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Determination of Antioxidant Activity for Seven Types of Macroalgae

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Abstract. The present study was conducted to evaluate the antioxidant activity of seven types of macroalgae extract from Malaysia. The extract was prepared with methanol respectively. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and reducing power were used to study their antioxidant activity while total phenolic content was measured using Folic-Ciocalteu method. The macroalgae extracts were compared with commercial antioxidant, butylated hydroxyanisole (BHA). Total phenolic content of macroalgae extracts were expressed in Gallic acid equivalent, mg/L. *Caulerpa racemosa* showed the highest total phenolic content with the value of 19.711 mgGAE/L ± 0.2546. In DPPH free radical scavenging activity assay, *Turbinaria conoides* showed the highest scavenging activity in 50 mg/ml extract concentration with the value of 73.57 % ± 0.5739 compared to another macroalgae extracts. The IC_{50} value of *Turbinaria conoides* extracts is 2.46 mg/ml. Low IC_{50} value will indicates the strong ability of the macroalgae extract to act as a DPPH scavenger. *Caulerpa lentillifera* showed the highest absorbance reading in 50 mg/ml extract concentration with the value of 0.603 ± 0.0015 in reducing power assay. Increased absorbance of the reaction mixture indicates greater reducing power.

Keywords: antioxidant, DPPH, Macroalgae, Reducing Power, Total Phenolic Content

1. Introduction

Macroalgae are included in the non-flowering plants those whose body is generally undifferentiated into leaves, stem and roots. Marine macroalgae extracts have been demonstrated to have strong antioxidant properties [1], [2]. Macroalgae are known as functional food because of their richness in lipids, minerals and certain vitamins and also several bioactive substances like polysaccharides, protein and polyphenols [3]. Free radicals are responsible for aging and causing various human diseases. The antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases. The primary radicals are reduced to nonradical chemical compounds and then converted to oxidize antioxidant radicals by donating the hydrogen radicals [4]. This action helps in protecting the body from degenerative diseases. The macroalgae have been used for centuries in the preparation of salads, soups and also as low-calorie foods in Asia [5]. Most Malaysians exhibit little interest in consuming edible macroalgae, but it is still consumed by small pockets of the population along the coastal areas of Peninsular Malaysia and East Malaysia [6]. Six types of macroalgae such as *Caulerpa racemosa, Caulerpa lentillifera, Padina gymnospora, Sargassum baccularia, Sargassum binderi* and *Turbinaria conoides* reported contain a high nutritional value. From the result, *Turbinaria conoides* showed higher content of ash (4.7%), vitamin A (24.1 mg/kg), niacin (274 mg/kg), sodium (13085 mg/kg), potassium (21137 mg/kg), calcium (2353 mg/kg), magnesium (4026 mg/kg), copper (25.2 mg/kg) and zinc (49.1 mg/kg) [7]. Macroalgae constitutes a commercially important renewable resource. Macroalgae such as *Sargassum, Padina, Dictyota* and *Gracilaria* sp. can be used as fertilizers, food additives and animal feed [8]. This research was conducted to explore the ability of several types of macroalgae as a potential antioxidant resource by a recent interest of macroalgae as a source of natural and healthy food.

2. Materials and Methods

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2.1. Algal materials and preparations of macroalgae extracts

Collection of macroalgae was done along the shore line of Cape Rachado during the low tide. Cape Rachado is a fringing coral reef located at Port Dickson, Negeri Sembilan, West Coast of Peninsular Malaysia. Six types of macroalgae namely *Caulerpa racemosa* (Forsskal) J. Agardh, *Caulerpa lentillifera* J. Agardh, *Padina gymnospora* (Kützing) Vickers, *Sargassum baccularia* C. Agardh, *Sargassum binderi* Sonder and *Turbinaria conoides* (J. Agardh) Kützing were sampled in Cape Rachado. The edible red macroalgae namely *Eucheuma cottonii* were purchased from cultivation area at Sabah, Malaysia. All the samples were rinsed with distilled water to remove salt and debris and then dried. The dried samples were cut into small pieces and ground into fine powder using a grinder. The ground samples were sieved to get uniform particle size, then kept in an air-tight container and stored in a freezer (-20^0C) until further analysis. Each ground sample was weighed and transferred into a beaker. Methanol was added in a concentration of 50 mg/ml sample. The extract was separated from the residue by filtration through Whatman No. 1 filter paper. The residual solvent of methanolic extract was removed under reduced pressure at 40^0C using rotary evaporator.

