Screening for antioxidant activity in extracts of the marine macro algae Enteromorpha flexuosa (Wulfen) J. Agardh from South Aceh

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Abstract. Indonesia is the world’s largest archipelagic nation with high marine biodiversity that can provide added value for local communities through using renewable resources. Aceh is one of the provinces in Indonesia directly facing the Indian Ocean with many marine biota distributed along the coast. Marine macroalgae are marine biota with potential for medicines and foodstuffs that have long been consumed by local communities in Aceh. Many marine macroalgae in Aceh coastal waters, especially along the south coast, contain a wide range of bioactive compounds with potential antioxidant activity. The aim of this study was to screen for antioxidant activity in extracts of the green macroalgae Enteromorpha flexuosa. Green algae of E. flexuosa were taken from along the South Aceh coastal zone. The research methods included extraction followed by phytochemical and antioxidant activity assays. The rendement of E. flexuosa extracts were ethanol with value of 1.38%; ethyl acetate with value of 1.11%; and n-hexane with value of 0.42%. The phytochemical analysis showed phenol and flavonoid compounds in the E. flexuosa extracts. The CUPRAC method for determining antioxidant activity showed activity of 96.40 µmol troloks/g in the ethanol extract; 16.77 µmol troloks/g in the ethyl acetate extract; and 22.38 µmol troloks/g in the n-hexane extract. These results showed that the ethanolic extract possesses the antioxidant capacity strongly than other extracts. There are indications that phenol compounds influenced antioxidant activity in the E. flexuosa extracts. Using the FRAP method to determine the antioxidant activity of the extracts was: ethanol extract 474%, ethyl acetate 363.50% and n-hexane 239.50%. These levels of antioxidant activity in the extracts point to E. flexuosa as a potential source of antioxidants that could provide benefits when used in marine natural products.
1. Introduction
The marine habitat is provided several metabolite secondary that different with natural compound from terrestrial zone [1]. The unique chemical compound derived from marine biodiversity that have prospective in medicine and cosmetic industry. The secondary metabolite that contained from marine biota in discovering of marine natural product for pharmaceutical chemicals. One of the marine diversity that potential herbal drug from the ocean environment in marine natural are marine macro algae. The marine macro algae possesses high amount of polyphenols that indicated in potential natural antioxidant agent. The antioxidant in macro algae showed its capability to protect human’s body from oxidative stress due to environmental condition [2]. One of the marine macro algae that possesses potential antioxidant from South Aceh is Enteromorpha flexuosa (Wulfen). E. flexuosa were abundantly growing green algae at the coastal of South Aceh. Marine macro algae possesses various source of bioactive component and have been explored by scientist as source of marine natural drugs [3]. Nowadays, several study in marine bioprospecting stated that marine macro algae have a metabolite activities such as anti fungal, anti cancer, anti inflammations, anti-diabetic, anti-viral, anti-turmeric, cytotoxic and anti-neoplastic [4], for hypertensive patient [5-6], anti bacterial [7], tyrosinase inhibitor [8], antioxidant agent [9-10]. Commercially, marine macro algae are renewable resources that replant in prevail environmental condition to providing valuable new marine drugs to protect the infectius diseases such as cancer, microbial infections and inflammations [11]. Marine macro algae also utilized extensively by local community for food product and organic fertilizer [12]. Marine macro algae have several metabolite secondary such as alkaloid, terpenoid and flavonoids that generate antioxidant, anti microbial, anti-neoplastic and antiviral properties [13]. Recently, natural antioxidant have pay attention by scientist to prevent various diseases that caused by free radical that play pivotal role in human’s body [14]. The previous study, mostly terrestrial plants have a lot of phytochemical properties that toward to antioxidant activity that have been explored by many researcher with different spesies of plant such as cereals crops, oil seeds, vegetables and spices [15]. Flavonoids are polyphenolic compound that safe and non-toxic antioxidant. Many report regarding of natural phenolic is absolutely associated with reducing risk of some chronic diseases, cancer, diabetes, obesity and blood pressure [16-18]. Phenolic compound also play important role in plant and marine macro algae with contained various inorganic and organic substitutes which can be advantages for human’s body [19]. Reactive Oxygen Species (ROS) production from marine macro algae is stimulated by environmental stresses including high light levels, heavy metals, high salt concentrations UV radiation etc. Meanwhile, marine macro algae has indicated to possesses higher antioxidant activity due to a higher content of non-enzymatic antioxidant component such as ascorbic acid, reduced glutathione, phenols and flavonoids [20]. Brown, red and green algae are indicated have bioactive compound that possesses antioxidant, antiviral, antifungal, anti microbial, anti tumor and anti inflammatory activities which can be develop to be new herbal drug and foodstuff [21]. The antioxidant properties from various macro algae have already reported [22-23]. The preliminary study regarding the exploration of bioactive compound in macro algae species from the coast of South West Aceh including Halimeda macroloba [24], Chaetomorpha crassa [25], Halimeda opuntia [26], Halimeda spp [27] and Chaetomorpha antennina [28] have already been reported. However, the recent study on the antioxidant activity of E. flexuosa from the coast of South Aceh has no yet documented. Even though species of E. flexuosa is sufficiently abundant that wide distributed in the intertidal zone of South Aceh. Thus, to the fruitful insight, there is no information regarding the antioxidant activities of green algae E. flexuosa from South Aceh coastal zone that utilized for pharmaceutical industries. The present study aimed to screen the antioxidant activity of green macro algae from the coast of South Aceh for future application in marine natural product, dietary supplements, cosmetic and food industries.
2. Materials and Methods

