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Antioxidant Activities and Total Phenolic Content of Macroalgae from Central Coast of Vietnam

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Antioxidant activities and total phenolic content of ethanolic extract of 41 marine algae species including red, brown and green algae from central coast of Vietnam were evaluated. The extract of *Sargassum polycystum*, *Chnoospora implexa*, *Chnoospora minima* demonstrated great DPPH radical scavenging activity with EC_{50} values (0.08, 0.2; 0.26 mg dry extract/L; respectively), extract from *Dictyota dichotoma* was found to be highest total antioxidant capacity (19.6 mg ascorbic acid equivalent/g dry seaweed) and *Sargassum microcystum* exhibited highest ferric reducing activity (23.1 mg Fe^{2+} equivalent/g dry seaweed). We found that only green algae showed a linear correlation between total phenolic content and antioxidant activities.

Key Words: Antioxidant, Algae, Vietnam.

INTRODUCTION

Antioxidant has become a very prominent topic in health consumption. Reactive oxygen species (ROS) is generated in living organisms during metabolism[1]. Excessive amounts of reactive oxygen species may be harmful because they can initiate biomolecular oxidations which lead to cell injury and death and create oxidative stress which results in numerous diseases and disorders. In addition, oxidative stress may cause inadvertent enzyme activation and oxidative damage to cellular systems[2-4].

Due to concerns on the toxic and carcinogenic effects of synthetic antioxidants, the search for alternatives from natural sources has received much interest. Seaweeds belong to a group of plants known as algae. In recent years, many marine algae extracts have been demonstrated to have strong antioxidant properties[5-8], but there are no such reports on the antioxidant activities of seaweeds from Vietnam. Although, there are many kinds of seaweed growing in the central coast of Vietnam. The total number of seaweed species along the Vietnamese coast is estimated to be nearly 1000 spp. About 639 species of seaweeds (269 Rhodophyta, 143 Phaeophyta, 151 Chlorophyta and 76 Cyanophyta) have been identified[9]. Vietnamese seaweeds were expected to develop a very effective antioxidant defence system due to the strong UV radiation in the tropical environment.

The present study was undertaken in order to examine antioxidant effects of crude ethanol extracts obtained from 41 marine macroalgae species including 07 red algae (Chlorophyta), 29 brown algae (Phaeophyta) and 05 green algae (Rhodophyta) harvested from the central coast of Vietnam. Antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, total antioxidant ability and ferric reducing activity. Since polyphenol is known as the main antioxidant compound in marine algae, therefore, in this work, the total phenolic content and the relationship between total phenolic contents and antioxidant capacities of the seaweed extracts were also investigated.

EXPERIMENTAL

2,2-Diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide and ferric chloride were purchased from Sigma Aldrich Chemie. Folin Ciocalteu, phloroglucinol and ethanol were obtained from Merck (Germany). All other solvents and chemicals were of analytical grade.

Seaweed collection: Forty one seaweed species were harvested from the central coast of Vietnam in the period of April-June, 2008 and identified by Dr.Le Nhu Hau, Nhatrang Institute of Technology Research and Application. A voucher specimen of each is deposited in Nhatrang Institute of Technology Research and Application. The collected seaweed was washed with tab water in order to remove salt, epiphytes and sand attached to the surface of the sample and then dried by air in a shade. The dried seaweed was crushed and ground into a powder form and passed through a 40-mesh sieve and stored at room temperature.

Preparation of dried seaweed extracts: 1 g of each algal sample was weighed then extracted with 20 mL of 90 % ethanol for 48 h. The extraction was carried out at 100 rpm in a shaker at room temperature. Samples were filtered and centrifuged at 10,000 rpm for 15 min. Resulting ethanol extracts (prepare in duplicate) were evaporated to dryness using vacuum evacuator at 40 ºC. The extracts were kept in fridge until using for experiments.

Total phenolic content (TPC): Total phenolic contents of the extracts in 90 % ethanol was measured using Folin Ciocalteu method as described[10]. Folin Ciocalteu's phenol reagent (1.0 mL) and 7.5 % w/v Na_2CO_3 (2.0 mL) were added to sample extract (0.5 mL sample add 0.5 mL distilled water) and the reaction mixture was incubated in the dark for 0.5 h. The absorbance of the reaction mixture was then measured at 765 nm. TPC was expressed in terms of mg phloroglucinol equivalents (PGE)/g of dried seaweed. The calibration equation for phloglucinol was y = 20.3 x-0.05. (R^2 = 0.9969).

