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Potential Antioxidant Activity of Three *Kappaphycus* spp. Cultivars (Rhodophyta, Solieriaceae) Collected During Two Monsoon Seasons in Kolambugan, Lanao del Norte, Philippines

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ABSTRACT

Seaweeds are exposed to large amounts of environmental stressors which favor the generation of free radicals. To mitigate oxidative damage during these adverse conditions, these organisms produce powerful antioxidants. This study therefore seeks to investigate the potentiality of three *Kappaphycus* cultivars as natural sources of antioxidants. Antioxidant potential was evaluated by the basis of DPPH radical scavenging and ferrous ion chelating activities. The aim is to take advantage the presence of their antioxidants that can be significant for coastal community nutrition and for future economic commercialization. Results revealed that methanolic extracts from different *Kappaphycus* cultivars have exhibited good radical scavenging and chelating activities ranging from 24-37% at 1000 mg mL^{-1} and 31-57% at 7 mg mL^{-1}, respectively. NE monsoon grown cultivars have shown better antioxidant activity wherein the green "Sacol" strain possessed the highest DPPH radical scavenging activity while the reddish brown "Tambalang" strain showed the highest chelating activity. The differences in radical scavenging and chelating activities, however, did not significantly vary among cultivars and between monsoon seasons. The obtained IC50 values of antioxidant activities are lower compared to the reported values of other studies. Nevertheless, this provides good information considering that the extracts were derived from natural sources. Seawater temperature is the sole factor that showed the highest significant association to the radical scavenging and chelating activities of the cultivars. As part of a balanced diet, regular consumption of seaweeds will not only provide basic dietary benefits but also offers the goodness of their anticipated antioxidant capacities. To ensure traceability and high level of food safety and security, seaweeds destined for human consumption must be ideally derived from pristine, managed and sustainable resources. Further studies are strongly encouraged to validate the results as the findings of this study are not yet conclusive.

Keywords: Antioxidant activity, DPPH radical, *Kappaphycus* spp.

INTRODUCTION

Antioxidants have multiple functions in biological systems that includes defense against oxidative damage and participation in biochemical signaling of cells. One essential role of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species called free radicals. Free radicals are responsible for aging, and their presence in excess constitutes the cause of various human diseases (Kumar et. al., 2008). Seaweeds are always exposed to numerous abiotic stressors such as high irradiance, ultraviolet radiation, salinity and temperature fluctuations, heavy metals, pollution, and seasonal changes resulting to the gradual and continued accumulation of ROS. To cope with such stresses, seaweeds deactivate free radicals and ROS by utilizing a high cellular content of antioxidant compounds or by increasing the activity of antioxidant enzymes (Cornish and Garbary, 2010). Hence, seaweed extracts have demonstrated to have strong antioxidant properties (Yuan & Walsh, 2006), and also reported to possess considerably significant amounts of protein, vitamins, trace elements, and a wide range of secondary metabolites not found in other organisms (Hayashi and Reis, 2012). Because of this additional uses as a natural source of functional ingredients, this resource is particularly attractive (Ferraces-Casais et al., 2011; Senthil et al., 2011).

Intensive marketing programs and the popular health food press have raised the public profile of antioxidants considerably. Several synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and butylated hydroxyquinone (BHQ) are commercially available and currently used; however, their safety and toxicity are some problems of concern (Matanjun et al., 2008). Hence, sourcing and development of alternative antioxidants from natural origin has drawn more and more attention. Many studies have already found different types of antioxidants in various kinds of terrestrial plants and now research is advancing in using seaweeds such as *Kappaphycus* for the production of novel foods. The objective is to take advantage of their naturally occurring antioxidant compounds and other nutritive components (Cornish and Garbary, 2010).

Since *Kappaphycus* is used for human consumption, as a component in a wide assortment of food products, and in view of the potential application as a natural antioxidant source, it is therefore of great interest to study and determine potential antioxidant activities (AOAs) of this seaweed species. Further, there is no information on the association of physico-chemical factors to the antioxidant activities of *Kappaphycus* spp. in Kolambugan, Lanao del Norte. Thus, this study is also interested to determine if antioxidant activities are affected by seasonal variations brought by monsoons.

