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In Vitro Antioxidant Activity of Selected Seaweeds in the Philippines

Patricia Louise C. Arive, Ionna H. Inquimboy and Nancy Lazaro-Llanos
Chemistry Department, De La Salle University,
2401 Taft Avenue Manila 1004, Philippines.

(Corresponding author: Nancy Lazaro-Llanos)
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ABSTRACT: The increasing amount of free radicals in the environment from both exogenous and endogenous sources make intake of antioxidants essential. This study focused on investigating the antioxidant properties of acetone, chloroform, and ethanol extracts of four seaweeds commonly found in the Philippines, namely: *Caulerpa lentillifera, Caulerpa racemosa, Kappaphycus alvarezii*, and *Hydropuntia edulis* using the DPPH assay. Results show that all seaweed samples exhibited antioxidant activities in all solvents used. Results show that all seaweed samples showed significant antioxidant activity. Between the two batches of collection of *C. lentillifera* and *K. alvarezii*, batch 1, which was collected in October 2016, showed significantly higher antioxidant activity in comparison to the activity of batch 2, which was collected in January 2017. With regards to the solvents used, acetone extracts demonstrated the highest antioxidant activity among all seaweed species. Results of the study can help further establish the use of seaweeds as potential source of naturally occurring antioxidants.

Key words: Antioxidants; ROS, Free-Radical Scavenging Activity; Seaweeds, *C. lentillifera, C. racemosa, H. edulis, K. alvarezii,* DPPH Assay.

I. INTRODUCTION

The excess amount of reactive oxygen species (ROS) present in the body induces lipid peroxidation which can react with or alter the structure of biomolecules in the body causing cellular disorders or cell death. For example, damage to specific proteins like enzymes, receptors, transport and structural proteins can have adverse effect on normal body processes. On the other hand DNA damage can even be more fatal as this can cause mutations or may lead to the development of cancer (Lobo *et al.,* 2010). The amount of free radicals present in our environment has increased making antioxidant intake necessary to counteract the effects of free radicals on human health. Antioxidants are molecules that have free radical scavenging activity. Many food, pharmaceutical, and personal care products contain synthetic antioxidants. However, most synthetic antioxidants have unstable and carcinogenic character (Natl. Toxicology Program, 2016; Nimse and Pal, 2015). Therefore natural sources a have become more attractive to consumers as well as manufacturers since they are easily obtained and cost effective.

Seaweeds, as raw materials, have found a wide variety of applications: as gelling agents, stabilizers, and emulsifiers; as a constituent of pharmaceutical and personal care products; as feed for livestock; as fertilizer; as filtering agent in wastewater treatment; and many more. Seaweeds as food product have become popular and widely accepted by consumers as healthy food as many studies have shown that they are a rich in vitamins, minerals, essential fatty acids, and high in fiber. Moreover several researches have demonstrated in vitro antioxidant activity in various seaweed samples, which are attributed to the presence of novel antioxidants such as polyphenols, carotenoids, and certain polysaccharides (Boonchumi, *et al.* 2011; Budhiyanti, *et al.* 2012; De Lima, *et al.* 2016; Foon, *et al.,* 2013; Ismail and Hong, 2002; Karthikeyan, *et al.* 2015; Kokabi *et al.* 2013; Ling, *et al.* 2013; Lou, *et al.* 2010; Meenakshi, *et. al.* 2012; Moubayed, *et al.* 2017; Parthiban, *et al.* 2013; Saranya, *et al.* 2014; Seenivasan, 2013; Shanab, 2007; Souza, 2011; Taheri, 2016).

Seaweeds are widely cultivated in the Philippines. The export of seaweeds has been a significant income generating industry for the country and a source of livelihood for people living in coastal communities. Seaweeds that are exported to many Western countries are mainly used as raw material for the production carrageenan. In addition, many edible types of seaweed have been in demand in many restaurants as ingredient in appetizers, salads, and soups.

This study investigated the antioxidant properties of acetone, chloroform, and ethanol extracts of four seaweed species commonly found in the Philippines, namely: *Caulerpa lentillifera*, *Caulerpa racemosa*, *Kappaphycus alvarezii*, and *Hydropuntia edulis*. Results of the study may well contribute to the pool of information on their benefits to human health, specifically as a source of antioxidants.

