

# Comparative Study on Antioxidant Activities, Total Phenolic Compound and Pigment Contents of Tropical *Spirulina platensis*, *Gracilaria arcuata* and *Ulva lactuca* Extracted in Different Solvents Polarity

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**Abstract.** These study aimed to determine antioxidant activity, total phenolic compound and pigment content of tropical *S. platensis*, *G. arcuata* and *U. lactuca*. *S. platensis* was extracted with aquadest (SPA), while *G. arcuata* and *U. lactuca* were extracted with n-hexane (GAH and ULH), ethyl-acetate (GAE and ULE) and methanol (GAM and ULM). The IC<sub>50</sub> value was used to calculate the ability of extract to inhibit free radicals using DPPH (515 nm). The TPC was determined using Folin-Ciocalteu (725 nm). Phycocyanin (620 nm), phycoerythrin (652 nm), allophycocyanin (562 nm), carotenoid (470 nm) and chlorophyll (645 nm and 662 nm) were measured based on color intensity. The results indicated that SPA was able to inhibit free radicals (46.12±2.03%), contains TPC (26.64±0.16 mg/GAE samples) and dominated by phycocyanin pigments (0.301±0.09 mg/g). *G. arcuata* extracted with ethyl-acetate (GAE) showed the best value with IC<sub>50</sub> 136.267±0.28 ppm, TPC 303.507±1.155 mg/GAE samples, carotenoid 0.528 ±0.009 μmol/g and chlorophyll a 2.845 ±0.069 mg/g. The *U. lactuca* extracted with ethyl-acetate also showed the best value with IC<sub>50</sub> 462.560±2.44 ppm, TPC 210.129±1.387 mg/GAE samples, carotenoid 0.442 ±0.046 μmol/g and chlorophyll a 9.216 ±0.103 mg/g. This results concluded that macroalgae extracted with ethyl acetate had better activity which categorized as moderate antioxidant.

## 1 Introduction

Marine algae that grow in the tropical area are exposed in strong ultraviolet radiation and combination of light and oxygen throughout the year. To avoid ultraviolet damage, these algae have protective antioxidative defense by change their metabolism, synthesize pigment

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and produce some active compounds. So, that the oxydative damage does not influence the structural components [1]. Tropical algae may possess a large number of active compounds as antioxidants, UV-absorbing matrix-metalloproteinase inhibitors, anti-aging, and immunomodulatory activities [2].

*Spirulina platensis* is one of blue-green microalga, which has a complex composition. The nutritional value of Spirulina is well recognized by its high protein content (60-70% by dry weight) and its richness vitamins, minerals, essential fatty acids and other nutrients especially vitamin B<sub>12</sub>, β-carotene and minerals such as iron [3]. Spirulina is a source of chlorophyll, and carotenoids [4] and containing phycobiliprotein compounds, consists of phycocyanin, allophycocyanin, and phycoeritrin. Its components have positive benefits for human health indications and can be used as a source of antioxidant [5]. Besides microalgae, macroalgae such as *Gracilaria arcuata* and *Ulva lactuca* are the alternative source which also provides several bioactive compounds used in biological activities such as antibacterial, anti-inflammatory and antioxidant activity [6].

Antioxidant can slows or prevents the oxidative or damage from oxygen process caused by free radicals, significantly [7]. The free radicals include superoxide anions (\*O<sub>2</sub>), hydroxyl radicals (OH\*), alkoxy radicals (RO\*), peroxy radical (ROO\*), (OONO\*), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and singlet oxygen (<sup>1</sup>O<sub>2</sub>), which triggers a chain reaction [8]. This free radical is one of the main cause of metabolic injury, accelerating aging, cancer, cardiovascular diseases, neurodegenerative diseases and inflammation [9]. Antioxidants are classified as synthetic and natural antioxidants. Synthetic antioxidants can induced the carcinogenic effect if used excessively [10]. But, natural antioxidant from marine algae has not side effects and non-toxic in nature [11]. It makes the source of natural antioxidant compounds important to explore and develop.