MATERIALS AND METHODS

Site Description

Seaweed farming in Lanao del Norte has been very intensive for many years now. The seaweed cluster covers the two municipalities of Kolambugan and Tubod. Farming activities are very active in four barangays of Kolambugan namely; Simbuco, Manga, Tabigue, Mukas; and one barangay in Tubod, Tanguengueron/Lamalama. To date, there are about 1,000 growers spread around the five barangays, with total production capacity of 12,000 monolines utilized by farmers. Total area planted is approximately between 200-300 hectares with an annual production of 3,000-4,000 metric tons. The sampling area was located at Brgy. Manga. Kolambugan (Fig. 1). It was selected primarily because of active seaweed farming activity, potential variations in physico-chemical environment due to geographical location, and the willingness of the farm owners to partner in this study.

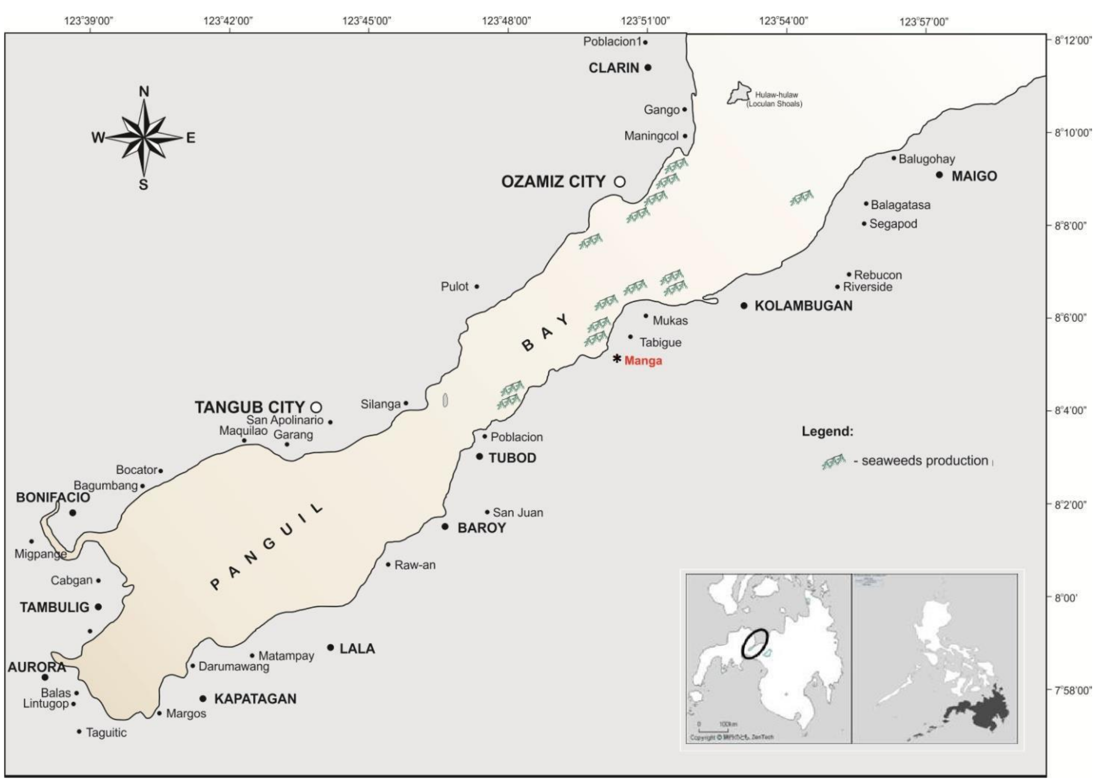

Figure 1. Location of culture site: Brgy. Manga, Kolambugan, Lanao del Norte (in asterisk) and other areas with active seaweed production in Panguil Bay, Mindanao, Philippines.

Determination of Physico-Chemical Parameters

Selected physico-chemical parameters were assessed weekly over the course of the cultivation period per growing season. All water samples were randomly collected during ebb (after peak tide) and flood (before peak tide) tides.

Time for water sample collection during ebb and flood tides was determined using a tide and current prediction program (WXTide32 version 4.7) with Iligan Bay: Misamis (Ozamiz City; 123°51.00'E, 8°9.00'N) as the reference station. All tests and analyses were replicated thrice.

Sample Materials

Kappaphycus cultivars namely; *K. alvarezii* var. *tambalang* (red brown strain), *K. alvarezii* var. *tambalang* (brown strain), and *K. striatum* var. *sacol* (green strain (Fig. 2) were used in this study due to their intensive utilization and availability for most part of the year for seaweed farming in Panguil Bay.