II. MATERIALS AND METHODS

A. Sample Collection

C. lentillifera and *K. alvarezii* were obtained from wet markets in Metro Manila on October 2016 (Batch 1) and January 2017 (Batch 2); while *C. racemosa* and *H. edulis* were collected on June 2016. Seaweeds were cleaned under running tap water for 5 min, followed by washing using distilled water for three repetitions. The samples were freeze-dried. The lyophilized samples were grounded using a blender.

B. Preparation of Extracts

The grounded, freeze-dried seaweed sample (2 g), were placed in a beaker and soaked in 20 mL of the following solvents: acetone, ethanol, and chloroform. Containers used were airtight and covered with aluminum foil. After 48 hours, samples were centrifuged for 10 min at 3000 rpm, 4°C. The supernatant was decanted into large test tubes, sealed with rubber stopper, and covered with aluminum foil.

C. DPPH Assay

The maximum absorbance for the methanol and DPPH solution was found to be at 520 nm using Hitachi U-2000 UV-Vis spectrophotometer and subsequently used in each run. A 3.3mM DPPH solution was according to Moubayed *et al.* (2017). DPPH radical scavenging activity was determined based on the methods of Duan *et al.* (2006) and Marinova and Batchvarov (2011). Seaweed extracts (100, 150, 200, and 250 ml) were diluted with methanol to a final volume of 3.00 mL in test tubes and covered with aluminum foil. Exactly 150 µL of DPPH solution were added to each test tube. The mixtures were vortexed and initial absorbance of each solution was immediately measured at 520 nm using methanol as blank. The solutions were left in the dark and final absorbance was measured after 30 minutes. The antioxidant activity was calculated by the formula used by Lachman *et al* (2009):

$$AA(\%) = 100 - [(A_{30}/A_0) \times 100]$$

where A_0 is the absorbance of the solution at 0 min, and A_{30} is the absorbance after 30 min.

D. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Using Microsoft Excel 2011, one-way analysis of variance (ANOVA) was the statistical test used to compare and analyze the data gathered in the experiment, and to determine whether the values are significantly different from one another or not. P-values less than 0.05 were considered statistically significant.

III. RESULTS AND DISCUSSION

The comparison of the antioxidant capacity of the different seaweeds species are presented in Table 1 and Figures 1, 2, and 3. Results show that seaweed extracts from different solvents showed varying antioxidant activities. Acetone extract of *K. alvarezii* showed the highest activity at 23.707 ± 4.811%, while *C. lentillifera* has the highest antioxidant activity at 23.656 ± 5.068% among all chloroform extracts. The *C. racemosa* exhibited the highest antioxidant activity at 15.425 ± 5.769 % in ethanol compared to the other three seaweed species. The values of obtained were statistically significant ($p<0.05$). Generally, it maintains an increasing percentage of antioxidant activity as the concentration is increased for all three solvents. The comparison of the antioxidant activities of *C. lentillifera* and *K. alvarezii* between seasons of collection are presented in Table 2.

Table 1: Comparison of the antioxidant activity among seaweed samples and extraction solvent used (%).

Seaweed	100 µL	150 µL	200 µL	250 µL
	Acetone Extracts			
C. lentillifera	15.584	21.22	23.165	24.628
C. racemosa	9.154	9.174	9.683	8.25
H. edulis	6.318	7.075	9.869	13.311
K. alvarezii	17.943	22.402	25.061	29.423
Seaweed	100 µL	150 µL	200 µL	250 µL
	Chloroform Extracts			
C. lentillifera	16.834	23.38	25.587	28.823
C. racemosa	10.373	13.16	18.187	26.622
H. edulis	2.7	8.612	13.752	14.028
K. alvarezii	2.992	0.136	1.84	1.28
Seaweed	100 µL	150 µL	200 µL	250 µL
	Ethanol Extract			
C. lentillifera	8.057	10.117	11.001	11.984
C. racemosa	3.635	5.462	8.738	10.33
H. edulis	7.794	14.502	18.254	21.149
K. alvarezii	---	0.924	1.062	10.172

Table 2: Comparison of the antioxidant activity between seasons of collection (%).

