Isolation of Potentially Active Compounds from *Halimeda* sp.

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Abstract

The secondary metabolites of marine organisms have proven over time to be an important source of structurally diverse and biologically active compounds.[1] These compounds have shown to be useful in the development of new classes of therapeutic agents, used specifically to fight various forms of cancer. The goal of this project was to extract and begin identification of these potentially useful compounds from a type of green marine algae (Chlorophyta) called *Halimeda*. This was done through extraction in organic solvents, separation through vacuum liquid chromatography (VLC), and analysis through both mass spectrometry and molecular networking and a brine shrimp bioassay. While certain identification and final conclusions cannot be reached without NMR and further research, a number of unique compounds were found to be present in *Halimeda* and have potential biomedical utility.

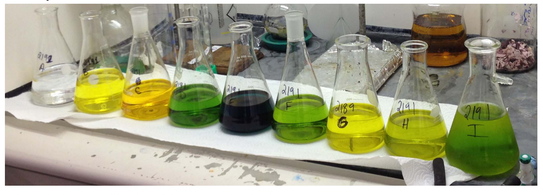
Sample of Halimeda collected from Saipan in February 2013.

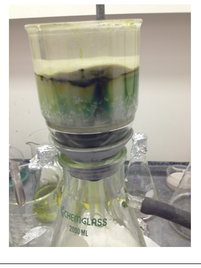
Extraction in Organic Solvents

The natural products of this alga were extracted from the sample using a solution of 2:1 dichloromethane and methanol. The goal of this process is to isolate as much compound as possible, while also avoiding any contamination of water in the solution. Hence, a vital part of this process is the back-extraction of any remaining compounds out of the water present in the first few extracted solutions. This is done by adding dichloromethane to the excess water, which binds to the organic compounds and separates itself from the water. The resulting extract was isolated using a rotational evaporator (rotovap), allowing the compounds to be properly weighed. The resulting amount of crude extract totaled to be about 11 grams from the original 2 liters of biomass. The original *Halimeda* sample was impure and contained other organisms including marine snails, starfish, and cyanobacteria. When the extraction was performed, compounds from these organisms were also likely obtained.

Vacuum Liquid Chromatography

Compounds from the crude extraction were then separated using Vacuum Liquid Chromatography (VLC): a method of separation based on the relative polarity of the compounds present. Solutions of varying polarity, starting with nonpolar hexane and ending with highly polar methanol were poured over a column containing the crude sample and vacuum applied through a porous filter to give fractions. The most vital of these fractions are the most polar (Fractions H and I) because they have historically yielded the most bioactive compounds.

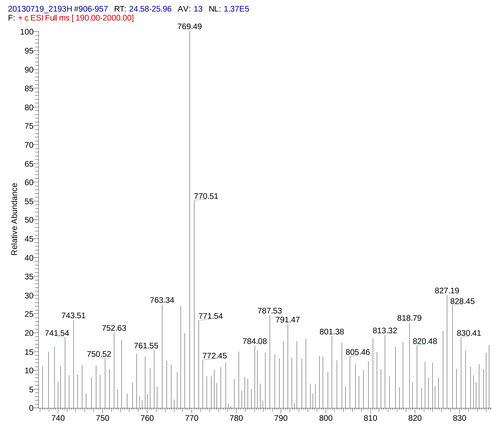




*above:*Final separated fractions *right:*A prepared VLC column

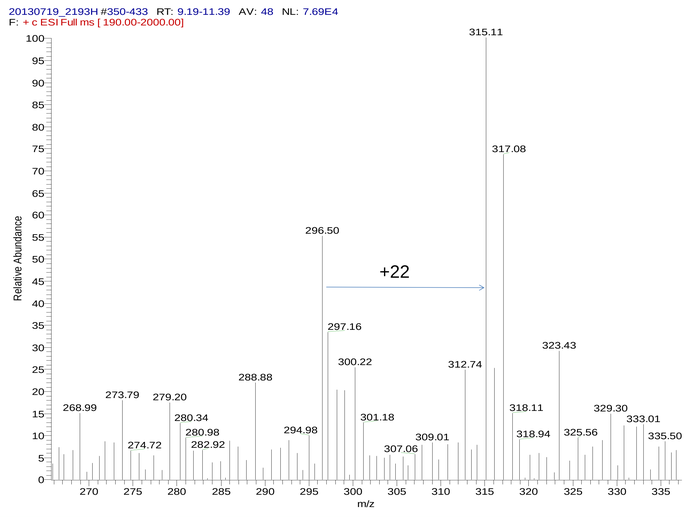
Liquid Chromatography-Mass Spectrometry

LCMS Preparation



Each fraction was dried down into a 10 mL vial and weighed. Based on this weight and the volume restrictions of the vial, each fraction was diluted to either a 10 mg/mL or 100 mg/mL solution in methanol. These solutions were then further diluted into an LCMS vial until the solution contained 1 mg/mL of methanol. LCMS was ran on every vial through 50% MeOH and water.

