Antioxidant Properties of Algae in the U.S. Virgin Islands

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ABSTRACT

Compounds high in antioxidant activity counteract the formation of free radicals, which are linked to many human diseases and cancers. The objectives of this study were to quantify the hydrophilic antioxidant activity (HAA), lipophilic antioxidant activity (LAA), and the total antioxidant activity (TAA) of algae collected in the US Virgin Islands. The three algae divisions collected are Rhodophyta, Chlorophyta, and Phaeophyta. We hypothesize that the HAA will be greater than the LAA for all algae species. We also hypothesized that Phaeophyceae would have the highest TAA compared to the other classes. In this study, 28 species of algae were collected on St. Croix, USVI. The ABTS, H_2O_2, horseradish peroxidase decoloration method was used to determine the antioxidant activity then scanned using a UV-VIS Spectrophotometer at 730 nm. The antioxidant activities were reported as µmol Trolox Equivalent (TE)/g Dry Weight (DW). For all of the samples tested, *Caulerpa prolifera* had the highest TAA (97.281 ± 18.475 µmol TE/g DW) while *Dictyota jamaicensis* had the lowest TAA (1.999 ± 0.889 µmol TE/g DW). Chlorophyta had the highest mean (23.754 ± 29.710 µmol TE/g DW) of antioxidant activity and Phaeophyta (12.595 ± 11.861 µmol/g DW) had the lowest mean. A one-way ANOVA test showed significant difference between the HAA and LAA ($p = 0.0509$). A one-way ANOVA test comparing all three groups revealed that there was no statistical difference between their TAA ($p = 0.621$). Based on this, we didn't find any significant difference among the antioxidant levels between the groups tested.

Introduction

Antioxidants are important to health. It's a good thing that it can be found in almost everything, fruits, vegetables, spices, vitamin supplements, and even in algae. Compounds booming with antioxidant properties appear to counteract the formation of free radicals in reactive oxygen species and reactive nitrogen species. These free radicals have been linked to many cancers, chronic and degenerative human diseases. The free radicals are known to destroy DNA, disrupt cell membranes, and cause enzymatic metabolic changes in cells. Two factors that increase the number of free radicals is exposure to high light and oxygen concentrations. All plants are subjected to these conditions; however, algae are not as adversely affected by the photodynamic change. This suggests that marine algae have a lot of antioxidant activity.

Background

Many genera of marine algae such as *Colpomenia*, *Gracilaria*, *Padina*, *Laurencia*, and *Turbinaria*, have been reported to have high levels of antioxidant activity (*2*). In order to determine the antioxidant activity in different complex samples including algae, many researchers have been using the FRAP (Ferric Reducing Antioxidant Power) assays or the 2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), H_2O_2, horseradish peroxidase (ABTS/H_2O_2/HRP)

decoloration method which determines both the hydrophilic (HAA) and lipophilic (LAA) antioxidant activity in each sample tested (*3*).

Even though there are many publications and research papers written on antioxidant activity in various genera of algae; there have been no reports of the testing of the antioxidant activity of marine algae found around the Virgin Islands. In previously studied places, Hawaii and the Yucatan Peninsula, have relatively similar marine ecosystems to that of St. Croix. It was found that there are different levels of antioxidant activity in several different species of algae. These studies have suggested that high antioxidant activity is shown to prevent forms of cancers, oxidative stress dysfunctions, and neurological diseases. Although there is a link between antioxidants and the pre stages of harmful diseases, the correct form of treatment using these antioxidants is still being determined and developed.

Objectives

The hydrophilic antioxidant activity (HAA), lipophilic antioxidant activity (LAA) and the total antioxidant activity (TAA) of the three algae families will be quantified. The three families are Rhodophyta (red algae), Chlorophyta (green algae), and Phaeophyta (brown algae). We will conclude which species of algae has the highest HAA, LAA, and TAA values. However, based on previous studies the HAA values are generally higher than the LAA values. So, we hypothesized that for all species of algae collected, the hydrophilic antioxidant activity will be higher than the lipophilic antioxidant activity.

Methods

To carry out our study, the 2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), H_2O_2, horseradish peroxidase (ABTS/H_2O_2/HRP) decoloration method was used. A Trolox solution was prepared so that a standard curve could be created to show the absorbance of the Trolox at 730 nm in wavelength in both the hydrophilic and the lipophilic solution. To create the Trolox standard curve, we injected 2, 5, and 10 μL samples (two of each sample size) of Trolox into cuvettes. Then scanned the cuvettes in the UV-VIS spectrophotometer at 730 nm and inserted the ABTS absorbency drop and the corresponding Trolox solution sample size. We then plotted the data in Excel to create a trend line that represented the equation that would then determine the antioxidant activity.