Ten kilograms of each fresh *Kappaphycus* cultivar were randomly harvested from a culture farm in Brgy. Manga, Kolambugan after 30-days culture period during a SW monsoon (August-September 2011) season and NE monsoon season (January-February 2012). The samples were thoroughly washed and cleaned from epiphytes, salt, sand and other impurities with filtered seawater. Samples were cut into small pieces and dried until constant weight. Dried samples were ground into fine powder using an osterizer blender, packed in sterile polyethylene bags and kept at room temperature for further use.

Figure 2. Cultivars of *Kappaphycus* used in this study: (a) *K. alvarezii* var. *tambalang* (reddish brown strain), (b) *K. alvarezii* var. *tambalang* (brown strain), and (c) *K. striatum* var. *sacol* (green strain).

Preparation of Extracts from *Kappaphycus* Cultivars

The extraction solution was prepared by diluting 80 mL of absolute methanol with distilled water to a volume of 100 mL (Aguinaldo et al., 2005). Preparation of extracts was based on the procedure of Aguinaldo et al. (2005) with slight modifications on the amount of ground dried samples used.

Fifty grams of dried ground sample per *Kappaphycus* cultivar, in an Erlenmeyer flask with stopper, were treated with sufficient 80% methanol to completely submerge the sample. The samples were kept soaked for 24 hrs. The mixture was then filtered through a Buchner funnel with gentle suction and the residue was discarded after completing the extraction. The filtrate was concentrated in vacuo using a rotary evaporator at 5 rpm and at temperature below 50°C until crude extract was obtained. The extract volume of the concentrated extract was then measured. The concentration of the stock seaweed extract was recorded as grams of dried seaweed sample per mL of the extract obtained. The crude extracts were kept at 4°C in sterile air tight amber glass containers.

Determination of Antioxidant Activities (AOAs) of Extracts

DPPH Radical Scavenging Assay

This assay is simple, rapid and convenient method to measure antioxidant capacity that involves the use of free radical, 2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl, (DPPH) (Aldrich). The assay is based on the theory that a hydrogen donor is an antioxidant. The antioxidant effect is proportional to the disappearance of 2010).

Free radical scavenging capacity of the *K. alvarezii* extracts was evaluated in terms of the scavenging activity of DPPH by measuring the absorbance at 517 nm based on the method used by Peteros and Uy (2010). The crude methanolic extracts were redissolved methanol at various concentrations (10, 50, 100, 500 and 1000 mg mL^{-1}). The mixture for assay contained a total volume of 1 mL, consisting of 500 mL of extract, 125 mL of freshly prepared 1mM in methanol DPPH solution and 375 mL of 8% methanol (solvent). The mixtures were shaken vigorously in a vortex mixer for 10 seconds, allowed to stand at room temperature for 30 min under light protection by wrapping the container with aluminum foil and the absorbance were measured at 517 nm using a spectrophotometer (Fisher Scientific Spectro Master Model 415). All assays were carried in quadruplicates. L-ascorbic acid was used as a standard. The intensity of suppressing DPPH corresponds to the radical scavenging activity of extract from each cultivar of *Kappaphycus*. The DPPH radical-scavenging activity (RSA) is calculated and expressed as percentage inhibition: where A517 control is the absorbance of control reaction of DPPH and methanol only while A517 sample is the absorbance in the presence of the seaweed extract in methanol and DPPH.

The inhibitory concentration of sample required to scavenge the DPPH radical by 50% (IC50) is determined by nonlinear regression analysis of dose-response curve between the %inhibition and concentration using a global sigmoidal (4-parameter logistic) model of curve-fitting (Mamadalieva et al.,2013):