2.2. Total phenolic content (TPC)

The total phenolic content of macroalgae extracts was measured using Folin Ciocalteu method [9]. A 0.02 ml aliquot of crude extracts dissolved in water was pipetted into a test tube containing 1.58ml of distilled water and 0.1ml of Folin-Ciocalteu's reagent. After mixing the contents, 0.3ml of saturated sodium carbonate (Na_2CO_3) was added to the mixture. The contents were vortexed for 15 seconds and then left to stand at 40^oC for 30 min. Absorbance measurements were recorded at 765nm using a spectrophotometer. Estimation of the phenolic compounds was carried out in triplicate. The results were mean values and were expressed as mgGAE (gallic acid equivalents)/L. The calibration equation for gallic acid was $y = 0.073x + 0.057$ ($R^2 = 0.914$).

2.3. Reducing power assay

The reducing power of the prepared extracts was determined according to reducing power assay [10]. Each extract (10 mg, 20mg, 30mg, 40mg and 50mg) was dissolved in 1.0 ml of distilled water to which was added 2.5 ml of a 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of a 1% (w/v) solution of potassium ferricyanide (Sigma). The mixture was incubated in a water bath at 50^0C for 20 min. Then, 2.5 ml of a 10% (w/v) trichloroacetic acid solution (Sigma) was added and the mixture was then centrifuged at 650 x g for 10 min. A 2.5 ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 0.5 ml of a 0.1% (w/v) solution of ferric chloride. Absorbance of the reaction mixture was read spectrophotometrically at 700nm. Mean values from three independent samples were calculated for each extract.

2.4. DPPH free radical scavenging assay

The scavenging activity of macroalgae extracts were measured using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical assay [11]. An aliquot of 100-975µl of macroalgae extract (0-50 mg/ml), were mixed with 0 - 875µl of methanol. The mixture was then added to 1 ml of 25µl DPPH (8 mg/ml). The reaction mixture were incubated at room temperature and allowed to react for 30 minutes. The optical density was measured at 520nm using a UV-Vis spectrophotometer. BHA was used as a positive control. DPPH was expressed in terms of ascorbic acid equivalent antioxidant capacity which was calculated based on its concentration of extract required to reduce DPPH radicals by 50%. The capability of macroalgae extracts to scavenge the DPPH radical was calculated by using Eq.1:

$$\text{Scavenging activity (\%)} = 1 - \left[\frac{Absorbance\ of\ sample\ at\ 520nm}{Absorbance\ of\ control\ at\ 520nm} \right] \times 100 \quad (1)$$

IC_{50} value was determined from the plotted graph of scavenging activity versus the concentration of macroalgae extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH scavenged. IC_{50} value of macroalgae extracts was calculated based on tread line equation $y = 7.038x + 1.383$ ($R^2 = 0.949$).

3. Results and Discussions

There is a considerable interest in the food industry as well as pharmaceutical industry for the development of antioxidants from natural sources such as marine flora and fauna. The marine macroalgae represent one of the richest sources of natural antioxidant [12]. In this study, we found that the all seven types of macroalgae extract contain an antioxidant properties. Thus, the extracts can be recommended for its application as a safe antioxidant in food processing industry.

3.1. Total phenolic content (TPC)

The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate. It is used for the colorimetric assay of phenolic antioxidants and polyphenol antioxidants [9]. The amount of the substance being tested needed to inhibit the oxidation of the reagent was measured in this assay [13]. Total phenolic compounds are found to be well correlated with antioxidant potential [14]. BHA act as a positive control, showed the highest total phenolic content (48.654 mgGAE/L ± 0.4194) and *Caulerpa racemosa* also show high total phenolic content with the value 19.711 mgGAE/L ± 0.2546 compared to another types of macroalgae. (Table 1).