*S. platensis* was able to inhibit 77.47 % free radicals and has antioxidant activity at 1000 mg/100 ml concentration. *S. platensis* contains a number of natural pigments such as chlorophyll, β-carotene, phycoerythrin and phycocyanin [12]. *Gracilaria* sp. had an IC<sub>50</sub> value of 72.9 μg/mL and had total phenolic content of 1.509 ± 0.023 mg GAE/g. *Gracilaria* species are important for the industrial and biotechnological because they have bioactive compounds such as carotenoid, feofotin, chlorophyll a, chlorophyll b, xanthophyll, alkaloid, flavonoid, saponin, fenol, triterpenoid [13].

*Ulva lactuca* was able to inhibit 29.4 % free radicals at concentration 50 μg/mL and had 0.45 mg/100 g of total phenols in summer session [14]. *U. lactuca* contains bioactive compounds such as polyphenols, flavonoids [14], neoxanthin steroids and fatty acids [15], xanthophyll β-carotene, lutein and lycopene which can be used as a source of natural antioxidant [16]. This study aimed to determine antioxidant activity, total phenolic content and pigment content of tropical *S. platensis*, *G. arcuata* and *U. lactuca*.

## 2 Materials and methods

### 2.1 Sample extraction

The *S. platensis* powder sample was collected from Brackishwater Aquaculture Development Centre, Jepara. Extraction of the *S. platensis*. was done by maceration using aquadest (SPA). One gram *S. platensis* powder macerated with warm aquadest and boiled for 2 hours. The sample was then macerated in room temperature for 24 hours and filtered. Filtrate then centrifugated in 3500 rpm for 15 minutes and evaporated by cool dried method.

The *G. arcuata* and *U. lactuca* sample was collected from Krakal Waters, Yogyakarta. In the laboratory, the samples were washed using fresh water and dried up at room temperature. Extraction of *G. arcuata* and *U. lactuca* was done by gradual maceration using solvents with

different polarity ie. n-hexane (non-polar) (GAH and ULH), ethyl acetate (semi-polar) (GAE and ULE) and methanol (polar) (GAM and ULM) [18]. A 250 gram dry sample was cut into small pieces ( $\pm 5$  mm) and macerated using 1000 mL n-hexane solvent for 24 hours at  $\pm 27^{\circ}\text{C}$ , then filtered. The residue is remacerated for 24 hours and refiltered. The n-hexane filtrate was concentrated using a rotary evaporator at  $35^{\circ}\text{C}$ . The seaweed residue was re-extracted with ethyl acetate and methanol solvent in a similar manner [19]. The extract was then concentrated and cool dried and stored in the refrigeration for the next test.

## 2.2 Determination of DPPH maximum absorbance

As much as 4 mg DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in 100 ml of methanol to produce a DPPH (2,2-diphenyl-1-picrylhydrazyl) solution with a concentration of 0.101 mM. The absorbance of this solution was observed using a spectrophotometer (Shimadzu UV-1280) at a wavelength of 400 - 800 nm [20].

## 2.3 The assessment of antioxidant activity

Antioxidant activity determination was carried out using spectrophotometric methods (Costa *et al.*, 2018). A total of 3 mL of the test solution was added with 1 mL of DPPH 0.1 mM. The solution was incubated for 30 minutes and then measured the absorbance at the maximum wavelength. The percentage of inhibition is calculated using the formula [21].

$$\text{Inhibition percentage} = \frac{(\text{Absorbance of DPPH} - \text{Absorbance of DPPH} + \text{Extract})}{\text{Absorbance of DPPH}} \times 100 \% \quad (1)$$

The radical scavenging activity of *S. platensis* water extracts were expressed as inhibition percentage of DPPH. While, the inhibition percentage data of *G. arcuata* and *U. Lactuca* was plotted to constructed the linear regression equation and determined the  $\text{IC}_{50}$  value.