Ferrous Ion Chelating (FIC) Assay

This assay is used to evaluate the capability of antioxidants to bind with metal ions. An extract with higher binding ability would inhibit reaction that generates reactive hydroxyl radicals (Chew et al., 2008). The FIC assay was based on the method used by Chew et al. (2008). Equal volumes (1 mL) of 0.1 mM $FeSO_4$, extracts of various dilutions (concentration range of 1.0-7.0 mg mL^{-1}) and 0.25 mM ferrozine (3-(2-Pyridyl)-5, 6-diphenyl-1, 2, 4-triazine-4′, 4″-disulfonic acid sodium salt) (Sigma-Aldrich) were combined. The reaction mixtures (in quadruplicates) were allowed to equilibrate for 10 min before measuring the absorbances at 562 nm. Ethylenediaminetetraacetic acid (EDTA) was used as the standard. The ability of the extract to chelate ferrous ion is calculated relative to the control (consisting of iron and ferrozine only) using the following equation (Singh and Rajini, 2004): where Acontrol and Asample are the absorbances at 562 nm of the control and test sample, respectively. The IC50 was determined using nonlinear regression analysis following the aforementioned dose-response curve model.

Statistical Analysis

Normality (Shapiro Wilk's test) and homogeneity of variance (Levene's test) tests were used to verify parametrical testing. Non-parametric data were transformed according to Zar (1996). The differences in antioxidant activities among Kappaphycus cultivars were identified using single factor ANOVA; while the differences of the same aforementioned parameter among *Kappaphycus* cultivars between monsoon seasons were verified using factorial ANOVA following a 2x3 design. To provide specific information on which means are significantly different from each other, Post Hoc Test was also conducted. Data were expressed as the mean of a quadruplicate ± % RSD (Relative Standard Deviation. The association of physico-chemical factors on the antioxidant activities were tested by conducting multiple stepwise regression tests, after proof of significant correlation (Pearson r test). PAST 2.09 Paleontologia Electronica and Microsoft Excel Analysis ToolPaK were the statistical softwares used in this study.

RESULTS

Environmental Factors

Variations in physico-chemical factors during two monsoon seasons in two selected culture areas of Panguil Bay are shown in Table 1. Salinity values significantly varied between monsoon seasons (T-test $p<0.05$) with the highest values recorded during NE monsoon season ranging from 19.7 to 29.3 ppt. Increase in salinity during NE monsoon is primarily due to the absence of frequent precipitation that normally occurred in the preceding monsoon. The pH range did not change significantly, with the lowest value (6.6) during the SW monsoon season and the highest value (8.7) recorded during NE monsoon season. The primary nutrients such as nitrogen and phosphorus are present in inorganic form in seawater, mostly as nitrate and phosphate ions. Nitrate and phosphate levels are relatively higher during NE monsoon season than during SW monsoon. However, only the concentration of phosphates significantly differed between monsoon seasons (T-test, $p<0.05$). Turbidity values are relatively higher during NE monsoon season than during SW monsoon. However, such recorded variation does not differ significantly between monsoon seasons (T-test, $p>0.05$). Total suspended solids ranged from 1.2 to 2.3 mg L^{-1} and 0.004 to 0.1mg L^{-1} during SW and NE monsoon seasons, respectively. These values did not significantly differ between monsoon seasons (T-test, $p>0.05$). The levels of suspended solids are relatively low when compared to the limits set by the DENR standards. Water transparency is typically deeper in Kolambugan, ranging from 2.9 to 3.8 m and 3.1 to 4.7 m for SW and NE monsoon seasons. The recorded variation significantly varied between monsoon seasons (T-test, $p<0.05$). There is no strong seasonal variation in seawater temperature and the recorded variations between monsoon seasons are not significantly different (T-test, $p>0.05$).

Table 1. Mean values for physico-chemical factors in Brgy. Manga, Lanao del Norte.

Parameter	SW Monsoon	NE Monsoon
Salinity (ppt)	22.88	23.33
pH	7.26	7.24
Nitrate (mg L^{-1})	0.12	0.15
Phosphate (mg L^{-1})	0.04	0.06
Turbidity (NTU)	54.92	61.25
Total Suspended Solids (mg L^{-1})	0.13	0.04
Water Transparency (m)	3.30	3.96
Seawater Temperature (°C)	29.40	29.50
Water Motion (DIF)	21.04	15.80

3.2. Radical Scavenging Activity of *Kappaphycus* Extracts

DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger. This assay is used to test the ability of the antioxidative compounds functioning as proton radical scavengers or hydrogen donors (Singh & Rajini, 2004).

Figure 3 showed that the radical scavenging activity of cultivars increased with concentration. Methanolic extracts from cultivars grown during NE monsoon showed higher percent DPPH inhibition than extracts obtained from SW monsoon-grown cultivars. Radical scavenging activities of *Kappaphycus* spp. ranges from 24-29% and 31-37% at 1000mg mL^{-1}, respectively, for SW and NE monsoon seasons.