Seaweed	100 µL	150 µL	200 µL	250 µL
	Acetone Extracts			
C. lentillifera (Batch 1)	15.584	21.22	23.165	24.628
C. lentillifera (Batch 2)	9.566	11.942	14.962	16.322
K. alvarezii (Batch 1)	17.943	22.402	25.061	29.423
K. alvarezii (Batch 2)	5.699	3.105	9.414	4.334
Seaweed	100 µL	150 µL	200 µL	250 µL
	Chloroform Extracts			
C. lentillifera (Batch 1)	16.834	23.38	25.587	28.823
C. lentillifera (Batch 2)	1.631	10.239	4.09	13.53
K. alvarezii (Batch 1)	2.992	0.136	1.84	1.28
K. alvarezii (Batch 2)	2.673	1.975	0.732	0.664
Seaweed	100 µL	150 µL	200 µL	250 µL
	Ethanol Extracts			
C. lentillifera (Batch 1)	8.057	10.117	11.001	11.984
C. lentillifera (Batch 2)	5.983	10.12	9.85	9.449
K. alvarezii (Batch 1)	---	0.924	1.062	10.172
K. alvarezii (Batch 2)	4.226	5.63	3.487	6.752

C. lentillifera extracts batch 1 consistently show higher antioxidant activity, at 21.149 ± 3.963%, 23.656 ± 5.068%, and 10.290 ± 1.672% respectively, compared batch 2 at 13.198 ± 3.035, 7.373 ± 5.473%, and 8.851 ± 1.931. Acetone extracts of *K. alvarezii* from batch 1 exhibits higher antioxidant activity at 23.707 ± 4.811% compared the acetone extract of the same seaweed in batch 2 at 5.638 ± 2.731%. For chloroform and ethanol extracts of *K. alvarezii* batch 1, values are at 1.882 ± 1.257% and 4.053 ± 5.300% respectively; and 1.511 ± 0.981% and 5.023 ± 1.455% respectively for batch 2. Results from batch 1 are statistically significantly different from the batch 2 values ($p<0.05$). Deviation in antioxidant activities of different extracts can be attributed to the variation in composition of seaweeds obtained in different seasons. Several environmental factors such as water temperature, salinity, light, and nutrients can affect seaweed composition. (Marinho-Soriano *et al.*, 2006) Changes in ecological condition due to transition of seasons can also induce or inhibit the production of certain nutrients. (Lobban *et al.*,1985). Comparing among the different solvent used for extraction (Fig. 1-3), the following exhibited the highest activities: chloroform (*C. lentillifera*,) at 23.656 ± 5.068%; ethanol (*C. racemosa*) at 15.425 ± 5.769%; and acetone (*K. alvarezii*) at 23.707 ± 4.811% respectively. The difference in polarities of the seaweed components can affect the efficiency of the extraction.

