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The potency of green algae *Chaetomorpha crassa* Agardh as antioxidant agent from the coastal of Lhok Bubon, West Aceh

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Abstract. The coastal of Lhok Bubon at West Aceh district, Aceh Province is ideal habitat for macro algae, such as *Chaetomorpha crassa*. The objectives of this paper is to analyse the antioxidant activity of *C. crassa*; to determine their antioxidant activities correlation with total phenolic contents with different extract of marine macroalgae. The *C. crassa* samples were collected from the coastal area of Lhok Bubon, West Aceh District (latitude 4011'51.07"N; longitude 9601'42.33"E) Aceh Province, Indonesia in June 2017. Several assay by laboratory approach including chemical composition, extraction, phytochemical screening, DPPH, CUPRAC, FRAP assays and measurement of total phenolic content (TPC) was used. The result showed that the antioxidant activities of three extract of green algae *C. crassa* clearly indicated that they possess antioxidant activity in different method including DPPH, CUPRAC and FRAP. The correlation has indicated to correlate positively between the total phenol content and antioxidant activity with three methods of antioxidant activity.

Keywords: algae, antioxidant, coastal

1. Introduction

Most marine macroalgae distributed in West Aceh waters are attached on the dead coral and sandy substrates. It is easy for them to be exposed to UV radiation from sunlight. Green algae is one of the species that sufficiently dominant in the coast of Lhok Bubon, West Aceh District. This coastal area also became a tourism site and *meugang* (Aceh wisdom) for the local community because it is interesting for recreation with their family. These recent studies have already been motivated us to investigate other species of marine macroalgae particularly green algae. Therefore, our research regarding the potency of green algae *Chaetomorpha crassa* as an antioxidant agent from the coast of Lhok Bubon was highly recommended.

The coastal of Lhok Bubon at West Aceh District, Aceh Province, Indonesia would be the ideal natural habitat for seaweed growth and development. Preliminary survey showed that, at least, 30 macroalgae species were found in this coastal area. Exploration on antioxidant activity in brown algae from this area have already been conducted [1] but research on antioxidant content from *C. crassa* has not been conducted yet. The objectives of this paper is to analyse the bioactive compound of *C. crassa* extracts



with antioxidant activity and their correlations with total phenolic contents in order to found their potential of biopharmaca sources for human health. Total antioxidant potential of marine green macroalgae will be examined by using FRAP, CUPRAC and DPPH assay.

Marine macroalgae has the potential to be developed as the source for antioxidant compounds. Macroalgae have antioxidant systems to resist environmental pressure, and possesses phytochemical properties such as carotenoids, alkaloids, polyphenols, phycocyanins, terpenes and several enzymes [2]. Recently, many novel of prevent reactive oxygen radical conciliated oxidative stress of natural antioxidants have been isolated from macroalgae [3]. Indonesia has the second longest coastline that is suitable for marine macroalgae growth and development. Indonesia have high marine macroalgae biodiversity. Marine macroalgae are consist of green macroalgae (Chlorophyta), brown macroalgae (Phaeophyta) and red macroalgae (Rhodophyta). Marine macroalgae offer a wide variety of natural compounds as natural antioxidants [4]. Many marine macroalgae have been already assayed for identifying new and effective antioxidant compounds, as well as mechanisms of cell proliferation and apoptosis elucidation [5].

Antioxidant content in marine macroalgae is associated with marine macroalgae's habitat which is generally waters that are continually exposed to UV from sunlight and oxygenated air. Those will generate free radical or reactive oxygen species (ROS). Oxidative damages are caused by reactive oxygen species are protected effectively by antioxidant [6]. Marine macroalgae's structural component will not experience oxidative damage being exposed continually to ROS. This shows a protective system against oxidative stress is inside marine macroalgae cells. By donating an electron, an antioxidant compound can neutralize the present of free radical or ROS [7]. A large number of reactive oxygen species formation could be a main factor due to oxidation of biomolecular. These will cause significant degradation of cell structure, and then will stimulate various degenerative disease and degenerative process such as ageing [8]. Several studies have been reported that there are some toxic and carcinogenic from synthesized antioxidants including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG) [9]. Marine macroalgae is more safe and interesting natural antioxidant for medicine and functional food ingredients then synthesised antioxidants. There are many phytochemical compound in macro algae which is rich with bioactive compound include antioxidant, anti-aging, anti-inflammatory and anticancer. *Hijikia fusiformis* and phlorotannins in *Sargassum kjellmanianum* are examples of macro alga which is rich with antioxidant [10]. Nowadays, antioxidant is intensively focused to be studied due to high pharmaceutical demand.