## 2.4 Measurement of total phenolic content

Determination of total phenolic compound of samples was performed using Folin-Ciocalteu and gallic acid reagents as standard [22]. A 5 mg gallic acid is dissolved in 5 mL ethanol to obtain a 1000 ppm stock solution. The stock solutions were diluted using methanol p.a. to obtain some series of concentrations (5, 10, 20, 30 and 40 mg/L). A 200  $\mu\text{L}$  of each concentration was taken, and then followed by the addition 10 mL of aquadest and 1 mL of Folin-Ciocalteu reagent. The solution was left for 5 minutes then added 1 mL of 5%  $\text{Na}_2\text{CO}_3$  solution and incubated at room temperature for 1 hours in dark conditions [23]. Absorbance was measured using a spectrophotometer (Shimadzu UV-1280) at a wavelength of 725 nm [24]. The value of total phenolics is expressed in mg Galic Acid Equivalent (GAE)/1000 g [25].

## 2.5 Assessment of chlorophyll a and carotenoids compounds

Each extract was dissolved with acetone p.a at 100 ppm and measured at a wavelength of 645 nm, 662 nm and 470 nm [26]. Most carotenoids absorb lights in the region between 400 and 500 nm, while chlorophylls absorb light at 645 and 662 nm at room temperature [27].

The content of chlorophyll a and carotenoids is calculated based on the following formula [28]:

$$\text{Chlorophyll a} = 11.75 \times A_{662} - 2,350 \times A_{645} \quad (2)$$

$$\text{Carotenoid} = \frac{1000 \times A_{470} - 2.270 - 81.4(18.61 A_{645} - 3.960 A_{662})}{227} \quad (3)$$

## 2.6 Assessment of phycobiliproteins compounds

Phycobiliproteins was extracted using the freeze thawing method from dry biomass of *S. platensis* powder. The sample was macerated with 1.5% CaCl<sub>2</sub> (1:20) (w/v) and homogenized with vortex for 1 minute. Samples are freezing in the freezer (-4 °C) for 12 hours, followed by thawing for 12 hours at room temperature [29]. The freeze thawing process is carried out 2 cycles. Filtrate was centrifuged at 4000 rpm for 10 minutes, so that crude extract is obtained. The crude extract is analyzed by the content of Phycocyanin using a formula [24].

$$\text{Phycocyanin} = \frac{A_{620} - (0.474 \times A_{652})}{5.34} \quad (4)$$

## 2.7 Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) at the level of significance of 0.05. A multiple comparison (LSD) test was used to examine significant differences among treatments using IBM SPSS Statistics 20 Computer Software. Before the analysis, the raw data were normalized using some transformation depend on the type of data.

## 3 Results and discussion

*S. platensis* was macerated using aquadest to dissolve the phycobiliprotein pigment, because phycobiliprotein is a natural pigment which is soluble in polar solvents [30]. While *G. arcuata* and *U. Lactuca* extracted step by step with n-hexane (non polar), ethyl acetate (semi-polar) and methanol (polar) solvents. This is intended to get the maximum extraction. This because each solvent has the ability to dissolve compounds with the same polarity [31]. The general principle in solvent extraction “like dissolve like” means that suitable solvent only dissolve suitable substance with similar polarities [32].

It is necessary to measure wavelengths which have maximum absorbance value of DPPH, caused the type and specificity of the instrument used and the time of observation can cause the difference of maximum wavelength [33]. In this study, maximum absorbance value of DPPH is reached at a wavelength of 515 nm. [34] [35].

Based on results of the antioxidant activity, SPA extract was able to inhibit 46.12±2.03 % (Table 1) of free radicals. In this present research, the result of inhibition percentage was slightly lower than 77.47 % [7]. Its because the extract produced is not a pure compound and presence of impurities. Furthermore, SPA was extracted in the form of dry powder. Actually, fresh samples will have antioxidant activity better than dry sample [36], since drying process are reported to have a negative effect to antioxidant compounds.

**Table 1.** Radical Scavenging Activity, Total Phenolic Compound, Pigment of SPA

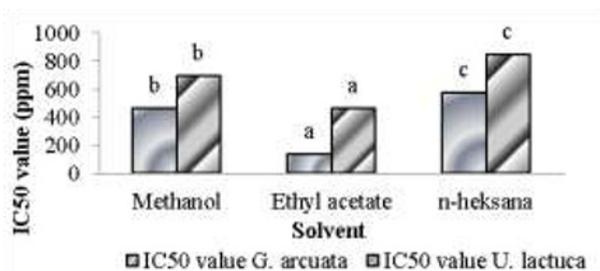
Content	Unit	Value
RSA	%	46.2±2.03
TPC	mg GAE /g sample	26.64±0.16
Phycocyanin	mg/g	0.30±0.09
Allo-Phycocyanin	mg/g	0.372±0.01
Phycoerytherin	mg/g	0.247±0.02

Radical scavenging capacity of extract related to their concentration of total phenolic content (TPC). Many polyphenols contribute significantly to the antioxidant activity and act as a high effective free radical scavengers. These are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals [17].