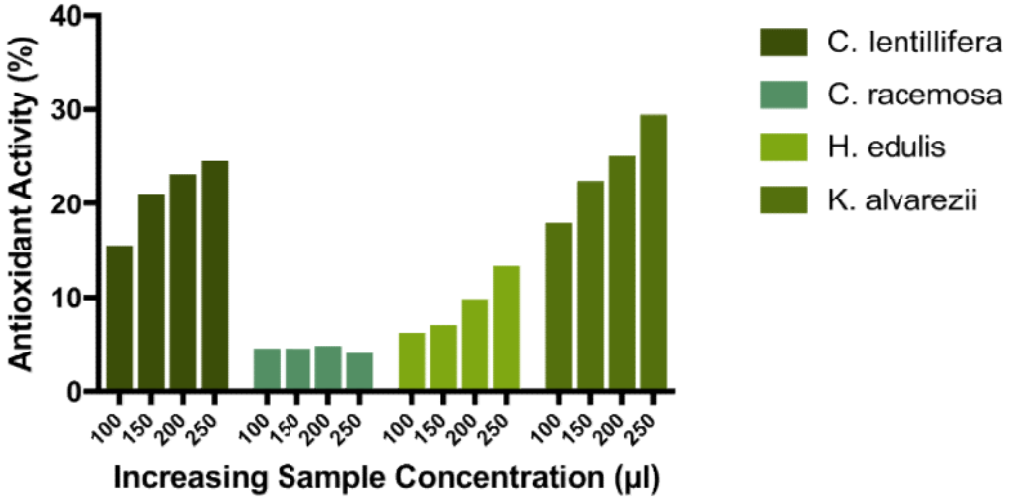

Fig. 1. Comparison antioxidant activity among seaweed samples (acetone extracts).

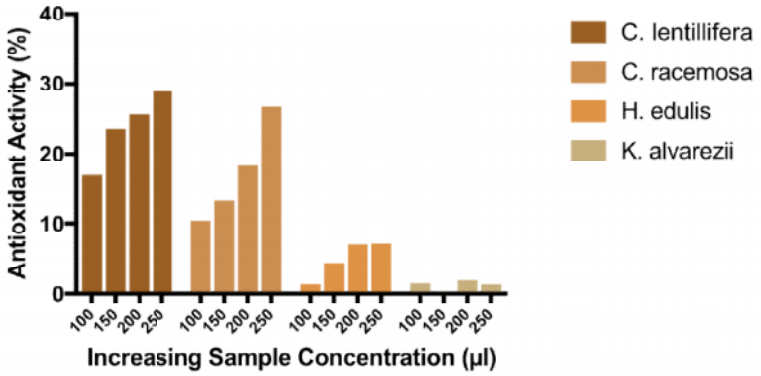

Fig. 2. Comparison of the antioxidant activity among seaweed samples (chloroform extracts).

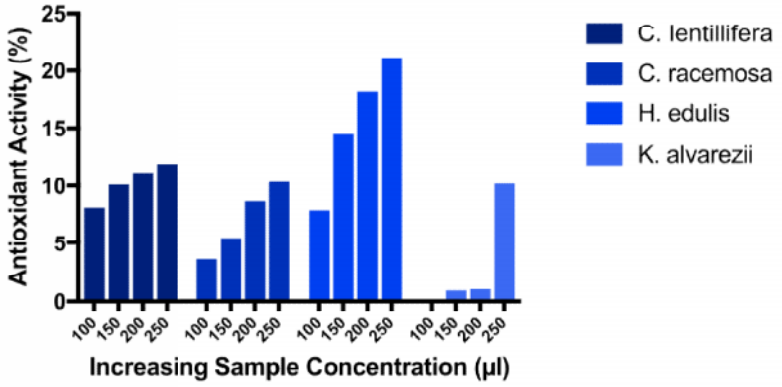

Fig. 3. Comparison of the antioxidant activity among seaweed samples (ethanol extracts).

The *C. lentillifera* species have mostly nonpolar and slightly polar antioxidants since it showed higher antioxidant activity in the chloroform and acetone solvents. The *C. racemosa* exhibited nonpolar antioxidants since its highest antioxidant activity is in its chloroform extract. Since the *H. edulis* generally has the same levels of antioxidant activity in all solvents, it can be said that it has both polar and nonpolar antioxidants. It is only slightly more polar due to its antioxidant activity being slightly higher in ethanol. Lastly, the *K. alvarezii* showed significantly higher antioxidant activity in the acetone solvent. All solvents have statistically significantly different antioxidant properties from the seaweeds according to the one-way ANOVA analysis ($p<0.05$).

V. CONCLUSION

All seaweeds samples exhibited significant antioxidant activity based on the DPPH assay. Among the solvents, acetone extracts exhibited the highest free radical scavenging activity. Results of this study can help further establish seaweeds as a healthy alternative ingredient in food and other consumer products. Further studies still need to be done particularly the isolation and identification of specific compounds exhibiting antioxidant properties. In addition, for better data analysis and more conclusive results, research directions point to increased number of trials and the use of more seaweed samples that includes green, red, and brown macroalgae.

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