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PRELIMINARY PHYCOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF SOME BROWN ALGAE SARGASSUM SPECIES FROM LAWAAN, EASTERN SAMAR

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ABSTRACT – Methanolic extracts of some Philippine Sargassum species (S. crassifolium, S. polycystum, S. gracillimum, S. hemiphyllum and S. cristaefolium) were evaluated for their phycochemical constituents and antioxidant properties. The presence of alkaloids, flavonoids, tannins and saponins was qualitatively screened. Total phenolic content was determined using Folin-Ciocalteu reagent in terms of gallic acid equivalents (GAE). Antioxidant activity was evaluated using diphenyl-1,2-picryl hydrazyl (DPPH) free radical scavenging activity assay, and Fe^{2+} chelating ability. Phycochemical studies showed presence of flavonoids, saponins and alkaloids in S. cristaefolium. Highest total phenolic content was observed in S. cristaefolium (40.8 $\pm$ 2.3 mg GA/100 g dry weight). At their highest concentration (100 mg/mL), all algal extracts showed considerably lower free radical activity than ascorbic acid (91%) and butylatedhydroxyanisole (BHA) (74.7%). S. hemiphyllum, S. polycystum, and S. cristaefolium showed strong Fe^{2+} chelating ability at 61.2%, 54.0%, and 51.8%, respectively. The results further revealed that the Fe^{2+} chelating ability of the extracts was dose-dependent and positively correlated to their phenolic content.

Keywords: Sargassum, free radicals, antioxidants, methanolic extracts, phycochemical

INTRODUCTION

The brown algae, Sargassum sp. are found mostly in tropical countries and, in the Philippines, 72 species have been recorded (Ortiz and Trono 2000). They are used in the production of animal feed, liquid fertilizer and source of alginates (Trono 1999). A survey of folk uses revealed that Sargassum species are also being used as flower inducer, insect repellant, and fish and other marine animal wrapper to preserve freshness (Montaño et al. 2006). Like all photosynthesizing plants, they are constantly exposed to direct sunlight. Free radicals and other oxidizing agents are readily produced under such conditions (Aguilera et al. 2002; Zubia et al. 2007). This suggests the presence of a protective antioxidant mechanism in seaweeds. Aside from antioxidants, brown algae have been important sources of metabolites with various pharmacological activities such as antitumor and antiinflammatory (El Gamal 2010).

Antioxidants are substances when present at low concentrations compared with those of the oxidizable substrate, considerably delay or inhibit oxidation of the substrate (Gutteridge 1995). Several studies have reported the antioxidant properties of some Sargassum species including S. siliquastrum (Lim et al. 2002), S. dentifolium (Shanab 2007), and S. boveanum (Zahra et al. 2007). Corpuz et al. (2013) had recently reported the free-radical scavenger potential of S. siliquosum collected from Batangas, Philippines. In fact, some secondary metabolites with antioxidant activity such as

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meroditerpenoid and monoterpene lactone were isolated from S. siliquastrum (Jung et al. 2008) and S. ringgoldanum (Yang et al. 2011).

In this study, five Sargassum species S. polycystum, S. hemiphyllium, S. gracillimum, S. cristaefolium, and S. crassifolium were screened for their phycochemical constituents and were evaluated for their antioxidant activity. Antioxidants are known to have different modes of action such as free radical scavenger, metal chelater and oxygen scavenger (Tiwari 2001). We utilized DPPH-radical scavenging activity and Fe^{2+}-chelating ability assays to better characterize the antioxidant activity of the brown algae. To date, there are limited studies on the potential of the Philippines brown seaweeds as sources of natural antioxidants. Antioxidant activities of S. polycystum (Anggadiredja et al. 1997) and S. hemiphyllum (Hwang et al. 2010), which were collected from Indonesia and Taiwan, respectively, were already reported. But still, it is important to screen Philippine seaweeds for its antioxidant activity as chemical constituents of brown algae are known to exhibit geographical and seasonal variations (Plouguerné et al. 2006).

MATERIALS AND METHODS

Algal Sample Collection

The brown algae S. polycystum, S. hemiphyllium, S. gracillimum, S. cristaefolium, and S. crassifolium were collected from the intertidal portions of Brgy. Bulusao, Lawaan, Eastern Samar, Philippines in January 2010. The samples were carefully cleaned from epiphytes, washed several times with tap water and air-dried at room temperature for 4 wk. Dried algal samples were powdered and kept in an airtight container. Characterization of the species was based on Algae Base (www.algaebase.org, Guiry and Guiry, 2010) and Trono (1997).

