Evaluation of antioxidant and anti-cancer activity of Fucose-containing sulfated polysaccharide (FCSPs) from Hawaiian Marine Algae

Ashley Fukuchi[2], Mayuramas Sang-ngern[1], Nathan Sunada[1], Santi Phosri[1], Ghee Teng Tan[1] and Leng Chee Chang[1].
[1]Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, Hilo, HI 96720 USA,
[2] Department of Chemistry, University of Hawai'i at Hilo, Hilo, HI 96720, USA

Abstract

Fucoidan is a group of sulfated polysaccharides isolated from marine algae. Fucoidan exhibits a variety of biological activities which are related to their chemically distinct sulfated fucose backbone. The structures of select fucoidans vary by species, and the structure and bioactivity remains unclear. The fucoidan of *Padina sp., Asparagopsis taxiformus*, and Nishime Kombu were extracted using four different methods using water at different temperatures. Method 2 gave the highest yield of fucoidan by ^{1}H NMR analysis, whereas method 4 yielded FCSPs with anti-cancer activities. The crude fucoidan of *Padina* sp. was further purified using ion-exchange chromatography, resulting in the highest yield of fucoidan at 2.5 M NaCl.

Cell viability was determined using sulforhodamine B (SRB) assay in five cancer cells (LNCap, PC-3, MCF-7, Caco-2, and LU-1). Both standard and Kombu-extracted fucoidan showed no effects on prostate (LNCaP and PC-3), and breast (MCF-7) cancer cells, but inhibited the growth of colon (Caco-2) and lung (LU-1) cancer cells by 43-74% at concentrations in the range of 50-400 µg/mL. These crude FCSPs also exhibited antioxidant activity. The FRAP values of Nishime Kombu, U-Fn, and Fucoidan Force, were 586.44 ± 107 µM/mg extract, 2922.38 ± 981 µM/mg, and 4212.03 ± 745 µM/mg, respectively.

Introduction

Natural products are great sources of potential compounds for commercial and pharmaceutical use.

Fucoidan, is a naturally occurring group of sulfated polysaccharides present in brown marine algae cell-wall matrices [1]. They consist of sugars such as L-fucose, L-xylose, L-galactose, L-mannose, and uronic acids [2]. Fucoidan has been widely studied due to its wide range of bioactivity, high bioavailability via oral administration, and low toxicity. Research has shown fucoidan to have anti-inflammatory [3,4], anticoagulant [5], antioxidant [6,7,8], and antiviral properties [9].

The brown algal genus, *Padina*, is found around the world in tropical climates. *Padina* are commonly present in intertidal or subtidal zones along coral reefs. Studies of Hawaiian marine algae shown greater antioxidant activities of three brown algae, over other algae, which establishes *Padina* as a potential candidate for evaluation of antioxidant activity.

Limu kohu (*Asparagopsis taxiformis*) is a popular red edible algae that was also incorporated into this study due to its accessibility and use in Hawaiian cuisine. Various algae obtained in the Hawaiian islands were extracted and analyzed for antioxidant and bioactivities.

Figure 3: *Padina spp.*

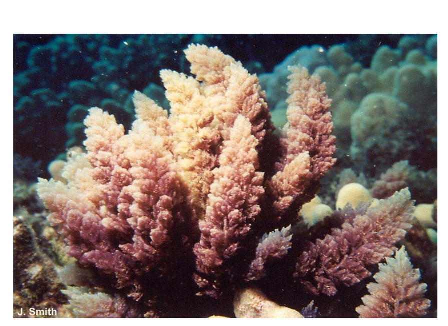


Figure 4: *Asparagopsis taxiformis*

Results

The highest yield of precipitates (method 4) containing crude fucoidan were from the Shirakiku Wakame. However, based on the ^{1}H NMR spectra analysis, there were no NMR signals identical to the fucoidan standard. The highest yielding extraction with the greatest intensity in their respective ^{1}H NMR spectrum was obtained for *Padina sp.* and Shirakiku Nishime (SM) using extraction methods 2 and 4, respectively. DEAE-column purification of *Padina sp.* Md. 2 extraction (87.2 mg) yielded in the highest elution concentration of 2.5M NaCl (12.1 mg), followed by 1.0M (11.1 mg).