Several studies have been done for green macroalgae that composed important natural resources, easy to cultivated abundance and wide distribution in natural environment and performed on antioxidant compounds [11]. Their solvent-partitioned fractions and ethanol extracts of *Enteromorpha compressa*, *Capsosiphon fulvescens*, *Chaetomorpha moniligera* and *Ulva pertusa* present plenty of potential antioxidant related with such bioactive compound including flavonoid and phenolics total [12].

2. Materials and Methods

2.1. Sampling method

The *C. crassa* samples were collected from the coastal area of Lhok Bubon, West Aceh District (latitude 4°11'51.07"N ; longitude 96°1'42.33"E). Aceh Province, Indonesia in June 2017 (figure 1). The green macroalgae *C. crassa* were colleted with hand and washed with seawater while remove the epiphytes, sand particles, and others. The clean sample was put in the cool box and transported. Then, the samples was identified in the Laboratory of Fisheries, Teuku Umar University [13].

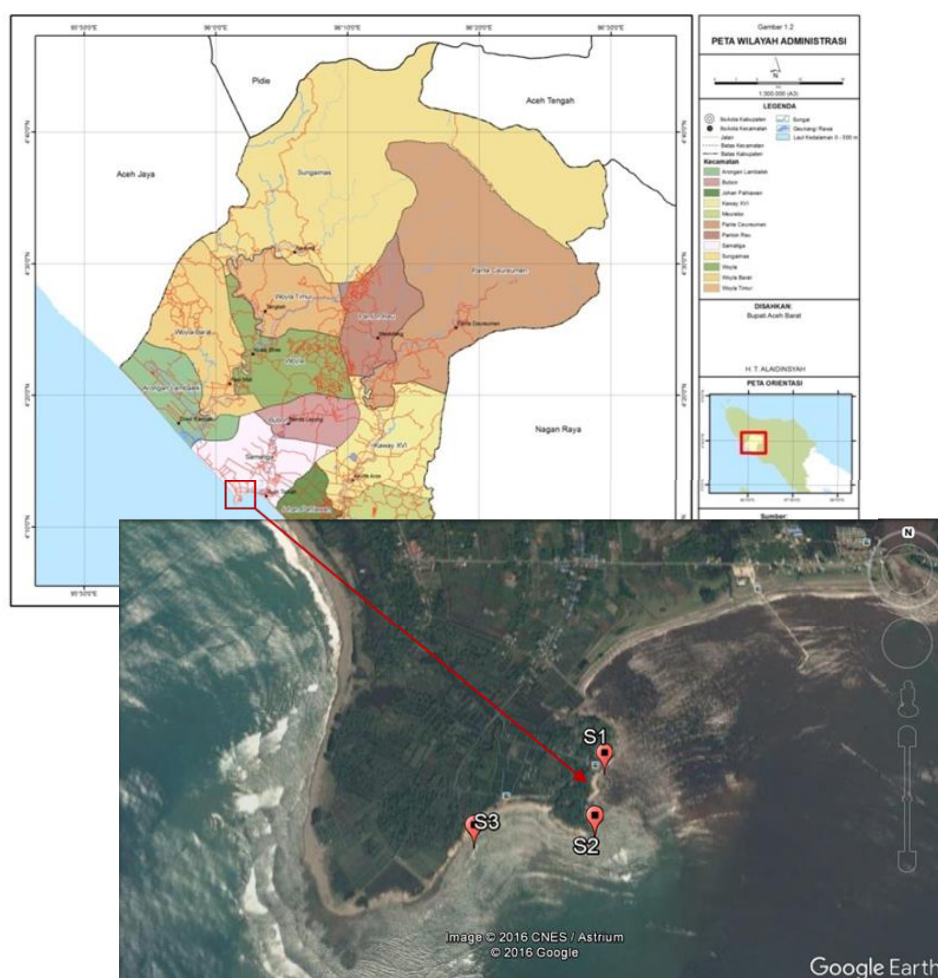


Figure 1. Sampling site.

