 (*Turbinaria ornate*) sample was an extract containing the most saponins, phenolic hydroquinone, and steroids or terpenoids compared to the other six extracts. The MA6 (*Turbinaria ornate)* extract had the lowest IC_{50} (75.25 g/mL), the highest IAA value (0.66), and the highest antioxidant activity indicating the strongest radical scavenging ability among all test samples. Thus, macroalgae scattered in Moudolung waters are very prospective to be developed in the food and non-food industries.

Conflict of interest

The authors declare no conflict of interest.

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