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# *In vitro* Antioxidant Activity of the Hexane and Methanolic Extracts of *Sargassum baccularia* and *Cladophora patentiramea*

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**Abstract:** *In vitro* antioxidant effects and phenolic contents of the hexane and methanolic extracts of *Sargassum baccularia* and *Cladophora patentiramea* were tested in this study. The antioxidant activity was evaluated with the DPPH (2, 2-diphenyl-1-picrylhydrasyl) method and the reducing power. The hexane extracts of *Sargassum baccularia* had shown good DPPH radical scavenging activity. The methanolic extract of *Cladophora patentiramea* exhibited promising result at higher concentration. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. BHT was used as standard antioxidant and positive control. *Cladophora patentiramea* exhibited higher reducing power than *Sargassum baccularia*. The reducing power of extracts was carried out with ascorbic acid as a standard reducing agent. At the same time the phenolic content of the extracts was determined using Folin-Ciocalteau reagent to evaluate their contribution to total antioxidant activity. The hexane extracts of *Sargassum baccularia* and the methanolic extracts of *Cladophora patentiramea* had higher phenolic content. In these two marine macro algae extracts there was a remarkable concentration dependent DPPH scavenging, reducing power and phenolic content was exhibited.

**Key words:** DPPH, reducing power, total phenolic content

## INTRODUCTION

In living organisms the Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are known to cause damage to lipids, proteins, enzymes and nucleic acids leading to cell or tissue injury implicated in the processes of aging as well as in wide range of degenerative diseases including inflammation, cancer, atherosclerosis, diabetes, liver injury, Alzheimer, Parkinson and coronary heart pathologies, among others (Duan *et al*., 2006). It is commonly recognized that antioxidants can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutations and therefore, help prevent cancer or heart disease.

Recently, there is a growing interest on the discovery of natural antioxidants, mainly for two reasons: (1) there are epidemical and clinical evidences suggesting that consumption of vegetables and fruits reduces the risk of developing chronic disease, e.g., cancer and (2) phytochemicals are generally safer than synthetic chemicals (Dastmalchi *et al*., 2007). Therefore, the search for natural antioxidants as alternatives to synthetic ones is of great interest among researchers.

Seaweeds have been use as a novel food with potential nutritional benefits and in industry and medicine for various purposes (Santoso *et al*., 2004). Recently, much attention has been paid on the anti-tumor activity, anticholesterolemic activity and antioxidant activity of seaweed constituents. Consequently, antioxidant activity is intensively focused due to the currently growing demand from the pharmaceutical industry where there is interest in anti-aging and anticarcinogenic natural bioactive compounds, which possess health benefits. Almost all photosynthesizing plants including seaweeds are exposed to a combination of light and high oxygen concentrations, which lead to the formation of free radicals and other strong oxidizing agents, but they seldom suffer any serious photodynamic damage during metabolism. This fact implies that their cells have some protective antioxidative mechanisms and compounds (Matsukawa *et al*., 1997). Marine algae are considered to be a rich source of antioxidants (Cahyana *et al*., 1992). Recently, the potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin and carotenoid etc.) and polyphenols (phenolic acid, flavonoid and tannin etc). Those compounds are widely distributed in plants or algae and are known to exhibit

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higher antioxidative activities. The activities have been reported through various methods of reactive oxygen species scavenging activities and the inhibition of lipid peroxidation (Siriwardhana *et al*., 2003).

Therefore, the current communication focussed on the investigation and evaluation of the antioxidant activities of hexane and methanolic extracts that were derived from *Sargassum baccularia* and *Cladophora patentiramea*. Furthermore, the total phenolic content of all extract were also determined.

## MATERIALS AND METHODS

This research has been conducted from 4th August to 7th November 2008.

**Plant material:** Marine macro algae samples of the selected species viz., *Sargassum baccularia* and *Cladophora patentiramea* were collected from the coastal regions of Peninsular Malaysia and species authentication were done by the algae researchers in UM. The seaweeds will be cleaned thoroughly, air-dried and stored at -20°C until further use.