2.2. Chemical composition

Chemical composition including water content, ash content, carbohydrate, protein, fatty acid was determined based on proximate analysis that refer to [14].

2.3. Extraction of green algae *C. crassa*

The sample dried under sunlight for ± 4 days. The dried sample is minced and milled by using a blender until it became simplisia powder. The simplisia is weighed as much as 50 g and put it in the erlenmeyer glass. The maceration was undertaken with ratio 1:3 by using three solvents including ethanol (polar), ethyl acetate (semi-polar) and n-hexane (non-polar) respectively and was soaked for 2x24 hours. The submersion was functioned for taking out organic compounds from the simplisia. The solution was filtered by using filter paper and concentrated in a vacuum concentrator until dry extract was obtained.

2.4. Phytochemical screening

Phytochemical constituents analysis including alkaloids, steroid and triterpenes, anthraquinones, flavonoids, saponins, cyanogenic glycosides, cardiac glycosides and tannins followed the methods described previously [15].

2.5. DPPH method

Antioxidant assessment was carry out by using DPPH method [16]. DPPH reagent (Merck) was treated by diluting 3 mg in 5 mL MeOH (p.a). Diluted 160 μ L dry seaweed extract of a methanol was poured into 96-well microplate. Then a 40 μ L of DPPH reagent was added (A). In this evaluation four reagent such as extract control (containing 160 μ L of seaweed extract and 40 μ L of MeOH) (B), negative control (160 μ L MeOH and 40 μ L of DPPH reagent) (C) and a blank (200 μ L MeOH) (D) were also used.

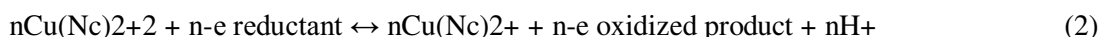
Absorbance of each well was measured by using a spectrophotometer UV-VIS. DPPH free radical inhibition percentage was analysis using the following equation.

$$[(C-D)-(A-B)]/(C-D) \times 100\% \quad (1)$$

Inhibition concentration 50 (IC₅₀) value was calculated using probit analysis. In this evaluation, positive control that by using Vitamin C and prepared in the concentration at the range of 1-10 µg/mL.

2.6. CUPRAC method

The final volume of 4 mL was made using CUPRAC method [17]. The CUPRAC method is based on the reduction of a cupric neocuproine complex (Cu(II)–Nc) by antioxidants to the cuprous form (Cu(I)–Nc). Colour development in the CUPRAC assayed is based on the following reaction [17].



2.7. FRAP method

Antioxidant evaluation by using Ferric Ion Reduction Antioxidant Power (FRAP) method was conducted refer to [18]. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution and 20 mM FeCl₃.6H₂O in the ratio 10 : 1 : 1. Upon usage, FRAP reagent was incubated in 37°C for less than one minute. Solution of acetate buffer was prepared by diluting 3.1 g of sodium acetate trihydrate in 16 mL acetic acid glacial then aquabidest was added up to the volume of 1 L. TPTZ solution was prepared by diluting TPTZ in 40 mM hydrochloric acid solution. In this analysis, FRAP reagent of 150 µL was poured into 96-well microplate and added with seaweed extract (1 mg/mL dose) for 20 µL. The microplate was incubated in a dark room at a temperature of 27-28°C for 8 minutes, then its absorbance was measured in microplate reader (Thermo Scientific) at the wavelength of 595 nm. Ferrous (Fe²⁺) standard curve was constructed simultaneously at FeSO₄.7H₂O concentration range of 50 - 1000 µM. FRAP value (in µM) was determined using the equation formulated from the standard curve.