Determination of total antioxidant capacity (TAC): Total antioxidant capacity was determined by the method of Prieto *et al.*[11]. The tubes containing 0.5 mL of seaweed extract, 0.5 mL distilled water and 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95 ºC for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank by an ELISA Reader (Bio-Rad, USA). All the results were expressed as mg ascobic acid equivalent (AAE) per gram of dry seaweed.

Determination of DPPH radical scavenging activity: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured following the method of Yen and Chen[12] 0.2, 0.4, 0.6, 0.8 and 1.0 mL of seaweed extract up to 1 mL by distilled water was added with 3.0 mL of 0.16 mM DPPH in methanol. The mixtures were then mixed vigorously and allowed to stand at room temperature in the dark for 0.5 h. The mixtures were then mixed vigorously and allowed to stand at room temperature in the dark for 0.5 h. The absorbance of the sample mixture was then measured at 517 nm using the UV-VIS spectrophotometer (ELISA Reader, Bio-Rad, USA). The control sample was prepared in the same manner as the preparation of sample mixtures except that deionised water was used instead of the extract. The blank sample was handled in the same manner but deionised water was used instead of a DPPH solution. Percentage of DPPH radical scavenging activity:

$$Sc\ (\%) = \left[1 - \frac{(A_{sample} - A_{blank})}{A_{control}}\right] \times 100$$

Here, $A_{control}$: Absorbance of control, A_{sample}: Absorbance of samples, A_{blank}: Absorbance of blank.

The EC_{50} values were calculated from dose-response curve by table curve program.

Positive control such as ascorbic acid, α-tocopherol, BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) were also measured and the EC_{50} of its control were calculated from five measurements.

Determination of ferric reducing activity (FRA): The reducing power was determined according to the method of Zhu *et al.*[13]. 1.0 mL of phosphate buffer (pH 7.2) and 1.0 mL of 1 % (w/v) potassium ferricyanide were added to 0.2 mL of polyphenol extract and 0.8 mL distilled water. The reaction mixture was then incubated at 50 ºC in a water bath for 20 min. Subsequently, 1.0 mL of acid trichloroacetic acid (10 %) was added to the solution. The solution was mixed with distilled water (0.6 mL) and 0.16 mL of ferric chloride. The absorbance of the mixture was measured at 655 nm by the UV-VIS spectrophotometer (ELISA Reader, Bio-Rad, USA). All the results were expressed as mg Fe^{2+} equivalent per gram of dry seaweed.

Statistical analysis: All experiments were performed in triplicate. The values are presented as mean ± SD. The means were compared by analysis of variance (ANOVA). Differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Total phenolic content (TPC): The result of TPC determination of 90 % ethanol extracts of 41 algae species were summarized in Table-1. Total phenolic contents of 41 extracts were ranged from (0.8-7.0 mg phloroglucinol equivalents/g dried seaweed depending on seaweed species. *Caulerpa cupressoides* had the highest TPC and brown algae generally contained higher levels of polyphenols than red and green ones. Comparing TPC of our samples with other seaweed resources in the world, we found that TPC from the seaweed harvested at central coast of Vietnam is similar with that of seaweed at Quingdao coast, China[14] and less than that of seaweed at Mexican sea[15].

DPPH Radical scavenging activity: DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger. This assay was used to test the ability of the antioxidative compounds functioning as proton radical scavengers or hydrogen donors[16].