The half maximal inhibitory concentration (IC50) of the radical scavenging activity of crude methanolic extracts of different *Kappaphycus* cultivars and L-ascorbic acid (synthetic antioxidant) grown during two monsoon seasons in two selected culture areas are shown in Table 2. Since RSA did not reach at least 50% DPPH inhibition, the half maximal inhibitory concentration (IC50) values were mathematically estimated and reported as being greater than the highest concentration of inhibitor tested (>1000 mg mL^{-1}).

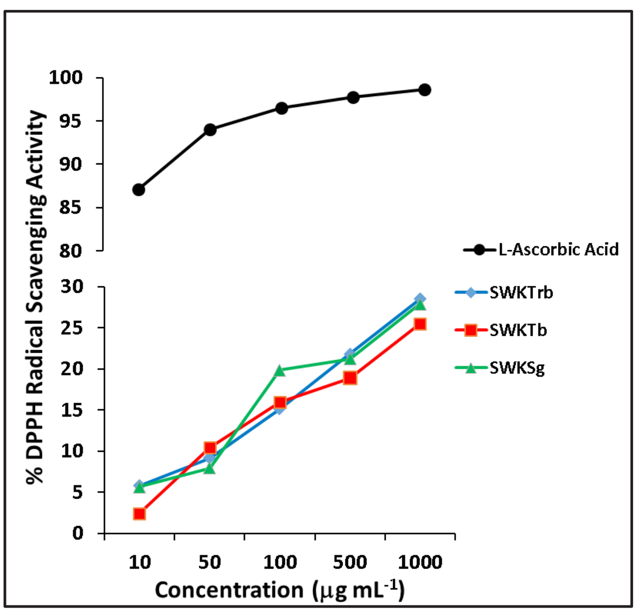

Figure 3. DPPH free radical scavenging activity of crude methanol extracts of three Kappaphycus cultivars grown during two monsoon seasons in Kolambugan, Lanao Del Norte. Trb - *K. alvarezii* var. *tambalang* (red brown strain), Tb - *K. alvarezii* var. *tambalang* (brown strain), Sg - *K. striatum* var. *sacol* (green strain), SW– Southwest monsoon, NE – Northeast monsoon.

The lower the IC50 values, the higher the radical scavenging activities. The highest radical scavenging activity is shown by the NE monsoon-grown green "Sacol" strain (IC50, 4.44±1.86 mg mL^{-1}) while the lowest radical scavenging activity is recorded from the SW monsoon-grown brown "Tambalang" strain (IC50, 18.30±8.29 mg mL^{-1}). However, the obtained results are not comparable to the radical scavenging activity of L-ascorbic acid (IC50, $29.32x10^{-3}±8.99x10^{-3}$ mg mL^{-1}). ANOVA (Table 3) revealed that the radical scavenging activities among cultivars did not significantly differ (p>0.05). Monsoon seasons have no significant effect on the radical scavenging activities of the cultivars (p>0.05).

3.3. Chelating Ability of *Kappaphycus* Extracts

This assay is used to evaluate the binding of the antioxidant components to metal ions. An extract with higher binding ability would prevent or inhibit reaction such as Fenton's type reaction, which generates reactive hydroxyl radicals (Chew et al., 2008), implicated in many diseases (Halliwell & Gutteride, 1990).

Table 2. Half maximal inhibitory concentration (IC50) of radical scavenging activity of methanolic extracts of three *Kappaphycus* cultivars grown during two monsoon seasons in Kolambugan, Lanao del Norte.

Monsoon	Cultivar/ Standard	IC_{50}	
		mg mL^{-1}	mg mL^{-1}
SW	Trb	12.2x103	12.2±7.7
	Tb	18.3x103	18.3±8.3
	Sg	14.2x103	14.2±7.6
NE	Trb	4.7x103	4.7±0.7
	Tb	6.9x103	6.9±1.7
	Sg	4.4x103	4.4±1.9
	L-Ascorbic Acid	29.3	29.3E-03 ±8.9E-03

Trb - *K. alvarezii* var. *tambalang* (red-brown strain), Tb - *K. alvarezii* var. *tambalang* (brown strain), Sg - *K. striatum* var. *sacol* (green strain), and Sb - *K. striatum* var. *sacol* (brown strain).), SW– Southwest monsoon (Habagat), NE – Northeast monsoon (Amihan). Data are expressed as the mean of a quadruplicate ± % RSD.