First, we started with the hydrophilic antioxidant activity assays. We had to prepare a phosphate buffer, a hydrogen peroxide dilution, and an HAA reagent. We weighed out 3.45 g NaH_2PO_4 and dissolved it in 400 mL of distilled water to make the buffer. Then, we brought it close to a volume of 500 mL and adjusted the pH to 7.5 by adding the strong base NaOH. Which brought the total volume to 500 mL in a volumetric flask, and we stored the phosphate buffer in a Nalgene bottle. To prepare the hydrogen peroxide dilution, we first measured out 2,363 μL of phosphate buffer and added 30 μL of 30%, 9.8 M H_2O_2. This was mixed and used immediately after being prepared. Lastly, the HAA reagent was prepared by measuring out 27.3 mL of the phosphate buffer (pH 7.5) and adding three 10 mg ABTS tablets that were dissolved in distilled water and ethanol. Then 0.3 mg of horseradish peroxidase and 10 μL of freshly diluted H_2O_2 were added to the solution.

In order to conduct an HAA assay, we had to do a hydrophilic extraction first. To do so, we weighed out 1 g of each algae sample and placed it in 2 mL of the sodium biphosphate buffer using the conversion factor of 1 g = 2 mL. We did this to be able to determine the amount of buffer being used. Once it was soaked overnight, we filtered the solution using a syringe with a filter attached and filtered out as much of the solution as possible. We then put the sodium biphosphate buffer and the HAA reagent into a cuvette and inserted it into the spectrophotometer to get the initial reading, then added a certain amount of the extract to the cuvette and monitored the absorbency drop after 5 minutes.

Finally, we did lipophilic antioxidant activity assays. We had to make the LAA reagent in which we measured out 40 mL of reagent alcohol, then added 326 μL of 86% phosphoric acid, 22 mg of ABTS powder, and sonicated to dissolve. We then added 3.6 μL of diluted H_2O_2 and added 40 μL of HPLC water to increase the enzyme solubility. To conduct an LAA assay, we had to do a lipophilic extraction. We weighed out 2 g of each algae sample and placed it in 4 mL of reagent alcohol using the conversion factor of 1 g = 2 mL to determine the amount of reagent alcohol. We let it soak overnight, then we filtered the solution using a syringe and an attached filter. We put reagent alcohol and the LAA reagent into a cuvette and put it into the spectrophotometer to get the initial reading, then added a certain amount of the extract to the cuvette to record the drop in absorbency every 30 seconds for 5 minutes.

Results

Caulerpa prolifera had the highest TAA value (97.281 ± 18.475 µmol TE/g DW) while *Dictyota jamaicensis* had the lowest TAA (1.999 ± 0.889 µmol TE/g DW). The highest HAA value was *Champia salicornioides* (red algae), followed by *Halimeda incrassata* (green algae), and lastly *Lobophora variagata* (brown algae). The highest to lowest LAA, *Caulerpa prolifera* (green algae), *Hinksia mitchelliae* (brown algae), and *Dicturus occidentalis* (red). The results showed that the HAA was generally higher than LAA. A one-way ANOVA test showed significant difference between the HAA and LAA ($p = 0.0509$). So, our hypothesis was correct. However, a one-way ANOVA test compared all three groups and revealed that there was no statistical difference between their TAA ($p = 0.621$).

Conclusion

In conclusion, *Caulerpa prolifera* had the highest TAA and LAA and *Champia salicornioides* had the highest HAA. These results showed that different algae had varying amounts of antioxidant activities. However, the HAA value was generally higher compared to LAA in each species. Unfortunately, we didn't find any significant difference between the groups for antioxidant levels.

Recommendations

We would like for the antioxidant activity in algae in the Virgin Islands to be a possible source of help for healthcare. Especially for diseases like cancer and some neurological ailments. If given the time and resources, we would like to extract and identify the particular compound in each sample that's responsible for the antioxidant activity using high-performance liquid chromatography (HPLC).

References

1. Vadlapudi, V. Antioxidant activities of marine algae: A review. Medicinal Plants as Antioxidant Agents: Understanding Their Mechanism of Action and Therapeutic Efficacy. 2012, 9, 189-203.

2. Cornish, M. L.; Garbary, D. J. Antioxidants from macroalgae: Potential applications in human health and nutrition. Algae. 2010, 25, 155-171.

3. Alcolea, J. F.; Cano, A.; Acosta, M.; Arnao, M. B. Hydrophilic and lipophilic antioxidant activities of grapes. Nahrung/Food. 2012, 46, 353-356.

4. Vijayavel, K.; Martinez, J. A. In vitro antioxidant and antimicrobial activities of two Hawaiian marine limu: Ulva fasciata (Chlorophyta) and Gracilaria Salicornia (Rhodophyta). J. Med. Food. 2010, 13, 1494-1499.

5. Kelman, D. Antioxidant activity of Hawaiian marine algae. Mar. Drugs. 2012, 10, 403-416.

6. Zubia, M.; Robledo D.; Freile-Pelegrin, Y. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. Journal of applied phycology. 2007, 19, 449-458.

7. Arnao, M. B.; Cano, A.; Acosta, M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry. 2001, 73, 239-244.

8. Tuitt, O.; Yabba, E. Antioxidant activity in commercial spices. 2014, 1-2.