EC_{50} values are summarized in Table-1. In this assay, all seaweed extracts have shown activity in DPPH radical scavenging suggesting that tropical macroalgae may develop an effective antioxidant defence system which may reflect an adaptation to high solar radiation. The extract from *Sargassum polycystum*, *Chnoospora implexa*, *Chnoospora minima*) exhibited great antioxidant potential with very low EC_{50} values (0.08, 0.2; 0.26 mg dry extract/L, respectively). This value is equivalent to EC_{50} of three commercial antioxidants were tested α-tocopherol (0.30 ± 0.03 mg/L), BHT (0.15 ± 0.02 mg/L), ascorbic acid (0.09 ± 0.03 mg/L). While the lowest antioxidant activity was observed in extract from *Gracilaria bailinae* with EC_{50} 29.89 mg/L. In accordance[15], various species from genus Gracilaria exhibited antioxidant activities with EC_{50} in the range of 28.9-72.7 mg/L. The green seaweed also exhibited a quite high antioxidant potential with EC_{50} values from 1.36-1.91 mg dry extracted seaweed/L). Among tested brown seaweed, Sargassum genus showed a big difference of antioxidant potential from species to species (EC_{50} in the range from 0.08-9.37 mg/L), while Padina and Dictyota genus has low activity and EC_{50} values were not so different among species. The same findings were reported[17,18].

Total antioxidant capacity (TAC) and ferric reducing activity (FRA): The results of total antioxidant capacity and

TABLE-1
TOTAL PHENOLIC CONTENT, TOTAL ANTIOXIDANT CAPACITY, FERRIC REDUCING ACTIVITY AND EC_{50} VALUE OF ETHANOL EXTRACTS OF 41 ALGAE SPECIES

No.	Species	TPC (mgPGE/g dry seaweed.)	TAC (mgAAE/g dry seaweed)	FRA (mgFe^{2+}/g dry seaweed)	EC_{50} (mg dry extract/L)
		Brown seaweed (Phaeophyta)			
1	*Padina boryana*	1.2 ± 0.3	7.6 ± 0.5	10.5 ± 0.5	2.02 ± 0.5
2	*Sarrgasum.binderi*	2.16 ± 0.3	5.13 ± 0.8	10.2 ± 0.7	1.47 ± 0.4
3	*Chnoospora implexa*	0.8 ± 0.1	5.1 ± 1.0	10.14 ± 0.8	0.2 ± 0.1
4	*Sargassum swartzii*	1.44 ± 0.5	3.63 ± 0.5	7.2 ± 0.4	9.37 ± 0.5
5	*S. cristaefolium*	3.0 ± 0.4	3.9 ± 0.7	7.5 ± 0.1	0.37 ± 0.1
6	*S. mcclureii*	2.34 ± 0.1	4.5 ± 0.3	8.1 ± 0.3	1.25 ± 0.3
7	*S. crassifolium*	3.6 ± 0.2	4.44 ± 0.5	7.8 ± 0.2	1.08 ± 0.4
8	*S. serratum*	1.04 ± 0.6	1.34 ± 0.4	5.85 ± 0.1	5.35 ± 1.0
9	*S. feldmannii*	0.3 ± 0.1	4.01 ± 0.2	6.3 ± 0.5	2.55 ± 0.7
10	*Turbinaria ornata*	1.41 ± 0.2	6.04 ± 0.4	7.4 ± 0.2	0.94 ± 0.3
11	*Padina australis*	0.98 ± 0.3	4.55 ± 0.7	5.07 ± 0.5	12.12 ± 0.8
12	*Hormophysa articulata*	0.88 ± 0.4	3.93 ± 0.1	5.3 ± 0.8	1.19 ± 0.4
13	*S. dentifolium*	4.4 ± 0.5	9.2 ± 0.2	14.4 ± 1.0	1.71 ± 0.2
14	*S. duplicatum*	3.06 ± 0.6	10.9 ± 0.3	19.1 ± 0.8	1.74 ± 0.3
15	*S. aemulum*	2.34 ± 0.2	6.7 ± 0.2	10.35 ± 0.6	1.57 ± 0.5
16	*S. kuetzingii*	3.4 ± 0.6	6.4 ± 0.2	13.7 ± 1.1	9.03 ± 1.0
17	*S. baccularia*	3.6 ± 0.1	7.92 ± 0.3	13.1 ± 1.2	1.93 ± 0.4
18	*S. henslowianum*	1.02 ± 0.4	6.16 ± 0.5	11.1 ± 0.5	1.34 ± 0.3
19	*Dictyota dichotoma*	1.34 ± 0.5	19.65 ± 0.4	9.4 ± 0.6	8.4 ± 0.2
20	*Dictyota linearis*	1.52 ± 0.8	18.36 ± 0.2	9.5 ± 0.3	8.0 ± 0.3
21	*Spatoglossum vietnamense*	0.98 ± 0.2	6.52 ± 0.5	9.9 ± 1.0	3.41 ± 0.4
22	*Turbinaria*	0.88 ± 0.2	8.81 ± 0.4	15.5 ± 1.0	2.03 ± 0.3
23	*S. microcystum*	2.54 ± 0.4	5.9 ± 0.5	13.6 ± 0.5	4.82 ± 0.5
24	*S. polycystum*	3.4 ± 0.02	5.4 ± 0.5	14.0 ± 0.5	0.08 ± 0.05
25	*S. serratum*	2.14 ± 0.1	2.92 ± 0.3	9.3 ± 1.0	1.44 ± 0.4
26	*S. microcystum*	0.98 ± 0.3	8.0 ± 0.7	23.2 ± 0.5	0.67 ± 0.3
27	*S. oligocystum*	1.52 ± 0.1	6.6 ± 0.3	14.9 ± 0.8	1.09 ± 0.4
28	*S. denticarpum*	2.2 ± 0.7	3.42 ± 0.4	13.1 ± 1.0	1.53 ± 0.3
29	*Sargassum. vietnamese*	3.4 ± 0.5	3.4 ± 0.2	7.6 ± 1.0	5.4 ± 0.8
		Red seaweed (Rhodophyta)			
1	*Gracilaria bailinae*	2.2 ± 0.1	2.4 ± 0.7	4.4 ± 0.8	29.89 ± 1.0
2	*Gracilaria firma*	1.7 ± 0.2	3.0 ± 0.5	3.7 ± 0.6	9.65 ± 0.2
3	*Acanthophora spicifera*	0.9 ± 0.2	2.52 ± 0.3	4.9 ± 0.5	7.8 ± 0.5
4	*Gracilaria salicornia*	1.7 ± 0.8	2.7 ± 0.4	4.2 ± 0.5	5.6 ± 0.5
5	*Callithamnion ramossisima*	2.2 ± 0.1	4.6 ± 0.8	4.56 ± 0.3	1.63 ± 0.3
6	*Porphyra Vietnameusis*	1.0 ± 0.2	2.1 ± 0.4	2.3 ± 0.1	2.2 ± 0.4
7	*Chnoospora minima*	2.0 ± 0.3	1.54 ± 0.3	2.8 ± 0.3	0.26 ± 0.1
		Green seaweed (Chlorophyta)			
1	*Ulva reticulata*	1.3 ± 0.04	3.96 ± 0.6	4.86 ± 0.6	1.36 ± 0.1
2	*Caulerpa cupressoides*	7.0 ± 0.5	11.1 ± 0.5	9.5 ± 0.04	1.91 ± 0.5
3	*Ulva papenfussii*	1.18 ± 0.2	3.9 ± 0.04	2.1 ± 0.2	1.54 ± 0.2
4	*Ulva intestinalis*	0.9 ± 0.1	3.36 ± 0.2	5.44 ± 0.5	1.71 ± 0.4
5	*Caulerpa rasemosa*	2.5 ± 0.1	5.6 ± 0.1	6.36 ± 0.2	1.63 ± 0.2