2.1. Preparation of samples
The samples of *E. flexuosa* were collected in April 2017 at the Lhok Pawoh coastal area, South Aceh, Aceh province (Figure 1). The species of *E. flexuosa* were identified in the Laboratory of Marine Science, Faculty of Fisheries and Marine Science, Teuku Umar University. The collected samples were cleaned and rinsed with pure water and throw all particle that attached in our macro algae. Then, we can analysed our samples at Department of Aquatic Product Technology Laboratory, Faculty of Fisheries and Marine Science, IPB University.

![Figure 1. Sampling Location](image)

2.2. Materials
All materials that we used in our experiment such ethyl acetate pa (Merck), methanol pa (Merck), ethyl acetate pa (Merck), n-hexane (Merck), mercuric chloride, Dragendorff's reagent, 4,6-tri(2-pyridyl)-s-triazine (TPTZ), amyl alcohol (Merck), ammonium acetate (Merck), Neocuproine (Sigma–Aldrich), potassium iodide, iodine (Sigma–Aldrich), hydrochloric acid (HCl), sulfuric acid (H₂SO₄), chloroform, ammonia, glacial acetic acid, sodium hydroxide (NaOH), CuCl₂·2H₂O (Merck), FeCl₃·6H₂O (Merck) and potassium peroxodisulfate. The analytical equipment include glassware, micropippet (Gilson*), vacuum rotary evaporator and UV-VIS spectrophotometer.
2.3. Analytical procedures
The dried samples were cut off and crushed by using a blender until it to be simplicia. Then, the powder of sample were weighed 70 g and put it in the glass. The extraction was undertaken with 1:3 uses three solvents (n-hexane, ethyl acetate, and methanol). Subsequently, our filtrate were soaked for 3x24 hours. The solution were Whatman paper and were concentrated by using a vacuum rotary evaporator.

2.4. Phytochemical Assay
Phytochemical analysis in this research with ethanolic extract were tested the properties of phytochemical including phenol, alkaloids, steroid and triterpenes, flavonoids, saponins, and tannins. The qualitative result is expressed as (+) for detected and (-) not detected [29].