Total phenolic content of SPA extract is 26.64±0.16 mg GAE/g samples (Table 1.) This result is much higher than 2.936±0.063 mg/g GAE samples [37] and slightly lower than 31.9 mg/g GAE sample [7]. The different result occurs because the total phenolic content of SPA are varied, depends on many factors (algal species, origin, growth conditions, etc.) [38]. The phenolic compounds was detected in spirulina is catechin, epicatechin, pyrocatechol [38], pyrogallol, gallic, protocatechuic and salicylic [39].

Antioxidant activity can also be affected by pigment content. Natural pigments phycobiliproteins which occur in SPA are phycocyanin and allophycocyanin [40]. SPA have total phycobiliprotein content consisting of phycocyanin 1.3 mg/g, allo phycocyanin 4.1 mg/g and phycoerytherin 2.83 mg/g (Table 1).

The free radical scavenging activity of *G. arcuata* and *U. lactuca* extract was assessed by the DPPH assay. Based on results of the antioxidant activity of *G. arcuata* and *U. lactuca* extract, each solvent performed different IC<sub>50</sub> values (Figure 1). A significant decrease in the concentration of DPPH radical was observed due to the scavenging ability of the seaweeds. This study showed the difference of solvent polarity (methanol, ethanol, ethyl acetate, and n-hexane) exhibited the different antioxidant activity. The results showed that the best IC<sub>50</sub> values in GAE and ULE were 136.3±0.28 ppm and 462.6 ±2.44 ppm, respectively. These indicate that the extract contents was dominated by semipolar compounds and polyphenol group such as tannin, chlorophyll and carotenoids [41].

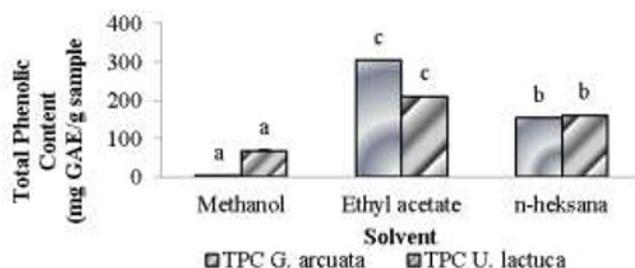


**Figure 1.** IC<sub>50</sub> value of *G. arcuata* and *U. lactuca* extract (ppm) macerated in different solvents. Bars with different letters indicate the significantly difference ( $p < 0.05$ ).

There was a significant difference in IC<sub>50</sub> values in two extract of *G. arcuata* and *U. lactuca*. GAE has moderate antioxidant activity with IC<sub>50</sub> value of 136.3±0.28, while extract of ULE has a very weak antioxidant activity with IC<sub>50</sub> values more than 400 ppm. Every species has a different compounds which affect its antioxidant activity and depends on extraction method, season, location and the species used in the study [42].

In this research, antioxidant activity of GAE was better than of *G. verrucosa* from Pok Tunggal water ( $IC_{50}$ = 188.53 ppm) [13], while antioxidant activity of ULE was still relatively low compared by *U. lactuca* extract [43] that have strong antioxidant activity ( $IC_{50}$ = 60.975 ppm). The different result occurs because the antioxidant activity are varied, depends on the species of seaweed [44], location [45] and seasons [14], respectively. Antioxidant compound act as a hydrogen atom donor [46]. Antioxidant compounds can inhibit free radicals by turning them into less dangerous free radicals [47].