Shirakiku Nishime (SM) and fucoidan standards at 400 µg/mL exhibited >70% inhibition of human colon (CaCo-2) and lung (LU-1) cancer cell growth. SM inhibited CaCo-2 by 68% and LU-1 by 74%, while the fucoidan standard inhibited these by 91% and 70%, respectively. Colchicine (control) exhibited inhibition of CaCo-2 and LU-1 by 71% and 80%. Other extractions, such as *Padina* sp., Shirakiku Wakame, and Shirakiku Dried Kelp did not have inhibitory effects against all five cancer cell lines.

Figure 1. ^{1}H NMR Spectrum of Extracted Samples .

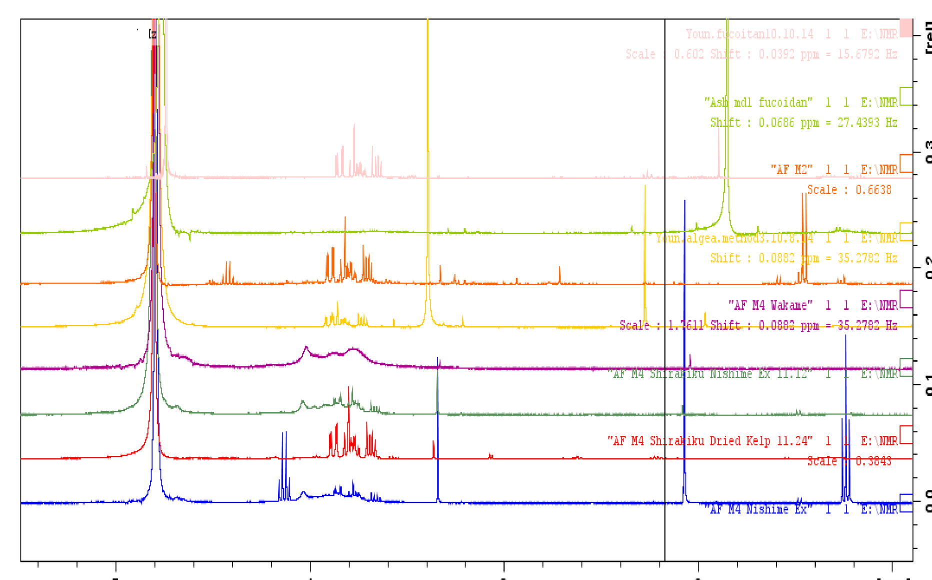


Figure 2. LU-1 SRB Assay Results.