Examples of compounds identified by LCMS. Above is a protonated compound and below is a sodiated compound.



Brine Shrimp Bioassay

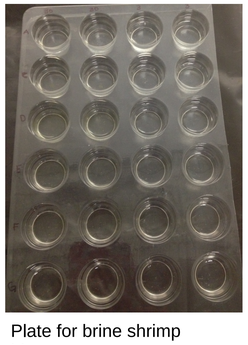
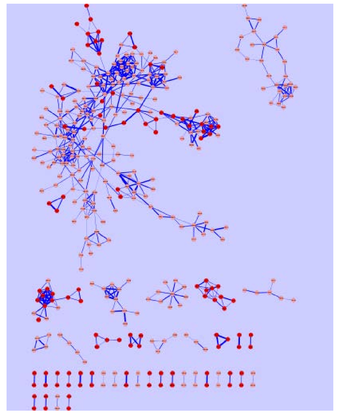


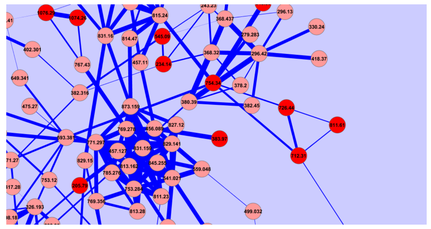
Plate for brine shrimp bioassay.

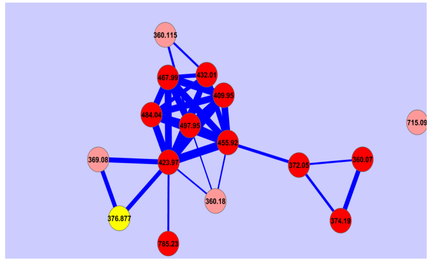
A brine shrimp bioassay was performed on each fraction to test for toxicity. This indicated whether or not the secondary metabolites of *Halimeda* have the capacity to be cytotoxic. The goal of this portion of the project was to kill the brine shrimp with compounds from the fractions. However, no shrimp were killed in any of the fraction wells. This shows that, while *Halimeda*'s secondary metabolites have yet to be identified, they do not have the capacity to be cytotoxic, but they may have other types of biological activity.

LCMS Analysis

LCMS graphs of each sample were analyzed with accompaniment of a Cytoscape molecular network. The goal of this analysis is to identify known compounds present in the extract by LCMS/molecular network in companion to queries of the Marinlit program. The program key searches published papers on marine compounds using their masses and respective taxonomy data. While each fraction had several peaks in the LCMS graph, no correlation was found in the molecular network and Marinlit database. This indicated that no known compounds were present in the extract but rather several other unknown *Halimeda*-specific compounds were present.







The molecular network shows no direct correlation between the pure compound library for cyanobacteria and other algae present in the Gerwick laboratory; however, structural similarities are present, as shown by the connections between the library (red) and *Halimeda* compounds (pink).

CONCLUSIONS AND FUTURE DIRECTIONS

- **The *Halimeda* extract consists of many previously unidentified compounds.**
- **These compounds are not brine shrimp toxic, but may have other biological properties.**
- **Secondary metabolites of marine organisms continue to be a useful source of biomedically-relevant compounds.**

- Identify the structure of the unknown *Halimeda* compounds through NMR analysis.
- Identify the potential other biological uses of the compounds present in the fractions.

ACKNOWLEDGEMENT

Thank you to Dr. Komives for allowing me to participate in the Research Scholars program.
Thank you to Evgenia Glukhov for helping me in the lab and being willing to explain about the goals of my project.
Thank you to Dr. Gerwick for allowing me to return to Scripps Institution of Oceanography and study in his lab.

References

1) Gerwick, W.H.; Moore, B.S.; *Chemistry and Biology,* **2012**, 19, 85