**Preparation of sample:** The dried samples were cut into small pieces and grind into fine power using a dry grinder. The ground samples were sieved to get uniform particle size, then kept in an air-tight container and stored in a freezer (-20°C) until further analysis.

**Preparation of extracts:** Each ground sample (10 g) was weighed and transferred into a beaker and extracted with hexane (150 mL) and stirred for 6 h with the aid of a magnetic stirrer. The extraction was repeated twice. The combined hexane extracts were evaporated under reduced pressure to a dark green semisolid. The extract got from the above process was stored in screw cap vials. The same procedure was repeated with polar solvent such as methanol.

**Antioxidant assay:** The antioxidant activity of seaweed extracts were determined by different methods such as, the DPPH free radical scavenging assay and reducing power methods. The different extracts were dissolved in methanol at the concentration of 2 mg $mL^{-1}$. All the assays were carried out in triplicate and average value was considered.

**DPPH radical-scavenging activity:** The scavenging effects of samples for DPPH radical were monitored according to the method of Yen and Chen (1995). Briefly, a 2.0 mL aliquot of test sample (in methanol) was added 2.0 mL of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark and its absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated using the follow equation:

$$\text{DPPH radical} - \text{scavenging}\,(\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right] \times 100$$

where, the $A_{control}$ is the absorbance of the control (DPPH solution without sample), the $A_{sample}$ is the absorbance of the test sample (DPPH solution plus test sample). Synthetic antioxidant, BHT was used as positive control.

**Reducing power:** The reducing power of all samples was determined as described by a literature report by Dorman *et al*. (2003). Generally, 1 mL of each sample dissolved in distilled water was mixed with 1.0 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of a 1% aqueous potassium ferricyanide [$K_3Fe(CN)_6$] solution. After 30 min incubation at 50°C, 1.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged for 10 min. Finally, 2.0 mL of the upper layer were mixed with 2.0 mL of distilled water and 0.5 mL of 0.1% aqueous $FeCl_3$ and the absorbance was recorded at 700 nm. As a control, ascorbic acid was used.

**Determination of total phenolic content:** Total phenols of the hexane and methanolic extracts were determined according to the Folin-Ciocalteu method (Velioglu *et al*., 1998). A 1.0 mL aliquot of sample was added to 1.5 mL of deionized water and 0.5 mL of 0.1 M Folin-Ciocalteu reagent and the contents were mixed thoroughly. After 1 min, 1.0 mL of 20% sodium carbonate solution was added and the mixture was again mixed thoroughly. The controls contained all the reaction reagents except the sample. After 30 min of incubation at 37°C, the absorbance was measured at 750 nm and compared to a gallic acid calibration curve. Total phenolics were estimated as Gallic Acid Equivalent (GAE).

## RESULTS AND DISCUSSION

### Antioxidant assay

**DPPH scavenging activity:** Like reducing power, the DPPH radical scavenging activity of the extract increases with increasing concentration, 50.90% DPPH racial scavenging. Nevertheless, it was 89.85% in the presence of 2 mg $mL^{-1}$ BHT (2, 6-di-tert-butyl-4-methylphenol) as standard (Table 1).

The methanolic extract of *Sargassum baccularia* was found to less effective than hexane extract. The DPPH racial scavenging activity of the extract increases with increasing concentration,only 36.27% DPPH racial scavenging. Nevertheless, it was 89.85% in the presence of 2.000 mg $mL^{-1}$ BHT (Table 2).

