The screening of bioactive compound of the green algae *Halimeda macroloba* (Decaisne, 1841) as an antioxidant agent from Banyak Island Aceh Singkil

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Abstract. Banyak Island is one of the outest island in western Indonesia which high biodiversity particularly macroalgae. One of the macroalgae which have potential food and herbal drug was *Halimeda macroloba*. The aim of this study was to screen the bioactive compound of the *H. macroloba* as antioxidant source. The sampling of *H. macroloba* were located at the coast of Banyak Island, Aceh Singkil. The research stages including the chemical composition, extraction, phytochemical screening, the antioxidant assays (DPPH, CUPRAC and FRAP). The result showed the highest rendement content was obtained from ethanol extract as much as 2.32%. Subsequently, the extract of the *H. macroloba* detected phenol compound. The antioxidant activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) method were obtained IC50 121.445 ± 1.03 mg/L, the n-hexane extract have IC50 181.945 ± 1.95 mg/L and ethyl acetate extract of IC50 228.67 mg/L. The antioxidant with ferric reducing antioxidant power (FRAP) method showed that the ethanol extract possesses the value of antioxidant capacity was 516.50±0.70 µmol trolox/g extract, ethyl acetate extract value was 482.00±1.41 µmol trolox/g extract and n-hexane extract was 323.50±0.70 µmol trolox/g extract. While, the CUPRAC (cupper ion reducing antioxidant capacity) with ethanol extract was 159.85±0.70 µmol trolox/g extract, ethyl acetate extract was 66.38±0.03 µmol trolox/g extract and n-hexane extract was 49.15±0.035 µmol trolox/g extract. The results from three methods of antioxidant showed the ethanol extract possesses high antioxidant capacity. Therefore, this research give fruitfull information regarding the potential green alga *H. macroloba* as new antioxidant sources for human health.

1. Introduction

Macroalgae plays an important role in the coastal ecosystem that provide various food and herbal drug sources. In revolution of 4.0 Industry, various diseases that caused by pollutant sources that derived from the air, water, and other sources. In Globalization era, the millenial generations have lack of movement in their metabolism that caused their antibody to be weak. Thus, we have already been explored and isolated the bioactive compound from the marine biota. One of the marine biota that possesses an antioxidant activity were macroalgae from Banyak Island, Aceh Singkil. Banyak Island is one of the outest of western Indonesia that administrative in Aceh Province with high
marine biodiversity. The bioactivity exploration of macroalgae from along the South West of Aceh coastal area including green algae (*Chaetomorpha crassa*) [1], brown algae (*Sargassum* sp) [2], green algae (*Chaetomorpha antennina*) [3], brown algae (*P. australis*) [4], green algae (*Udotea* sp) [5]. The exploration of the bioactive compound from *H. macroloba* from Banyak Island Aceh Singkil has not yet investigated. Therefore, our research regarding the screening of the bioactive compound of the green alga *H. macroloba* as an antioxidant sources was highly recommended. Humans are impacted by a lot of free radicals both from inside our body and surrounding environments, particularly reactive oxygen species (ROS). Furthermore, free radical also produced by several other pathwayss (exogenous sources) ionizing radiation, UV light, cigarette smoke, industrial waste and pollutants. The free radicals can be scavanged by antioxidant present in tissue ([6];[7]. Natural antioxidant are found in vegetables, fruits and a variety of other foods [8]. Antioxidant has a potential to prevent cancer and cardiovascular diseases. The nature of these substances varies a lot whereas the most powerful antioxidant are polyphenols, phycobiliproteins, vitamin C, α-tocopherols and some carotenoids (xanthophylls). Furthermore, the macroalgae contains a high concentration of polysaccharides of various structure and functionality. The indigestible polysaccharides of macroalgae could be important sources of dietary fibres which may potentially be exploited as prebiotic for applications in both human and animal health [9]. Among the most relevant in macroalgae are antioxidant which have attracted major interest due to their positive effects in human’s body. The beneficial effect of antioxidant are due to their capacity to scavenge and neutralize reactive oxygen species (ROS) [10]. An excessive ROS production can cause oxidative damage to biomolecules such as proteins, lipids and DNA [11]. Antioxidant may reduce ROS production by scavenging free radicals through various mechanism [12]. The antioxidant system from macroalgae that interact with environmental stresses, and produce bioactive compounds including polyphenols, alkaloids, terpenes, phycocyanins, carotenoids, and various enzymes [13]. Antioxidants are effective in protecting living organisms against oxidative damages that caused by Reactive Oxygen Species (ROS). Therefore, commercial antioxidants have been in high prospect in global market. But, most of them are synthesised *butylated hydroxyanisole* (BHA), *butylated hydroxytoluene* (BHT) and *propyl gallate* (PG). However, these synthetic antioxidants have already been contained carcinogenic matter [14]. Thus, we did exploration natural antioxidant from marine biota that have potential antioxidant sources are macroalgae which are distributed in the alongside of intertidal zone with various substrats including sandy, rocky, and muddy. Recently, macroalgae have also attracted by many scientist for searching the natural antioxidants to develop functional food ingredients and new sea drugs. The aims of this research was to screen the bioactive compound of green alga *H. macroloba* as an antioxidant from Banyak Island Aceh Singkil. In addition, we has not yet found this journal publication regarding the potency of green algae *H. macroloba* from Banyak Island Aceh Singkil.