Sample Preparation

Five (5) g of each powdered sample was macerated with 25 mL pure methanol and then allowed to stand with the solvent for 24 h in the dark at room temperature. The mixture was filtered using Whatman no. 1 filter paper. The residue was again extracted with 25 mL methanol, allowed to stand for 24 h and filtered. The filtrates were then combined and the volume was adjusted to 50 mL. The combined filtrate with final concentration of 100 mg dried sample weight/mL methanol was used as the methanolic extract in this study and stored in refrigerator until analysis.

Phycochemical Screening

The methanolic algal extracts were analyzed for the presence of alkaloids, flavonoids, saponins and tannins using the method of Mojab et al. (2003).

Alkaloids. Ten (10) mL of the methanolic extracts was evaporated to dryness in water bath. Five (5) mL of 2 N HCl was added to the residue and heated on the water bath for 10 min. The mixtures were cooled, filtered and divided into two equal portions. One portion was treated with few drops of Mayer's reagent (0.1 g $HgCl_2$ in 0.6 mL dis. H_2O combined with 0.5 g KI in 0.1 mL dis. H_2O then diluted to 10 mL with dis. H_2O) and the other portion with Wagner's reagent (0.2 g iodine + 0.6 g KI in 10 mL dis. H_2O). Turbidity or precipitation indicated the presence of alkaloids.

Flavonoids. Ten (10) mL of the methanolic extracts was evaporated to dryness on water bath. The residue was dissolved with 5 mL 80% ethanol and filtered. Few drops of concentrated HCl were

added to the filtrate, then with 0.5 g magnesium powder. The development of pink or magenta-red color within 3 min indicated the presence of flavonoids.

Tannins. The methanolic extract (10 mL) was evaporated to dryness on water bath and the residue was dissolved in 10 mL hot 0.9% NaCl solution. Few drops of 15% $FeCl_3$ solution were added to 5 mL of this mixture. Development of blue, blue-black, green or blue-green color and precipitate indicated the presence of tannins.

Saponins. Ten (10) mL of the methanolic extracts were evaporated to dryness on water bath. The residue was dissolved in 10 mL distilled water and shaken vigorously to froth and allowed to stand for 15-20 min. Formation of froth which persisted for 30 min was indicative of the presence of saponins.

Total Phenolics Assay

Total phenolic content was determined using Folin-Ciocalteu method as described by Chan et al. (2008). Briefly, 1.5 mL of Folin-Ciocalteu reagent was added to a test tube containing 300 µL of different concentrations of the extract. Then, 1.2 mL of Na_2CO_3 (7.5% w/v) was added. The mixture was allowed to stand for 30 min. The absorbance was measured at 765 nm (Shimadzu UV-210A Double-beam Spectrophotometer, Japan). Phenolic content was expressed in milligrams per 100 gram of plant sample based on a standard curve of gallic acid (GA) (where $y = 0.007x - 0.007$, $R^2 = 0.991$).

Antioxidant Activity Assay

Antioxidant activities were evaluated using the DPPH radical scavenging activity and ferrous-ion chelating ability presented by Chan et al. (2008).

DPPH Radical Scavenging Assay

Appropriate dilutions (0-100 mg/mL) of the extracts, abscorbic acid, and BHA were prepared in methanol. One mL of the test samples was mixed with 1 mL 0.4 mM methanolic DPPH radical solution. The mixture was shaken and allowed to stand for 30 min. The absorbance of the resulting solution was measured at 517 nm using Shimadzu UV-210A Double-beam Spectrophotometer (Japan). The ability to scavenge DPPH radical was calculated using the following equation:

$$\text{Scavenging Activity (\%)} = [A_0\text{-}A_1/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample extract.

Fe^{2+} Chelating Ability

One (1) mL of different concentrations of the algal extracts (0-100 mg/mL) was mixed with 1 mL of 2 mM $FeSO_4$ and 1 mL of 5 mM ferrozine. The resulting solution was measured at 562 nm (Shimadzu UV-210A Double-beam Spectrophotometer, Japan). The capability to chelate Fe^{2+} was calculated using the following equation:

$$\text{Chelating Ability (\%)} = [A_0\text{-}A_1/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample extract.

Statistical Analysis

All analyses were run in triplicates and the data was expressed as mean ± standard deviation (s.d.). Data were subjected to analysis of variance using IRRISTAT (IRRI 2005). Least significant

difference was employed to evaluate the difference between means. Differences at $P<0.05$ were considered to be significant. Simple linear regression was used to determine correlation.