Table 1: Antioxidant activity of hexane extract of *Sargassum baccularia*

| | Absorbance (517 nm) | | Activity (%) | |
|---|---|---|---|---|
| Concentration (mg mL$^{-1}$) | Sample | Standard | Sample | Standard |
| 0.125 | 0.538 | 0.190 | 19.70 | 71.64 |
| 0.250 | 0.430 | 0.157 | 35.82 | 76.57 |
| 0.500 | 0.383 | 0.125 | 42.84 | 81.34 |
| 1.000 | 0.360 | 0.092 | 46.27 | 86.27 |
| 2.000 | 0.329 | 0.068 | 50.90 | 89.85 |

Table 2: Antioxidant activity of methanolic extract of *Sargassum accularia*

| | Absorbance (517 nm) | | Activity (%) | |
|---|---|---|---|---|
| Concentration (mg mL$^{-1}$) | Sample | Standard | Sample | Standard |
| 0.125 | 0.504 | 0.190 | 24.78 | 71.64 |
| 0.250 | 0.484 | 0.157 | 27.76 | 76.57 |
| 0.500 | 0.474 | 0.125 | 29.25 | 81.34 |
| 1.000 | 0.462 | 0.092 | 31.04 | 86.27 |
| 2.000 | 0.427 | 0.068 | 36.27 | 89.85 |

Table 3: Antioxidant activity of hexane extract of *Cladophora patentiramea*

| | Absorbance (517 nm) | | Activity (%) | |
|---|---|---|---|---|
| Concentration (mg mL$^{-1}$) | Sample | Standard | Sample | Standard |
| 0.125 | 0.619 | 0.190 | 7.61 | 71.64 |
| 0.250 | 0.570 | 0.157 | 14.92 | 76.57 |
| 0.500 | 0.544 | 0.125 | 18.81 | 81.34 |
| 1.000 | 0.513 | 0.092 | 23.43 | 86.27 |
| 2.000 | 0.470 | 0.068 | 29.85 | 89.85 |

Table 4: Antioxidant activity of methanolic extract of *Cladophora patentiramea*

| | Absorbance (517 nm) | | Activity (%) | |
|---|---|---|---|---|
| Concentration (mg mL$^{-1}$) | Sample | Standard | Sample | Standard |
| 0.125 | 0.448 | 0.190 | 33.13 | 71.64 |
| 0.250 | 0.422 | 0.157 | 37.01 | 76.57 |
| 0.500 | 0.397 | 0.125 | 40.75 | 81.34 |
| 1.000 | 0.387 | 0.092 | 42.84 | 86.27 |
| 2.000 | 0.344 | 0.068 | 48.66 | 89.85 |

The like reducing power, the DPPH racial scavenging activity of the extract increases with increasing concentration, only 29.85% DPPH racial scavenging was present for 2 mg mL$^{-1}$. This result found to be lower than that of hexane extract of *Sargassum baccularia*. Nevertheless, it was 89.85% in the presence of 2 mg mL$^{-1}$ BHT. Although this algae extract shows lower scavenging activity in comparison to BHT (Table 3). Marine algae extract exhibited antioxidative potential and increased concentration of algae extract has shown increased antioxidative potential.

The methanolic extract of *Cladophora patentiramea* was found to most effective than hexane extract (Table 4). The DPPH racial scavenging activity of the extract increases with increasing concentration exhibited only 48.66% DPPH racial scavenging was noted. Nevertheless, it was 89.85% in the presence of 2 mg mL$^{-1}$ BHT. These results suggest that hexane extracts of *Sargassum baccularia* exhibited little better than other extracts.

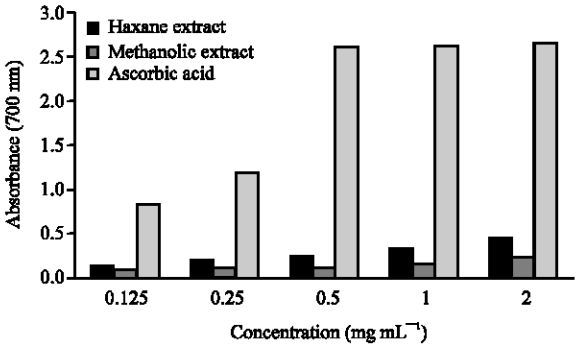


Fig. 1: Reducing power of hexane and methanolic extracts of *Sargassum baccularia*