2. Material and Methods

2.1. Sampling

Our samples were collected from the coast of Banyak Island, Aceh Singkil in July 2017 (Figure 1). Then, these samples were immediately washed with seawater to remove the foreign particles and others. Then, it was put in an ice box and brought to the Laboratory of Fisheries, Teuku Umar University for identifying the species [15].
2.2. The proximate analysis
The chemical composition are consist of the water content, ash content, carbohydrate, protein, fatty acid was determined based on proximate analysis with AOAC method [16].

2.3. Preparation and extraction
The wet samples were dried on sunlight for ± 2 days. The samples were minced and milled by using blender until to be simplisia powder. The simplisia were weighed as much as 125 g and put it in the erlenmeyer glass. The maceration was undertaken with ratio 1:3 by using ethanol, ethyl acetate and n-hexane respectively and was soaked for 3x24 hours. The submersion was functioned for taking out organic compound from simplisia. The solution was filtered used filter paper Whatman 25 and concentrated in vacuum rotary evaporator until dry extract obtained.

2.4. Phytochemical screening
The phytochemical assays are consist of alkaloids, steroid and triterpenes, anthraquinones, flavonoids, saponins, cyanogenic glycosides, cardiac glycosides and tannins followed the methods described previously [17].

2.5. The DPPH (1,1-diphenyl-2-picrylhydrazyl radical) Assay
The antioxidant activity assay used 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) method according to [Chan et al., 2007]. The crude extract samples were diluted respectively in n-hexane, ethyl acetate, and ethanol solvents with concentration 10, 20, 30, 40, and 50 ppm. Vitamin C was used as positive control with concentration 1, 2, 3, 4 and 5 ppm. The antioxidant activity in respectively samples were stated with percentage of free radical inhibition.

2.6. The CUPRAC Assay [19]
1 ml of extract was diluted in ethanol 96% and added 1 mL of CuCl$_2$H$_2$O 0.01 M; 1 ml ethanolic neocuproine 0.0075 M; 1 ml of the buffer of acetate ammonium pH 7 1 M; and 0.1 ml of aquadest. The
solution were incubated for 30 minutes and measured their absorbance in 453.4 nm. The blank was used the mix solution without extract. The standard calibration curve of trolox with several concentration. The antioxidant capacity was stated in µmol troloks/g of dried powder.

2.7. FRAP Assay
Antioxidant evaluation using Ferric Ion Reduction Antioxidant Power (FRAP) Assay method was conducted according to [Benzie et al. 2002]. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6- Tripyridyl-s-Triazine (TPTZ) solution in 40 mM HCl; and 20 mM FeCl$_3$.6H$_2$O in the ratio 10 : 1 : 1. As much as 150 µl of extract were added 4.5 ml of FRAP reagent and then incubated for 30 minutes in temperature 30 °C and measured its absorbance in 598 nm. The trolox solution with concentration was used to made calibration curve. Antioxidant capacity was stated in µmol troloks/g of dried powder.