RESULTS AND DISCUSSION

The present study evaluated the potential of S. crassifolium, S. cristaefolium, S. gracillimum, S. hemiphyllum, and S. polycystum as sources of antioxidants. Preliminary phycochemical investigation showed that the methanolic extract of S. cristaefolium was observed to contain alkaloids, flavonoids and saponins (Table 1). Flavonoids and saponins were present in S. crassifolium. The algal extract of S. hemiphyllum tested positive for alkaloids and flavonoids. Only saponins were present for S. polycystum and all the phycochemicals tested was not detected in S. gracillimum. The total phenolic content of the algal extracts measured by Folin-Ciocalteu reagent is presented in Table 2. Results revealed that S. cristaefolium had the highest total phenolic content (40.8 $\pm$ 2.3 mg GA/100 g), followed by S. hemiphyllum (35.9 $\pm$ 0.1 mg GA/100 g), then by S. polycystum (31.3 $\pm$ 1.1 mg GA/100 g). The lowest total phenolic content was observed in S. gracillimum (27.9 $\pm$ 0.7 mg GA/100 g) and S. crassifolium (26.9 $\pm$ 1.2 mg GA/100 g).

Table 1. Preliminary phycochemical screening on the methanolic extracts of some Sargassum species.

Sargassum sp.	Alkaloids		Flavonoids	Tannins	Saponins
	Mayer Reagent	Wagner Reagent			
S. cristaefolium	-	+	+++	-	+
S. crassifolium	-	-	+	-	+
S. gracillimum	-	-	-	-	-
S. hemiphyllum	-	+	+++	-	-
S. polycystum	-	-	-	-	+

(+)-trace; (++)-positive; (+++)-highly positive; (-)-not detected

Table 2. Total phenolic content of the algal extracts.

Methanolic Extract	Total phenolic content* (mg GA/100 g dry sample)
S. crassifolium	26.9 ±2.1 [d]
S. cristaefolium	40.8 ±3.9 [a]
S. gracillimum	27.8 ±1.6 [cd]
S. hemiphyllum	35.9 ±0.1 [b]
S. polycystum	31.3 ±1.8 [c]

*Values are expressed as mean±s.d. (n = 3)

Values with the same lowercase letters are not significantly different (P<0.05)

The antioxidant activities of the methanolic algal extracts were measured using two assays: DPPH free-radical scavenging assay and Fe^{2+} chelating ability. The DPPH method is based on scavenging of the DPPH from the antioxidants which causes fading of DPPH purple color and subsequently, decrease in absorbance (Ali et al. 2008). Low absorbance readings correspond to high amount of scavenged free radicals by the antioxidants. Figure 1 shows that the scavenging activities of the algal extracts were significantly lower compared to ascorbic acid and BHA. The highest scavenging activity recorded for the algal extracts was only 12.4 % for S. crassifolium at its highest dose of 100 mg/mL. This is followed by S. polycystum (8.3%), S. hemiphyllum (7.2%), S. gracilliumum (6.6%), and S. cristaefolium (4.7%). At the dose level of 0.2 mg/mL, ascorbic acid and BHA had a scavenging activity of 90.9% and 74.7%, respectively.

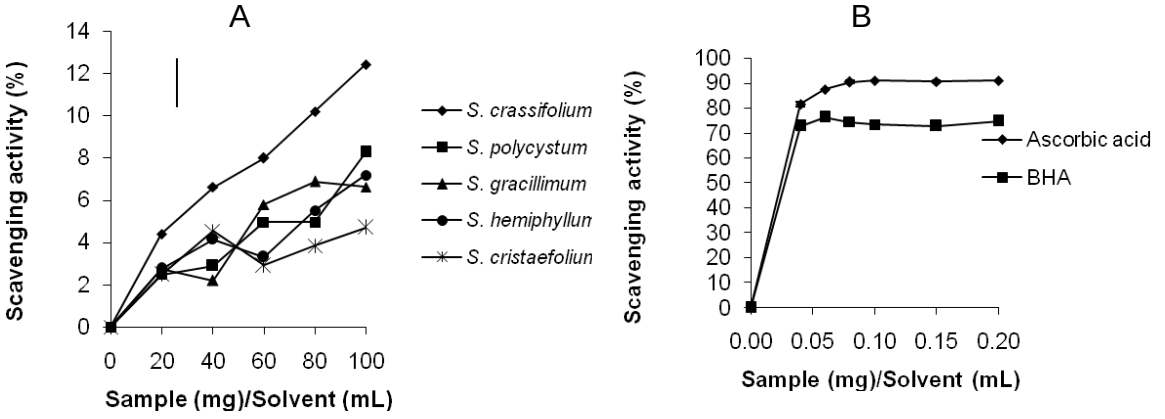

Figure 1. Free radical scavenging activities of (A) Sargassum methanolic extracts and (B) the positive controls ascorbic acid and BHA. Vertical bar indicates LSD value at P=0.05.