There are high correlation between antioxidant and total phenolic content. The ability to reduce free radicals is related to the hydroxyl groups present in phenol compounds [48]. The hydroxyl group function act as a contributor to hydrogen atoms. It will reacting with free radicals through the electron transfer mechanism. There is a strong correlation between total phenolic content and the antioxidant activity potential with correlation coefficients 0.93 [49], and known that 99% of antioxidant activity is the result of the phenol compounds contribution, whereas 1% is thought to be a contribution from other compounds that have antioxidant abilities [50]. It is well known that the antioxidant activity of seaweed extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [51][59].

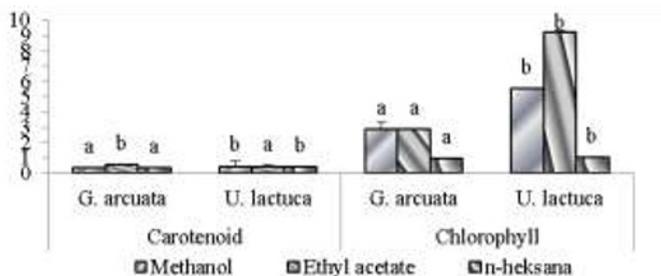


**Figure 2.** TPC value of *G. arcuata* and *U. lactuca* extract macerated in different solvents. Bars with different letters indicate the significantly difference ( $p < 0.05$ ).

Different extract had a different total phenolic compound (Figure 2.) Different phenolics can react differently to the Folin-Ciocalteu reagent, exhibiting lower absorption and hence leading to less estimation of phenolic compounds [52]. The highest content TPC were found in GAE ( $303,507 \pm 1,155$  mg GAE/g sample) and ULE ( $210,129 \pm 1,387$  mg GAE/g sample). This result is much higher than *G. gracilis* ethyl acetate extract which is  $35.53 \pm 1.47$  mg GAE/g sample [53]. And also higher than total phenolic compound of *U. lactuca* extract which is  $56.8 \pm 14.5$  mg GAE/g sample [54]. The high content of this compound in ethyl acetate extract is thought to have a soluble polyphenols biocompounds such as tannins and flavanols.

Antioxidant activity can also be affected by pigment content. Chlorophyll a and carotenoids are extracted using organic solvents such as methanol, ethyl acetate and n-hexane. It is because carotenoids are hydrophobic [55]. Hydrophobic is a property of a substance that repels water, lacking affinity for water, and tend to repel or not to absorb water. The results of *G. arcuata* and *U. lactuca* extract pigment (Figure 3.) shows that the highest chlorophyll a and carotenoid was reached by ethyl acetate. GAE extract contains  $2,845 \pm 0,069$  mg/g of chlorophyll and  $0,528 \pm 0,009$   $\mu$ mol/g carotenoids, while ULE extract contains  $9,216 \pm 0,103$  mg/g of chlorophyll and  $0,442 \pm 0,046$   $\mu$ mol/g carotenoids.

The interest in carotenoids found in plants over the last years is not only due to their A provitamin activity but also due to their reduction of oxidative stress in the organism by capturing oxygen radicals. Carotenoids can inhibit active radicals by transferring electrons, giving hydrogen atoms to radicals or attaching to radicals [56].



**Figure 3.** Pigment content of of *G. arcuata* and *U. lactuca* extract macerated in different solvents. Bars with different letters indicate the significantly difference ( $p < 0.05$ ).

Another pigment that have the same functions with carotenoid is chlorophyll. Chlorophyll is the main pigment found in seaweed and cooperate with carotenoid to absorb energy from the light [47]. Chlorophylls act as antioxidants to prevent oxidative DNA damage and lipid peroxidation both by chelating reactive ions and by scavenging free radicals [57]. The alterations in pigment concentration are determined by the interaction between two factors, light intensity and nutrient availability [58].

## 4 Conclusion

SPA extract was able to inhibit  $46.12 \pm 2.03$  % of free radicals, contains total phenolic content  $26.64 \pm 0.16$  mg GAE/g samples and dominated by phycocyanin pigments ( $0.301 \pm 0.09$  mg/g). The best  $IC_{50}$  values, total phenolic content, chlorophyll a and carotenoid in *G. arcuata* and *U. lactuca* reached by ethyl acetate. It can be concluded that macroalgae extracted with ethyl acetate categorized as moderate antioxidant.

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