3. Results and Discussions
3.1. The characteristic of H. macroloba
*H. macroloba* is a common widespread species in the Banyak Island, Aceh Singkil. *H. macroloba* several features including holdfast type, segment shape and size, node height with differences in pore size, the presence of two additional pattern of peripheral utricle as seen in surface view. *H. macroloba* have prolific thalus, stiff blade and calcareous. *H. macroloba* have yellowish green colour that grow in habitat with sandy substrat (Figure 2).

![Figure 2. The morphology of the algae H. macroloba from Banyak Island](image)

3.2. The rendement of crude extract
The rendement of *H. macroloba* is presented on Table 1. The rendement content of green algae *H. macroloba* obtained was 2.32% (ethanol), 1.26% (ethyl acetate), 1.03% (n-hexane).

| The solvents   | Rendement (%) |
|----------------|---------------|
| Ethanol        | 2.32          |
| Ethyl Acetate  | 1.26          |
| n-hexane       | 1.03          |
Table 1 showed that the highest rendement content was obtained from ethanol extract as much as 2.32%. It is indicate that the polar compound much more in the *H. macroloba*. According to Harborne, [1984], the ethanol is a solvent with polar attributes that have ability to extract active compound in extracelluler and intracelluler liquid. Ethanol is very polar molecule due to its hydroxyl (OH) group, with high electronegativity of oxygen allowing bonding to take place with other molecules.

### 3.3. Phytochemical constituent

To investigate the antioxidant of *H. macroloba* extracts. Previously, we carried out the phytochemical analysis with using various solvents including ethanol, ethyl acetate and n-hexane. The *H. macroloba* extracts were detected the presence of alkaloids, flavonoids, phenols, glycosides, and saponin used qualitative approach. The results were showed in Table 2.

#### Table 2. Phytochemical constituents of green algae *H. macroloba*

| Constituents       | Ethanol | Ethyl Acetate | n-hexane | Result of Positive Assays   |
|--------------------|---------|---------------|----------|-----------------------------|
| Alkaloids          | -       | -             | -        | -                           |
| Flavonoid          | -       | -             | -        | -                           |
| Fenol              | +       | +             | +        | Dark green/green             |
| Saponin            | -       | -             | -        | -                           |
| Tannin             | -       | -             | -        | -                           |
| Steroid            | -       | -             | -        | -                           |
| Triterpenoid       | -       | -             | -        | -                           |

Information: + = Detected, - = Not Detected

Based on Table 2 showed three extracts of *H. macroloba* have been detected the presence of fenol compounds. The present study showed the phytochemical screening of *H. macroloba* with different extract including ethanol, ethyl acetate and n-hexane extract had been showed variation in phytoconstituents present in respectively extracts. This *H. macroloba* extract have potential secondary metabolites like fenol. These constituents have a great medicinal value. They have been extensively used in the preparation of drug and medicinal industry [22]. We know that fenol compound is important for the survival of a plant in its environment. They regulate plant growth, inhibit or kill many bacterial strains, inhibit major viral enzymes and destroy some pathogenic protozoans [23]. Saponins are used as anti-inflammatory agent as well as it is used in a dietary product [24]. Tannins are used as antioxidant, antiviral and antibacterial agents. Steroids are used for its antimicrobial, anti-parasitic, cardio tonic properties.

### 3.4. Antioxidant activity of the DPPH method

DPPH method is nitrogen radical compound of DPPH will take hydrogen atom that got in compound, for instance phenol compounds. The mechanism occured this DPPH reaction through electron transfer. DPPH solution were violet colour to gave DPPH electron. This DPPH solution will oxidase the compound in plant extract. This processes signed with fading the colour of solution from violet to yellow. The result of the antioxidant activity were presented in Table 3.