The Fe^{2+} chelating ability was measured by adding the algal extracts to a solution containing ferrozine-Fe^{2+} complexes. In the presence of Fe^{2+} chelating agents, the complex formation is disrupted (Senevirathne et al. 2006) which would result to a decrease in the characteristic purple color of the complex. The algal extracts were able to decrease the absorbance shown by ferrozine and Fe^{2+} complex, suggesting they have Fe^{2+} chelating abilities (Figure 2). At their highest concentration of 100 mg/mL, S. hemiphyllum had Fe^{2+} chelation ability of 61.2%, S. polycystum (54.0%), S. cristaefolium (51.8%), S. gracillimum (33.1%), while S. crassifolium had 21.0%. Ascorbic acid and BHA showed no such chelating ability. The chelating ability of S. hemiphyllum, S. polycystum and S. cristaefolium are not significantly different ($P<0.05$). Moreover, these three algal extracts had more potential Fe^{2+} chelating ability than S. gracillimum and S. crassifolium. Also, the chelating ability of each algal extract increased with concentration. The Fe^{2+} chelating properties of the algal extracts may be attributed to their phenolic content as they showed strong positive correlation between the phenolic contents and their chelating abilities (Figure 3). Moreover, this study showed that S. hemiphyllum and S. cristaefolium had appreciable amount of flavonoids, which are known to effectively chelate trace metals such as free iron and copper (Tiwari 2001).

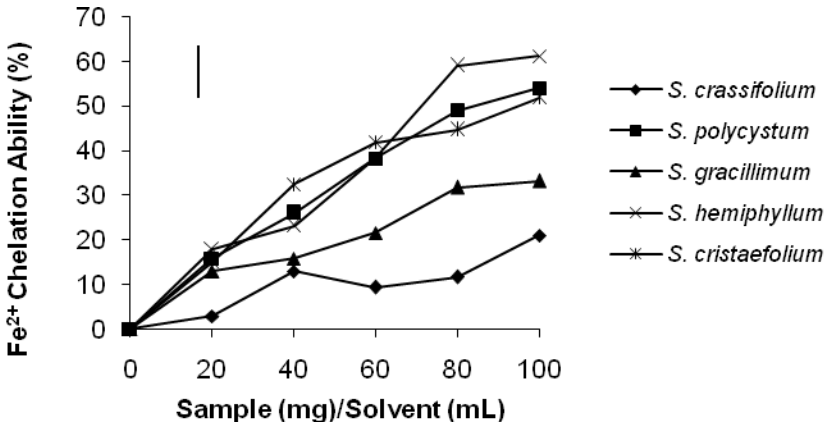

Figure 2. Fe^{2+} chelating ability of Sargassum methanolic extracts. Vertical bar indicates LSD value at P=0.05.

Antioxidants can be categorized based on their action such as free radical scavenger, metal chelater and oxygen scavenger (Tiwari 2001). The results of the present study suggested that the algal extracts could inhibit oxidation more through the metal chelation mechanism rather than free radical scavenging. Free ferrous ion in the cell can generate hydrogen radicals by reacting with hydrogen peroxide (H_2O_2) via Fenton reactions (Salgado et al. 2013). Chelation of the metal ion by the antioxidants makes the metal unavailable for the reaction. As a result, a decrease in metal concentration protects the cell from oxidative damage by inhibiting production of ROS and lipid peroxidation (Gutteridge 1995).

A previous study had showed that hot-water extract of S. hemiphyllum collected from Taiwan had been reported to have strong DPPH free-radical scavenging and Fe^{2+} chelating activities (Hwang et al. 2010). It was also reported that fresh sample of S. polycystum showed antioxidant activity using the thiocyanate method while the dried sample did not (Anggadiredja et al. 1997). The difference in the

activities between the previous reports and this study may be attributed to the difference in the extraction process. In S. wightii, the non-polar extracts showed the highest DPPH radical scavenging activity among the solvents used; and, the water extract showed higher activity than methanol (Syad et al. 2013). Furthermore, the antioxidant activity can also be due to other type of phycochemicals, not tested in this study, such as polysaccharides (Vijayabaskar and Vaseela 2012) and terpenoids (Syad et al., 2013) which were also reported to exhibit antioxidant activity. Thus, these factors must be considered in further works on Philippine seaweeds on their antioxidant potentials.