Table 1. Mean of total phenolic content (mgGAE/L) of BHA and seven types of macroalgae

Sample	Mean ± SD
BHA	48.654 ± 0.4194
Caulerpa racemosa	19.711 ± 0.2546
Padina gymnospora	15.320 ± 0.4334
Caulerpa lentillifera	14.423 ± 0.3331
Sargassum binderi	5.897 ± 0.0560
Turbinaria conoides	5.545 ± 0.0554
Sargassum baccularia	4.359 ± 0.2217
Eucheuma cottonii	2.82 ± 0.018

3.2. Reducing power assay

Fig. 1 shows the absorbance reading in 700 nm of seven types of macroalgae and BHA (positive control) on reducing power assay. From the result, it showed the highest absorbance reading (1.561 ± 0.0049) in 50 mg/ml BHA. BHA exhibited a stronger reducing power compared to macroalgae. The increased absorbance of the reaction mixture will indicates greater reducing power because the antioxidant compound are reducing agents and capable of donating a single electron or hydrogen atom for reduction.

3.3. DPPH free radical scavenging assay

DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger. The antioxidative compounds act as proton radical scavenger was measured by this assay [15]. DPPH has been used extensively as a free radical to evaluate reducing substances [16]. Table 2 shows the scavenging activity (%) of DPPH radical of seven types of macroalgae, ascorbic acid (standard reference) and BHA (positive control) at different concentration (mg/ml). For DPPH radical scavenging activity assay, BHA showed highest scavenging activity in 50 mg/ml concentrations with the value of 97.56 % ± 0.2145. while ascorbic acid also show high scavenging activity with the value 88.9 % ± 0.265 compared to the *Turbinaria conoides* that 73.57 % ± 0.5739. The comparison of the mean concentration for 50% free radical scavenging activity (IC_{50}) of ascorbic acid and macroalgae extracts also shown in Table 2. The IC_{50} of *Turbinaria conoides* is 2.46 mg/ml. Low IC_{50} value indicates strong ability of the extract to act as DPPH scavenger.

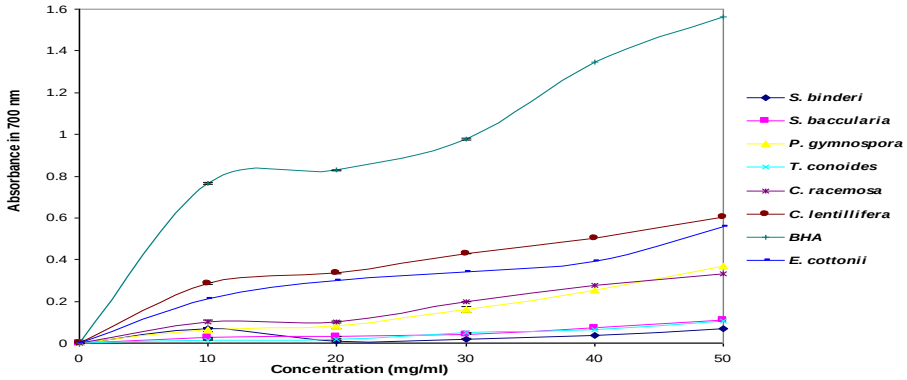

Fig. 1: Absorbance reading in 700 nm of seven types of macroalgae and BHA on reducing power assay

Table 2. IC_{50} value of seven types of macroalgae extracts and ascorbic acid

Sample/Extract	Scavenging activity (%)	IC_{50} value (mg/ml)
Ascorbic acid	88.9 ± 0.265	0.832
Turbinaria conoides	73.6 ± 0.574	2.46
Padina gymnospora	54.3 ± 1.157	4.89
Caulerpa racemosa	52.8 ± 0.578	4.91
Sargassum baccularia	45.9 ± 0.427	4.98
Sargassum binderi	44.1 ± 0.224	5.27
Caulerpa lentillifera	37.4 ± 0.815	6.74
Eucheuma cottonii	67.63 ± 0.153	7.3

3.4. Correlation between DPPH scavenging assay, reducing power and total phenolic content

The reducing power assay and total phenolic content have showed a positive significant correlation at the 0.01 level with the value of 0.889. At 0.05 levels, the reducing power assay and DPPH free radical scavenging activity assay have a positive correlation with the value of 0.661. It is difficult to decide in a screening for antioxidants from natural sources which of the macroalgae species studied can be considered as best, as each of them exhibits different antioxidant and/or scavenging activities.

4. Conclusion

The macroalgae have the potential as radical scavenger, power reducer and contained total phenolic compound. Seven types of macroalgae extracts showed moderate to good antioxidant properties, making it as potential health ingredient for human nutrition. More research is needed to establish the nutritional value of macroalgae especially in the fields of biochemical analysis that can contribute to human health.

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