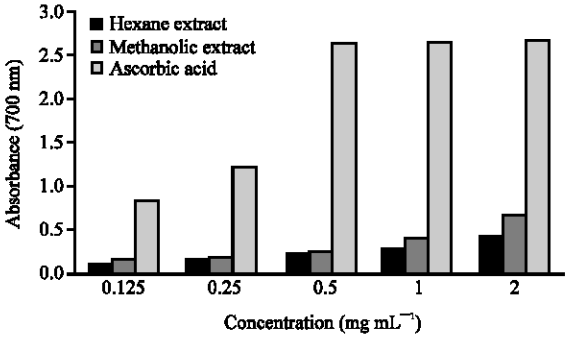


Fig. 2: Reducing power of hexane and methanolic extracts of *Cladophora patentiramea*

**Reducing power:** Different extracts of *Sargassum baccularia* exhibited good reducing power. The reducing power of extracts of *Sargassum baccularia* along with that of ascorbic acid at concentrations between 0.1250-2 mg mL$^{-1}$. High absorbance indicates high reducing power. The reducing power of algae hexane extract of *Sargassum baccularia* leaf as the amount of extract increases (Fig. 1). However, this reducing power is lower than that of ascorbic acid which was used as control. Therefore, the absorbance of ascorbic acid in a sample was (2 mg mL$^{-1}$) 2.661 while at the 2 mg mL$^{-1}$ hexane extract concentration it was 0.460. Nevertheless, the reducing power of methanolic extract of *Sargassum baccularia* was 0.223 and it was considerably lower than those of hexane extract (Fig. 1).

The reducing power of *Cladophora patentiramea* extract has shown good reducing power than *Sargassum baccularia*. As the amount of extract increase, the reducing power also increases (Fig. 2). However, this reducing power is lower than that of ascorbic acid which was used as control. Therefore, the absorbance of ascorbic acid in a sample was (2 mg mL$^{-1}$) 2.661 while at the 2 mg mL$^{-1}$ hexane extract concentration it was 0.421. Nevertheless the reducing power of methanolic

Table 5: Total amount of plant phenolic content

| | GAE±SD | |
|---|---|---|
| Seaweed | Hexane | Methanol |
| *Sargassum baccularia* | 18.78±1.20 | 5.616±0.236 |
| *Cladophora patentiramea* | 9.624±0.404 | 15.616±0.236 |

extract of *Cladophora patentiramea* was 0.675 (Fig. 2) than those of *Sargassum baccularia* (0.460). In both cases of *Sargassum baccularia* and *Cladophora patentiramea* there is a remarkable concentration dependent reducing power was exhibited. This variation in reducing activity may be due to crude nature of algae extracts and availability of different phytochemicals in these algae.

**Total phenolic content:** So far as seaweed phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected seaweed extracts. The content of phenolic compounds (mg $g^{-1}$) in hexane and methanolic extracts, determined from regression equation of calibration curve ($y = 3.648x + 0.01582$, $R^2 = 0.9948$) and expressed in Gallic Acid equivalents (GAE), varied between 5.616 and 18.78 (Table 5). The highest amounts were found in the hexane extracts of *Sargassum baccularia* and the methanolic extracts of *Cladophora patentiramea*. Total phenolic content of different extracts were solvent dependent.

## CONCLUSION

The present study demonstrated that the hexane extracts of *Sargassum baccularia* and the methanolic extracts of *Cladophora patentiramea* were the strongest radical scavengers in both DPPH and reducing assays among the algae screened. They are promising plants for more detailed investigation of their antioxidant properties, identification of the active components and the development of therapeutic products to protect against certain diseases, subject to toxicity evaluation. In addition, they could be considered for the future applications in medicine, food production or cosmetic industry.

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