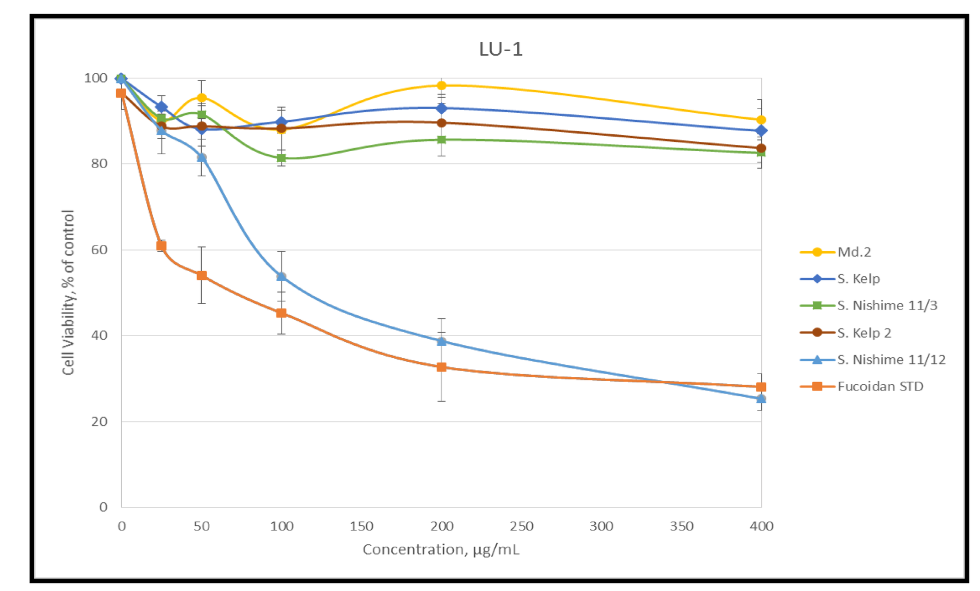
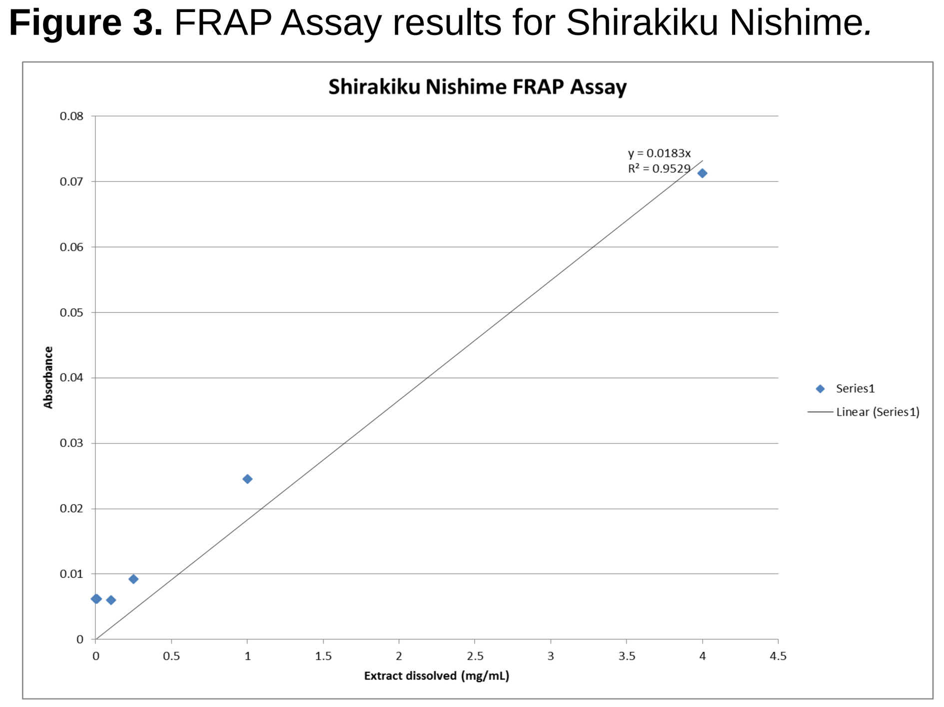
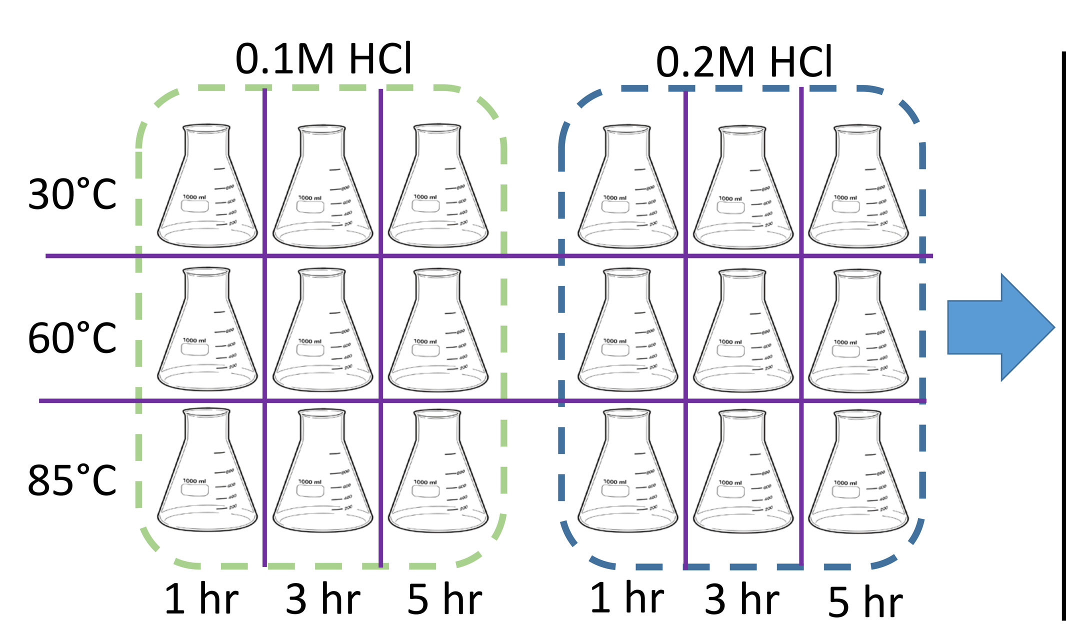