2.8. Total phenolic content measurement

The total phenolic content was estimated by using Gallic acid equivalents (GAE) according to Folin–Ciocalteu reagent [18].

3. Result and Discussion

3.1. Chemical composition

Chemical composition of *C. crassa* was presented on table 1. Moisture content of green algae *C. crassa* obtained here was 33.99%. The dried of *C. crassa* possesses ash content was 37.91%, protein content was 7.94% and fat content was 1.05%.

Table 1. The proximate composition of dried *C. crassa*.

Parameters (content)	Average (%) <i>C. crassa</i> (WB)
Moisture	33.99 ± 0.38
Ash	37.91 ± 0.29
Protein	7.94 ± 0.16
Fat	1.05 ± 0.04
Carbohydrate	14.4

Table 1 showed that moisture content obtained was 33.99%. It is indicated that *C. crassa* simplisia can be durable for storage, but not for a long time because moisture content from material was less than 10% that indicated optimum stable and the growth of microorganisms can be reduced. The determination of moisture content was aimed to know the resilience of a material in order to estimate the way of the best storing for sample to avoid the influence of the microorganism activity [19]. The determination of ash content is one of the way to determine the existing of mineral or inorganic compound in a material. The ash content of *C. crassa* was 37.91%. The high ash content of marine

macroalgae was influenced from existing of salt and other minerals that attached including Na, Ca, K dan Mg [20], the higher the mineral so the ash content also higher [20]. It is showed the high of ash content from *C. crassa* was related with mineral content. *Sargassum crassifolium* possesses the high ash content was 36.93% [21]. Thus, this result almost similar with the ash content of *C. crassa*. Marine macroalgae are carbohydrate, protein, fat sources. The fat content of green algae *C. crassa* was 1.05% and protein content green algae *C. crassa* was 7.94%. *S. polycystum* possesses fat content 0.23% and protein 3.64% [22]. Protein was formed from several amino acids that bonded by peptide bonds, protein content was different in macroalgae due to the amino acid content [23]. Protein is an important part of our diet [24]. Moreover, macroalgae also contained less fat content. Generally, macroalgae stored food from carbohydrate especially polysaccharides. The high fiber that caused by the high polysaccharides into macroalgae cell [25].

3.2. Phytochemical constituent

To investigate the antioxidant activity from three extracts, secondary metabolites were carried out using various solvents. The extracts of green macroalgae *C. crassa* were tested for determination of alkaloids, flavonoids, phenols, glycosides, and saponin by using qualitative phytochemical screening. The result (table 2) showed that different patterns of bioactive compound of the extracts.

Table 2. Phytochemical constituents of green algae *C. crassa*.

Constituents	Ethanol	Ethyl Acetate	n-hexane	Result of positive assays
Alkaloids				
a. Mayer	-	-	-	White sediment
b. Wagner	-	-	-	-
c. Dragendorff	+	+	-	-
Flavonoid	+	+	-	-
Phenol hydroquinon	-	-	-	Dark green/green
Saponin	+	+	-	-
Tannin	+	+	-	-
Steroid	+	-	-	-
Triterpenoid	+	-	-	Red

Information: + = Detected, - = Not Detected

Based on table 2 showed that two extracts (ethanol and ethyl acetate) of green algae *C. crassa* had detected the bioactive compound including alkaloids, flavonoid, saponin, tannin compounds. Moreover, the presence of steroids and triterpenoid was only detected in the ethanol extract. N-hexane extract has no detected of bioactive compound. The present study showed the phytochemical screening of green algae *C. crassa* with different extract including ethanol, ethyl acetate and n-hexane extract showed variation in phytoconstituents present in respectively extracts. This green algae *C. crassa* is rich in secondary metabolites like flavonoid, saponin, tannin, steroid, triterpenoid and alkaloids. These constituents have great medicinal value. They have been utilized in the drug and medicinal industry [26]. In the present investigation, there was also flavonoid, saponin, tannin, steroid, triterpenoid and alkaloid that were detected in the green algae *C. crassa* extract. The flavonoids compound are very important element for the plant to survive from their environment. This compound regulate plant growth, inhibit or kill many bacterial strains, major viral enzymes and destroy some pathogenic protozoans [27]. Saponins were used as an anti-inflammatory agent in a dietary product [28]. Tannins are used as antioxidant, antiviral and antibacterial agents. Steroids are used for its antimicrobial, anti-parasitic, cardio tonic properties.