2.5. Ferric reducing antioxidant power Method
In FRAP assay with modification, this analysis assay of the antioxidant activity total with used FeSO₄.7H₂O as standar. The made of the reagent FRAP was prepared by mixing 300 mM acetate buffer (6 mL CH₃COONa and 50 mL CH₃COOH) pH 3.6, 10 mM larutan TPTZ (2, 4, 6-tripyridyl-striazine) in 40 mM HCl and FeCl₃.6H₂O 20 mM. The solution were prepared in fresh condition with mixed 35 mL of acetate buffer, 3.5 mL of TPTZ and 3.5 mL of FeCl₃.6H₂O. The measurement of absorbance used 100 μL of sample, 600 μL of aquadest and 3000 μL of FRAP reagent. The mix of sample and FRAP reagent homogenized by using vortex, then incubated with waterbath for 30 minutes with temperature 37°C. The absorbance were measured in wavelength 593 nm. the standart curve was made with using FeSO₄.7H₂O solution with several concentrations [30]. For standar calibration, we used trolox with calibration curve standar solution in various concentrations. The standard solution and sample was reported as mg trolox equivalent (TE)/g extract [31-32].

2.6. Modified CUPRAC Method
In the CUPRAC method refer to Apak et al., [33] with modification. Previously, we begin with taking sample as much as 0.3 mL then diluted in ethanol 99.9% and added with 1 mL of mL CuCl₂.2H₂O 0.01 M ; 1 mL neocuproine of ethanol 0.0075 M; 1 mL of ammonium acetate buffer with pH 7 1 M and 0.8 mL of aquadest. The mix sample and reagent were vortexed then incubated in dark condition for 30 minutes. According to Baskan et al., [34] that the curve of calibration standar used trolox was depicted in absorbance and concentration and molar absorptivity that found from the slope of calibration line. Karaman et al., [35] stated the bis (2,9-dimethyl-1,10-phenanthroline: neocuproine) Cu(II) chelate cation was used in method of total antioxidant capacity (TAC) assay.

2.7. Statistical Analysis
All the data were expressed as means ± standard deviation (SD). The experiments were carried out in triplicates.

3. Result and Discussion
3.1. The Characteristic of E. flexuosa
The identification of the green macro algae E. flexuosa morphology is carried out through macroscopically observation. We found the green macro algae E. flexuosa at hard stone substrate, thus the hold fast play important role in attachment in substrate to withstand the big wave from open ocean because the Lhok Pawoh in front of the Indian ocean with the big wave. Characteristic of E. flexuosa species morphologically with unbranched filament like thread with chloroppyll pigmentation in their thallus. (Figure 2).
Figure 2. The *E. flexuosa* morphology from the Lhok Pawoh Coastal Area

3.2. Rendement analysis

Based on the result showed the rendement yield as much as 1.38% in ethanol extract, the ethyl 1.11% in ethyl acetate extract and the 0.42% extract as if depicted in Figure 3.

Figure 3. The rendement of *E. flexuosa* extracts

This is indicated that the polar compound was more dominant that other extracts. Based on the result above indicated that the highest rendement percentage derived from ethanolic crude extract. Nevertheless, n-hexane had the lower rendement than ethyl acetate extract. According to Harborne [36] that the ethanolic solvent have a polarity that able to absorb the bioactive compound.

3.3. The result of phytochemical

Bioactive component in living organisms particularly marine plant can be detected quantitatively by using phytochemical assay. The phytochemical assay in consisting of phenolics, saponin, tannin,
flavonoids, alkaloids and steroid. In our recent study, we have already been found one bioactive compound in species of *E. flexuosa*.

**Table 1.** The phytochemical assay result

| Phytochemical properties | Crude extract | Positive result According to the Reference |
|--------------------------|---------------|--------------------------------------------|
|                          | Ethanol       | Ethyl acetate | n-hexane |                                        |
| Alkaloids                | -             | -             | -        | The color change occurs from the control tube |
| Phenolic                 | +             | +             | +        |                                        |
| Saponin                  | -             | -             | -        |                                        |
| Triterpenoid/Steroid     | -             | -             | -        |                                        |
| Tannin                   | -             | -             | -        |                                        |

Note: (+) : detected, (-) : not detected

The phytochemical properties of crude extract revealed the secondary metabolites presences is phenolic compound (See Table 1). The metabolite properties properties were detected in our sample used quantitative analysis. Baskan et al., [37] reported that polyphenols are compounds group that have play important role as as potent antioxidant. Nevertheless, the determination of phenolic compounds in the marine macro algae extract were acceptable. Polyphenolic compounds have a ring of aromatic benzene with a substituted OH functional group that are able to absorb free radical and can chelate metal ions. It also catalyze the reactive oxygen species (ROS) formation, which promotes peroxidation of lipid. Among polyphenol, flavonoids have play pivotal role to against harmful illness in human organs. The flavonoids compounds are great antioxidant product depends on their molecular structures, the OH group position, and other features in its chemical structure.