Table 3. Summary of ANOVA results of the interaction of radical scavenging activity of different *Kappaphycus* cultivars, culture areas, and monsoon seasons.

Source of Variation	*SS*	*df*	*MS*	*p-value*
Between Cultivars	12.39	2	6.19	0.93
Cultivars x MS	10.34	2	5.17	0.94

MS –Monsoon Seasons
*Indicates significance ($p<0.05$)

Figure 4 showed that the chelating activity of cultivars increased with concentration. Methanolic extracts from cultivars grown during NE monsoon showed higher percent chelation than extracts obtained from SW monsoon-grown cultivars. Chelating activities of *Kappaphycus* spp. ranges from 31-38% and 46-57% at 7 mg mL^{-1}, respectively, for SW and NE monsoon seasons.

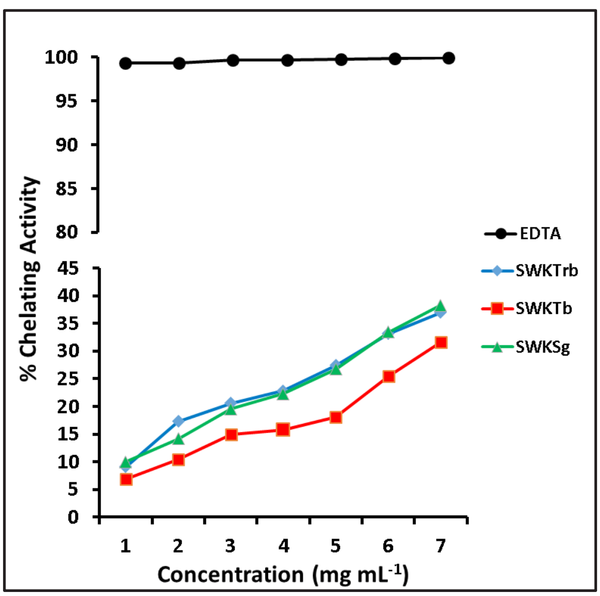

Figure 4. Ferrous ion chelating ability of crude methanol extracts of three Kappaphycus cultivars grown during two monsoon seasons in Kolambugan, Lanao Del Norte. Trb - *K. alvarezii* var. *tambalang* (red brown strain), Tb - *K. alvarezii* var. *tambalang* (brown strain), Sg - *K. striatum* var. *sacol* (green strain), SW– Southwest monsoon, NE – Northeast monsoon.

IC50 values of the chelating ability of methanolic extracts of different Kappaphycus cultivars and of Ethylenediaminetetraacetic acid (EDTA) (synthetic antioxidant) are shown in Table 4. Since CA did not reach at least 50% chelation, the half maximal inhibitory concentration (IC50) values were mathematically estimated and reported as being greater than the highest concentration of inhibitor tested (>7 mg ml^{-1}). The lower the IC50 values, the higher the chelating activities. The highest chelating activity is shown by the NE monsoon-reddish brown "Tambalang" strain (IC50, 8.15±1.04 mg mL^{-1}) while the lowest chelating activity is recorded from the SW monsoon brown "Tambalang" strain (IC50, 16.6±3.7 mg mL^{-1}). However, the obtained results are not comparable to the chelating activity EDTA (IC50, $7.96x10^{-4}±2.16x10^{-7}$ mg mL^{-1}). ANOVA (Table 5) revealed that chelating activities among cultivars did not significantly vary (p>0.05). Monsoon seasons did not show any signicant effect to the chelating activities of cultivars (p>0.05).

Table 4. Half maximal inhibitory concentration (IC50) of ferrous ion chelating ability of methanolic extracts of three *Kappaphycus* cultivars grown during two monsoon seasons in Kolambugan, Lanao del Norte.