3.3. Antioxidant activity of the DPPH method

DPPH method (2,2-diphenyl-1-picrylhydrazyl) is a nitrogen radical compound of DPPH that will take a hydrogen atom that entered the compound, for instance phenol compounds. The mechanism enabled this DPPH reaction through electron transfer. DPPH solutions were violet in colour to give DPPH

electron. This DPPH solution will oxidase the compound in plant extract. The results are presented in table 3.

Table 3. Antioxidant activity of *C. crassa* extract of DPPH method.

Extracts	Antioxidant activity (IC ₅₀) (mg/L)
Ethanol	260.977 ± 11.322
Ethyl acetate	70.64 ± 8.61
n-hexane	-
Vitamin C	1.30

The results showed that the three extracts and vitamin C possess distinct activities. The ethyl acetate possesses the strongest antioxidant activity with IC₅₀ value was 70.64±8.61 mg/L whereas the ethanol extract possesses the weakest antioxidant activity with IC₅₀ value was 260.977 ±11.322. Moreover, the n-hexane had no activity of antioxidant. The value of IC₅₀ less than 50 mg/L was the category that possesses the high antioxidant activity, 50-100 mg/L was the moderate category, 150-200 mg/L was the weak category and more than 200 mg/L was the weakest category [29]. The low IC₅₀ showed that the high capacity from the extract as the role of donor of hydrogen atom. The high capacity of scavenging related with hydroxyl group in phenolic compound [30].

3.4. Antioxidant activity of FRAP method

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

Table 4. Antioxidant Activity *C. crassa* extract of FRAP method.

Extracts	Antioxidant activity (μmol trolox/g extract)
Ethanol	62.71 ± 0.30
Ethyl acetate	22.32 ± 0.35
n-hexane	-
Vitamin C	1.30

The results in table 4 showed that ethyl acetate extract *C. crassa* possess a high ability to reduce ferric ion (Fe³⁺) was 22.32 ± 0.35 μM equivalent trolox/g extract whereas the ethanol extract possesses the reduction capacity was 62.71 ± 0.30 μM equivalent trolox/g extract. Moreover, the n-hexane had no antioxidant activity. It is depicted that two extracts possess antioxidant activity with the biggest ability to reduce ferric ion (Fe³⁺) shown in the ethyl acetate extract *C. crassa*. The assay result with FRAP method influenced by many reductions from iron compounds (III)-tripiridil-triazine (Fe(III)-TPTZ) to iron (II) -tripiridil triazine (Fe(II)-TPTZ) by reductant [31]. Based on the antioxidant capacity assay showed that ethanol extract was the best antioxidant.

3.5. Antioxidant activity of CUPRAC method

The antioxidant activity of CUPRAC Method follows as [32]. Table 5 shows the result of antioxidant activity of CUPRAC method.

Table 5. Antioxidant activity *C. crassa* extract of CUPRAC method.

Extracts	Antioxidant activity (μmol trolox/g extract)
Ethanol	167.39 ± 0.55
Ethyl acetate	6.75 ± 0.05
n-hexane	-
Vitamin C	1.30

The assayed of antioxidant activity with CUPRAC method also described the antioxidant properties positively. The value of antioxidant activity from ethanol extract was 167.39 ± 0.55 $\mu\text{mol trolox/g}$ extract whereas the antioxidant activity value of the ethyl acetate extract was 6.75 ± 0.05 $\mu\text{mol trolox/g}$ extract. Many assays of antioxidant capacity for phenolic compounds by using The CUPRAC method [33]. Therefore, from the three extracts were predicted to possess plenty of phenolic compounds content. The result of the antioxidant capacity assay of CUPRAC exhibited that the ethanol extract possess the best antioxidant properties.