ferric reducing activity are summarized in Table-1. It shows that all test seaweed species showing total antioxidant capacity and ferric reducing activity with various degrees. The greatest total antioxidant capacity is from Dictyota genus including two species: *D. dichotoma* and *D. linearis* (19.65 and 18.36 mg ascorbic acid equivalent/g seaweed, respectively). While, the greatest ferric reducing activity is from *S. microcystum* (23.1 mg Fe^{2+} equivalent/g seaweed).

Among the tested algae, the brown algae as a group had the highest total antioxidant capacity and ferric reducing activity followed by the green algae and red algae. Total antioxidant capacity of brown, green and red algae were 6.56 ± 4.03; 5.58 ± 3.19; 2.69 ± 0.95 mg ascorbic acid equivalent/g seaweed, respectively. Ferric reducing activity of brown, green and red algae were 10.8 ± 4.18; 5.65 ± 2.67; 3.84 ± 0.971 mg Fe^{2+} equivalent/g seaweed, respectively. Our result is in agreement with Dovi Kelman *et al.*[19], while green algae shown a highest DPPH radical scavenging activity, followed by brown and red algae with EC_{50} values are 1.63 ± 0.2, 4.88 ± 10.11 and 8.15 ± 10.18 mg/L. Our result indicated that red algae had the lowest antioxidant activity.