Monsoon	Cultivar/ Standard	IC50
		mg mL^{-1}
SW	Trb	13.8±1.1
	Tb	16.6±3.7
	Sg	12.4±1.6
NE	Trb	8.1±1.0
	Tb	11.2±1.9
	Sg	10.7±1.6
	EDTA	7.9E-04 ±2.2E-07

Trb - *K. alvarezii* var. *tambalang* (red-brown strain), *Tb - K. alvarezii var. tambalang* (brown strain), Sg - *K. striatum* var. *sacol* (green strain), and Sb - *K. striatum* var. *sacol* (brown strain). SW– Southwest monsoon (Habagat), NE – Northeast monsoon (Amihan). Data are expressed as the mean of a quadruplicate ± % RSD.

Table 5. Summary of ANOVA results of the interaction of radical scavenging activity of different *Kappaphycus* cultivars, culture areas, and monsoon seasons.

Source of Variation	*SS*	*df*	*MS*	*p-value*
Between Cultivars	61.05	2	30.52	0.79
Cultivars x MS	70.34	2	35.17	0.70

MS –Monsoon Seasons
*Indicates significance ($p<0.05$)

Table 6. RSA and CA findings in comparison to other studies.

Parameter	IC50 (mg/ml)	Reference
Radical Scavenging Activity	4.28 mg mL^{-1}	Kumar *et al.* (2008)
	11.80 mg mL^{-1}	Chew *et al.* (2008) 12.2–
	18.3 mg mL^{-1}	This study (SW Monsoon)
	4.4 – 6.9 mg mL^{-1}	This study (NE Monsoon)
Chelating Activity	3.08 mg mL^{-1}	Kumar *et al.* (2008)
	12.4 – 16.6 mg mL^{-1}	This study (SW Monsoon)
	8.1 – 11.2 mg mL^{-1}	This study (NE Monsoon)
% Chelation	67%	Kumar *et al.* (2008)
	28-57%	This study

MS –Monsoon Seasons
*Indicates significance ($p<0.05$)

DISCUSSION

Because of the commercial success of *Kappaphycus* for the carrageenan industry and its farming technology based on vegetative propagation, new potential applications are of interest in several branches of the different rheological properties occur as a response to unfavorable environmental conditions. In some rhodophytes, the physiological biochemical composition varies during exposures in pre-monsoonal, monsoonal, and post-monsoonal periods along with the physico-chemical parameters associated in a particular period (Goes and Reis, 2011).

Marine macroalgae have developed diverse stress-coping mechanisms to maintain homeostasis and these include antioxidant production and free radical scavenging activities (Nahas et al., 2007). The presence of these active compounds mitigate the negative effects of oxidative stress which results due to the excessive amounts of reactive oxygen species (ROS) in the system of the organism (Chew et al., 2008).

Research shows that many macroalgae possess considerable amount of antioxidant compounds and these are categorized into major groups which include carotenoids, phenolic compounds, phycobilin pigments, polyphenols, sulphated polysaccharides (e.g. carrageenan), and vitamins (Cornish and Garbary, 2010). The aforementioned compounds are all present in most rhodophytes especially *Kappaphycus alvarezii*.

Kappaphycus is rich in Vit. C, Vit. A and phenolic compounds which function as free radical scavengers, reducing agent and as well work as metal chelators Shahidi and Zhong, 2007). In an unpublished thesis work of Oon Xin Yan from the University of Malaysia Sabah, his results have shown that *Kappaphycus* possessed the highest DPPH radical scavenging activity and the highest total phenolic and flavanoid content in comparison to *Sargassum polycystum* and *Caulerpa lentillifera*.

Holdt and Kraan (2011) reported that the crude extracts and the polyphenol content of *Kappaphycus* spp. exhibited reducing power and hydroxyl radical scavenging activity higher than that of standard antioxidants. This antioxidant activity is of particular interest for cosmetic and pharmacological applications. Fayaz et al. (2005) analyzed the antioxidant activity of extracts of *K. alvarezii* and observed that the chloroform:methanol (2:1) extract had 82.5% hydroxyl radical scavenging activity at a concentration of 1000 ppm.

Kumar et al. (2008) observed that the methanol extracts had a radical scavenging activity with an IC50 of 4.28 mg mL^{-1} while Chew et al. (2008) reported an IC50 of 11.80 mg mL^{-1} for 50% methanol (v/v). Good ferrous ion chelating activity is observed for the methanol extract (IC50 3.08 mg mL^{-1}) at 5.0 mg mL^{-1}; this extract showed 67% chelation (Kumar et al., 2008). Hence, these extracts could be considered as natural antioxidants and may be useful for curing diseases arising from oxidative deterioration.