3.6. The result of total phenol content

The measurement of total phenol content was conducted with the addition of Folin-Ciocalteu reagent. Folin Ciocalteu is the in-organic reagent that forms complex solutions with phenol compounds. The colour will be detected by the absorbance with wavelength of 756.5 nm. Phenol compound is a secondary metabolite in plants that was potent as antioxidant agent. It is caused by hydroxyl functional groups in the phenol compound. The functional group was functioned as the contributor of the hydrogen atom when it reacts with radical compounds through the mechanism of electron transfer. Thus, the oxidation processes can be inhibited. The measurement of total phenol will be presented in table 6. Marine macroalgae are a rich source of secondary metabolites including phenols and polyphenols [34]. Antioxidant activity, an important tool for macro algae bioactive compounds, is associated with the ability to extract from reactive oxygen species, singlet oxygen cooling, reduce power, and chelating ability [35]. A few antioxidant components from marine macroalgae extracts make individual measurements different of each antioxidant [36].

Table 6. Total phenol content.

Extracts	Total phenol content (mg GAE/g)
Ethanol	1853.71 ± 67.02
Ethyl acetate	99.50 ± 28.9
n-hexane	-

The total phenolic content from three extract of *C. crassa* was significantly different. The ethanol extract had the highest mean TPC (1853.71 ± 67.02 mg GAE/g) whereas the ethanol extract had the lowest mean TPC (99.50 ± 28.9 mg GAE/g). Moreover, the n-hexane extract had no mean TPC.

3.7. The correlation

Phenol compound is a chemical compound that have potency as an antioxidant but overall antioxidant activity not only due to by phenol compound. *Pentacyclic triterpenes*, vitamin C, colour substance such as chlorophyll, sulfuric acid, nitrogen had roles as antioxidant agent [37]. The correlation analysis by using DPPH, CUPRAC and FRAP method for determination of the correlation between antioxidant activity and total phenol content. The results are shown in figure 2 until 4.

The correlation analysis has obtained that the correlation of total phenol content and antioxidant activity by using DPPH, CUPRAC and FRAP methods are 0.986; 0.998; 0.997. They correlated positively with three methods of antioxidant activity. From this result, it can be explained that antioxidant activity was caused by total phenol content from marine macroalgae. Direct relationship between antioxidant activity, total phenolic content and concentration in macroalgae [38]. There is correlated positively between phenol content and scavenging capacity in several species, similar to extracts from *Sargassum* sp [39], *Caulerpa sedoides* [40] and *E. arborea* [41, 42]. These results suggested that the antioxidant activity might be due to the content of phenolic compounds in three extracts.

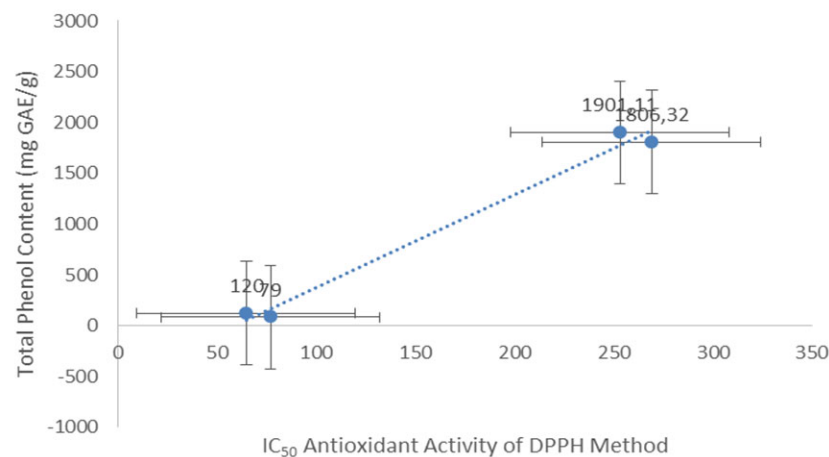


Figure 2. The correlation between total phenol content and antioxidant activity DPPH method.