#### Table 3. Antioxidant Capacity of *H. macroloba* extract of DPPH Method

| Extracts      | Antioxidant Capacity (mg/L) |
|---------------|-----------------------------|
| Ethanol       | 121.44 ±1.03                |
| Ethyl acetate | 228.67 ±1.42                |
| n-hexane      | 181.945 ±1.95               |
| Vitamin C     | 1.35                        |
The result showed the three extracts and vitamin C possess different activity. The ethanol possesses the strongest antioxidant activity with IC$_{50}$ value was 121.445 ±1.039 mg/L and the n-hexane have IC$_{50}$ 181.945 ±1.95 mg/L. However, the ethyl acetate extract possesses the weakest antioxidant activity with IC$_{50}$ value was 228.67 ±1.42 mg/L. [25] have already been reported that the value of IC$_{50}$ less than 50 mg/L was category to possesses the strong antioxidant activity, 50-100 mg/L was moderate category, 150-200 mg/L was weak category and more than 200 mg/L was the weakest category. The low IC$_{50}$ value showed the strong capacity from the extract to role as donor of hydrogen atom. The high capacity of scavenging related with hydroxyl group in phenolic compound [26].

3.5. **Antioxidant activity of (ferric reducing antioxidant power) FRAP method**

Reducing power determined by using FRAP (ferric reducing antioxidant power) that is based on the ability of the compounds in reducing iron compounds (III)-tripiridil-triazine to iron (II) -tripiridil triazine at pH 3.6. The absorbance was measured using the UV-Vis spectrophotometer at a wavelength of 598 nm.

| Extracts    | Antioxidant capacity (µmol trolox/g extract) |
|-------------|---------------------------------------------|
| Ethanol     | 516.50 ±0.70                               |
| Ethyl acetate| 482.00 ±1.41                               |
| n-hexane    | 323.50 ±0.70                               |
| Vitamin C   | 1,305                                       |

The antioxidant capacity of ethanol extract was higher than ethyl acetate and n-hexane extract with value 516.50 ±0.70 µmol trolox/g extract. Three extracts have antioxidant capacity but the biggest ability to reduce ferric ion (Fe$^{3+}$) showed in the ethanol extract. Based on the antioxidant capacity assay showed that ethanol extract was the best antioxidant. The result of measurement were different than with DPPH method that used IC$_{50}$ value but the ethanol extract possesses high antioxidant capacity than other extract. According to Ou et al (2002) that the measurement of antioxidant used FRAP method will be accurate if antioxidant compound can reduce Fe(III)TPTZ in reaction condition thermodynamically.

3.6. **Antioxidant capacity of CUPRAC (cupric ion reducing antioxidant capacity) method**

The CUPRAC (cupric ion reducing antioxidant capacity) method, bis neokuproin-copper(II) complex will oxidize antioxidant compound in extract and experience reduction to form the bis neokuproin-copper(I) complex. CUPRAC reagent are selective reagent because it have lower reduction potential value [28]. The result of antioxidant activity of CUPRAC method that presented in Table 5.

| Extracts    | Antioxidant Capacity (µmol trolox/g extract) |
|-------------|---------------------------------------------|
| Ethanol     | 159.85 ±0.70                               |
| Ethyl acetate| 66.38 ±0.03                                |
| n-hexane    | 49.15 ±0.03                                |
| Vitamin C   | 1.35                                        |

The result showed that the ethanol extract have the highest antioxidant capacity with value was 159.85 ±0.70 µmol trolox/g extract and followed by the ethyl acetate extract was 66.38 ±0.035 µmol trolox/g extract. While n-hexane extract possesses low antioxidant capacity with value was
49.15±0.035. The CUPRAC method was used many assays of antioxidant capacity for phenolic compounds [29]. Therefore, from this three extracts were predicted to possesses plenty of phenolic compounds content. The result of the antioxidant capacity assay of CUPRAC showed that the ethanol extract possesess the best antioxidant activity which suspected a hydroxycinnamic acids which are almost the most abundant phenolic component in the macroalgae.

4. Conclusion
The antioxidant activities of three extract of *H. macroloba* from the coastal of Banyak Island Aceh Singkil clearly indicated that they possess antioxidant activity in different method including DPPH, CUPRAC and FRAP. To our knowledge, this is the first report on antioxidant activity of *H. macroloba* extract as well as screening of antioxidant capacity and phenolic content of macroalgal species in the coastal of Banyak Island Aceh Singkil. These phenolic may play a role in the antioxidant activities observed in the ethyl acetate and ethanol extracts.

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