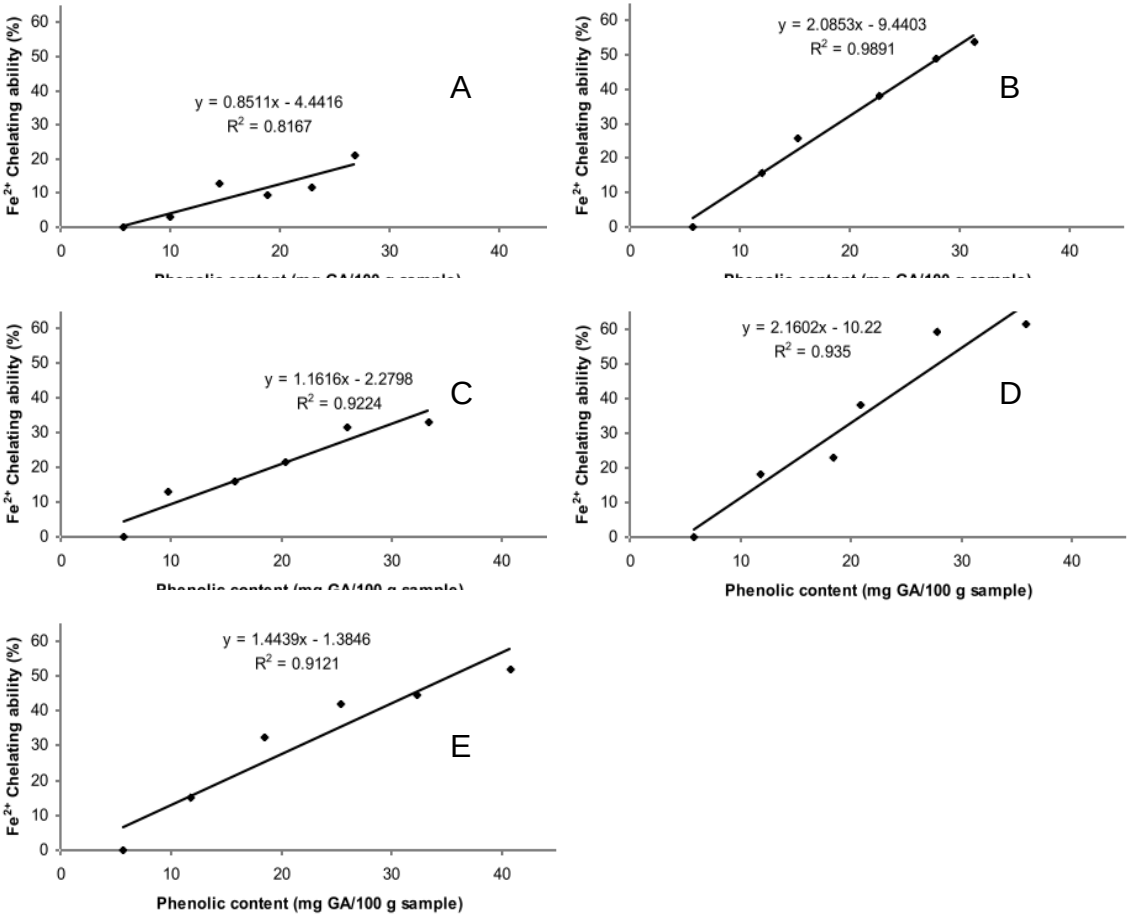

Figure 3. Simple regression correlation between the phenolic content and chelating ability of S. crassifolium (A), S. polycystum (B), S. gracillimum (C), S. hemiphyllum (D), and S. cristaefolium (E).

CONCLUSION

In this preliminary investigation of the antioxidant activity of Sargassum species from Lawaan, Samar, we showed that these brown algae, particularly S. hemiphyllum, S. polycystum, and S. cristaefolium can be potential sources of antioxidants against oxidative damage caused by Fenton reaction based on their strong Fe^{2+} chelating ability. The results further revealed that the Fe^{2+} chelating ability shown by the extracts was dose-dependent and positively correlated to their phenolic content. The algal extracts showed very low DPPH radical scavenging activity.

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STATEMENT OF AUTHORSHIP

BJRB did the sample collection, identification and extraction. BJRB and RGF conducted the phycochemical analysis and antioxidant activity assays. RGF prepared the draft and finalized the manuscript.

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