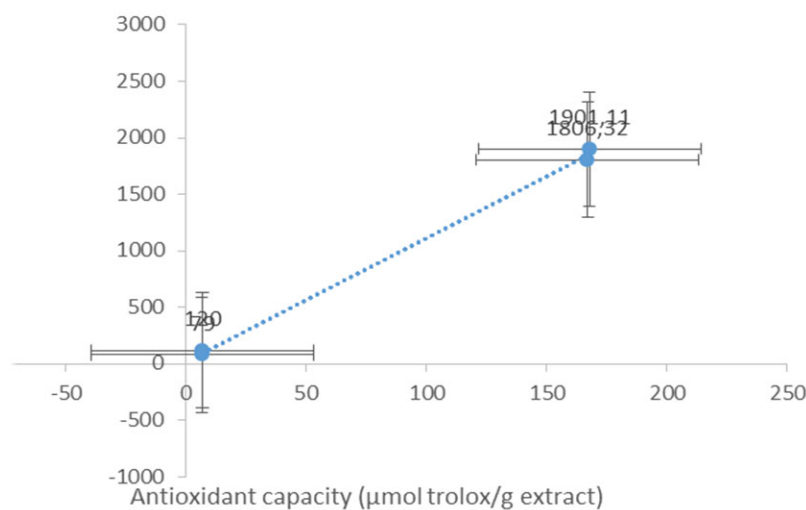


Figure 3. The correlation of total phenol content and antioxidant activity by using CUPRAC method.

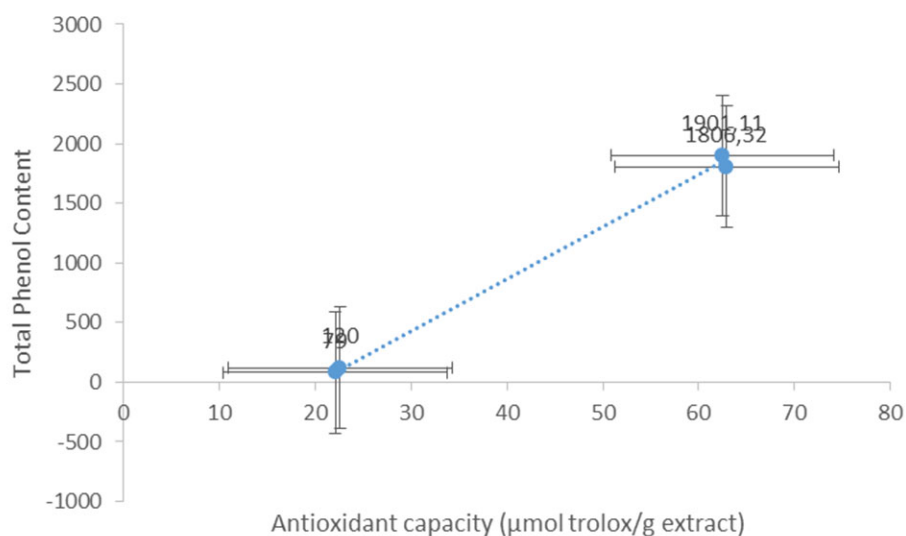


Figure 4. The correlation of the total phenol content and antioxidant activity by using FRAP method.

4. Conclusion

Three samples (extracts) of green algae *C. crassa* from the coastal of area of Lhok Bubon West Aceh clearly possesses antioxidant activity in distinct methods including DPPH, CUPRAC and FRAP. This is the first report on antioxidant activity of *C. crassa* extract as well as screening of antioxidant capacity and phenolic content of green algae *C. crassa* species in the coastal area of Lhok Bubon West Aceh. These phenolic may play a role in the antioxidant properties observed in the ethyl acetate and ethanol extracts.

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