3.4. The result of The cupric ion reducing antioxidant capacity method

The cupric ion reducing antioxidant capacity method is based on Cu(II)-Cu(I) reduction by antioxidants in the presence of neocuproine. The CUPRAC assay determine with routine compound as comparative compound. The activity of antioxidant was regarded as the capacity of The cupric ion reducing antioxidant capacity reduction was gained from the absorbance decreasing sample to control absorbance [38].

![Figure 4. The capacity of antioxidant of CUPRAC method](image-url)
Based on Figure 4 showed that antioxidant capacity of CUPRAC method where ethanolic extract value of 91.05±0.21 µM trolox/g, ethyl acetate crude extract value of 16.77±0.73 µM trolox/g and n-hexane extract value of 11.71±0.01 µM trolox/g. This result indicated that capacity of antioxidant from ethanolic crude extract is higher than ethyl acetate and n-hexane. The researcher assume that ethanolic crude extract have the stronger phenolic properties than ethyl acetate and n-hexane extracts. The CUPRAC method is based on transfer of electron that considered a good tools for determining of the total of antioxidant ability [39]. Apak et al. [40] reported that The cupric ion reducing antioxidant capacity method is more familiar used to antioxidant capacity for the phenolic compound. Therefore, this ethanol extract possesses the content of phenolic group compound. The marine macro algae E. flexuosa could be utilized as marine product to prevent free radicals.

3.5. The result of Ferric ion reducing power (FRAP) method
In this 2nd assay, the researcher used FRAP method as comparative assay. We used the FRAP method in which depends upon the ferric tripyridyltriazine (Fe(III)-TPTZ) complex reduction to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reduction at low pH.

![Figure 5. The capacity of antioxidant of FRAP method](image)

In the Figure 5 was described that marine macro algae E. flexuosa FRAP antioxidant activity was 474 ±11.3 µM trolox/g in ethanol crude extract whereas of FRAP value from the ethyl acetate extract was 363.5 ±0.70 µM trolox/g and 239.5±0.70 µM trolox/g from n-hexane extract FRAP. The ethanolic crude extract were yielded strong compound because the antioxidant capacity is 100-500 µmol Fe/g. According to Wong et al. [41], the compound will be category as very strong if the antioxidant ability is more than 500 µmol Fe/g, whereas the strong category if the antioxidant ability is 100-500 µmol Fe/g and weak indicator if the antioxidant ability value < 10 µmol Fe/g.

However, some disadvantage this assay of the FRAP does not react quickly with some antioxidants like glutathione [42]. The higher FRAP values indicated the higher antioxidant capacity because FRAP value is based on reducing ferric ion, where antioxidants are the reducing agent. Moure et al. [43] also reported that the quality of natural extracts and activities of antioxidant depend on the storage time, geographic origin, harvesting date and environment as well as technological factors. The FRAP method is to determine the antioxidant activity to measure the antioxidant capacity with reducing Fe³⁺ in complexes Fe³⁺- TPTZ to be Fe²⁺- TPTZ via electrons donation.
4. Conclusion
In these research, we concluded that the ethanol extract of *E. flexuosa* possesses marine herbal potential because the indication of phenolic component that play pivotal role in marine macro algae *E. flexuosa* that compared with both ethyl acetate and n-hexane extracts and it also showed the capacity of antioxidant in ethanol extract is suspected the potent antioxidant in macro algae *E. flexuosa* from South Aceh. Thus, it could be benefited as a source of marine natural medicine and cosmeceuticals.

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