Biochemical activities may vary from sample to sample, depending upon the sample history such as cultivation period, season of harvesting, and extraction procedures (Fleurence et al., 2005). Table 6 shows the comparison of RSA and CA findings of this study to other cited literature.RSA of extracts from NE Monsoon–grown cultivars are closer to the findings of Chew et al. (2008). The obtained AOA values might be lower compared to the reported values of Kumar et al. (2008). However, this only provides additional information of the potential application of these cultivars as a natural source for antioxidants. Additionally, this also suggests that the regular consumption of seaweeds as part of a balanced diet will not only provide fibre, protein, minerals, vitamins, and low fat carbohydrate content (Yuan and Walsh, 2006) but also offers the goodness of their anticipated in vivo antioxidant capacities and associated synergistic effects.

Among the physico-chemical parameters, seawater temperature is the sole factor that has the highest negative association to the radical scavenging and chelating activities wherein the period of minimal activity corresponds to the highest and lowest extremes of temperature. Temperature is indeed one of the most important factors affecting antioxidant activity (Réblová, 2012). This is true since most antioxidant compounds are in the form of enzymes which are very reactive to temperature. Generally, high and low temperatures cause an acceleration/ deceleration of the initiation reactions, and hence causing a decrease in the activity of the antioxidants (Pokorný, 1986).

Table 6. Summary of correlation coefficients among physico-chemical factors and radical scavenging activities of different Kolambugan-grown *Kappaphycus* cultivars grown during two monsoon seasons.

Season	Cultivar	Salinity	pH	Nitrates	Phosphates	Turbidity	TSS	Trans-parency	Max Temp	Min Temp	Water Motion
SW	Trb	-0.08	0.54	-0.27	0.12	0.03	-0.15	0.45	-0.81	-0.83	0.02
	Tb	-0.15	0.47	-0.03	0.21	0.26	-0.25	0.63	-0.81	-0.93*	-0.16
	Sg	-0.06	0.69	-0.09	0.13	0.07	-0.04	0.49	-0.93*	-0.86	-0.02
NE	Trb	-0.04	0.24	-0.21	0.55	0.18	-0.46	0.24	-0.28	-0.36	-0.72
	Tb	0.13	0.04	-0.33	0.50	0.01	-0.56	0.04	-0.12	-0.42	-0.73
	Sg	0.28	-0.06	-0.49	0.33	-0.12	-0.68	-0.06	-0.05	-0.58	-0.69

Trb - *K. alvarezii* var. *tambalang* (red-brown morphotype), Tb - *K. alvarezii* var. *tambalang* (brown morphotype), Sg - *K. striatum* var. *sacol* (green morphotype)

*Indicates significance at $p<0.05$.

Table 7. Summary of correlation coefficients among physico-chemical factors and ferrous ion chelating activities of different Kolambugan-grown *Kappaphycus* cultivars grown during two monsoon seasons.

Season	Cultivar	Salinity	pH	Nitrates	Phosphates	Turbidity	TSS	Trans-parency	Max Temp	Min Temp	Water Motion
SW	Trb	-0.22	0.39	0.03	0.27	0.36	-0.33	0.70	-0.94*	-0.76	-0.20
	Tb	-0.21	0.55	0.04	0.28	0.27	-0.23	0.67	-0.91*	-0.86	-0.09
	Sg	-0.14	0.55	-0.10	0.19	0.16	-0.19	0.57	-0.89*	-0.85	-0.06
NE	Trb	-0.10	-0.16	0.35	-0.41	-0.04	0.59	-0.13	0.17	0.50	0.76
	Tb	-0.28	0.09	0.49	-0.34	0.12	0.66	0.07	0.06	0.57	0.69
	Sg	-0.15	-0.04	0.37	-0.45	0.01	0.59	-0.04	0.12	0.48	0.74

Trb - *K. alvarezii* var. *tambalang* (red-brown morphotype), Tb - *K. alvarezii* var. *tambalang* (brown morphotype), Sg - *K. striatum* var. *sacol* (green morphotype)

*Indicates significance at $p<0.05$.

CONCLUSION

This study has illustrated the potentiality of *Kappaphycus* as a natural source for antioxidants. *Kappaphycus* spp. extracts have exhibited good radical scavenging and chelating activities ranging from 24-37%.

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