The statistical analysis revealed that in brown and green algae, there was no significant relationship between the ferric reducing activity, DPPH scavenging activity, total antioxidant capacity and total phenolic content ($p < 0.05$). The correlation coefficient between the total antioxidant and phenolic content

was found to be very small in brown seaweed (R^2 = 0.046) and in red seaweed (R^2 = 0.119). Only green seaweed showed a linear correlation between TPC and total antioxidant capacity, ferric reducing activity and DPPH scavenging activity (R^2 = 0.999, 0.732 and 0.578; respectively) as shown in Fig. 1. It was reported that[20] high antioxidant potential of green algae Caulerpa was due to high TPC and the presence of some bioactive compound mainly sesquiterpenoid and ditecpenoid. Our results suggest that, phenolic compounds are main antioxidant component in green seaweed and other compounds such as tocopherols, ascorbic acid, carotenoids, phospholipids or polysaccharides play a role in the antioxidant activity of brown and red seaweeds[21]. There have been few studies on the relationship between the antioxidant activity and TPC for algae and these studies demonstrated that the relationship between two parameters was very small[22,23]. In this study, there are scientific evidences for phenolic compound contribute total oxidant capacity in green algae.

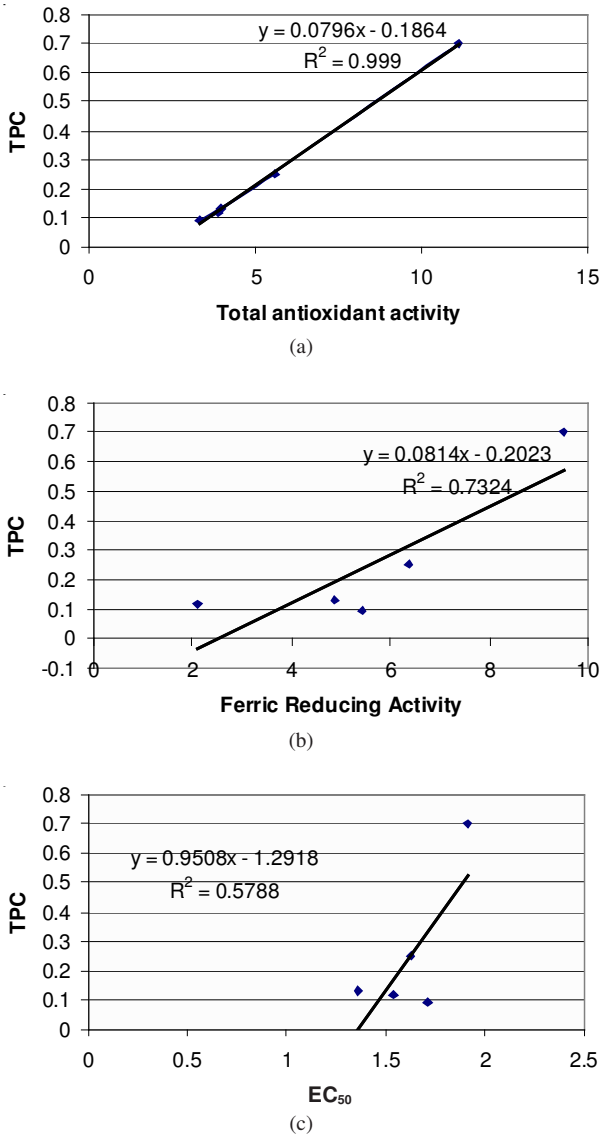

Fig. 1. Linear correlation between total phenolic content and total antioxidant capacity (a), ferric reducing activity (b) and DPPH scavenging activity (c) of green algae

Conclusion

It is the first significant assessment of the antioxidant activity of Vietnam algae. Present results indicate that 41 seaweed species were collected from the central coast of Vietnam present a significant capacity to show a variety of antioxidant activities, which makes them interesting for program screening natural products. Among tested algae, the brown algae were statistically the most active on total antioxidant capacity and ferric reducing activity and green algae showed a highest DPPH scavenging activity and a good linear correlation between total phenolic content and antioxidant activities.

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