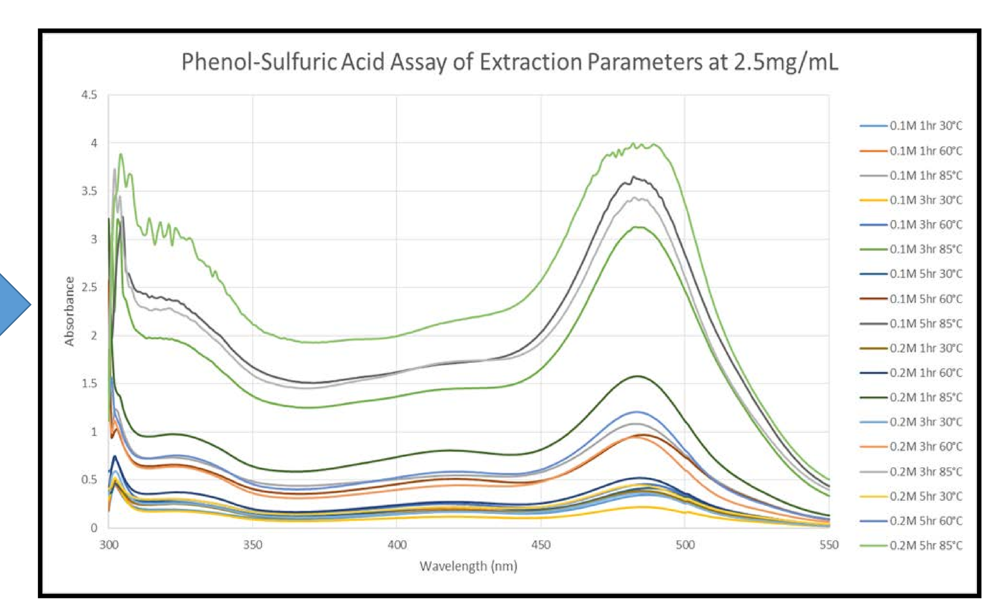
Figure 3. FRAP Assay results for Shirakiku Nishime.



The FRAP assay was conducted for all extracts. The FRAP values of Nishime Kombu, U-Fn, and Fucoidan Force, were 586.44 µM/mg extract, 2922.38 µM/mg, and 4212.03 µM/mg, respectively. The standard used for FRAP was iron (III) hexahydrate.

For the method optimization of the SM, temperature was the major factor which increased uronic acid and fucose content, followed by time of extraction and acid concentration.





Experimental Section

General Procedures: *Padina* sp. was collected at Richardson Beach, Hilo, Hawai'i in 2014. The lyophilized and dried *Padina* sp. (10g) were extracted using three methods. Preliminary evaluation of crude fucoidan was conducted using ^{1}H NMR analysis. Along with *Padina* sp., *limu kohu* was extracted using method 2. Other store bought seaweed (Shirakiku Nishime, Wakame, and Dried Kelp) were extracted with method 4.

Table 1. Extraction Methods for Crude Fucoidan

Methods	**1**	**2**	**3**	**4**
Procedure	Extraction with H_2O, 3 x 2hr at 70°C, precipitated with EtOH and $CaCl_2$	Water extraction at boiling temp 4 hr, 0.09M HCl at 4°C 2 hr, ppt with 85% EtOH	Pretreatment of MeOH-$CHCl_3$-H_2O (4:2:1), extracted with 2% $CaCl_2$ 5 hr, ppt with 20% EtOH.	Pretreatment of MeOH-$CHCl_3$-H_2O (4:2:1), 0.03M HCl, 4hr at 90°C.

FRAP (Ferric Reducing Ability of Plasma) assay: This assay was used to determine antioxidant activity of extracts. [6]

Phenol-sulfuric acid assay: Purification of the crude FCSPs was conducted using diethylaminoethyl (DEAE) Sephadex A-25 ion exchange column. Fucoidan fractions were separated with gradually increasing concentrations of NaCl. This assay was used to detect fucoidan and uronic acids containing fractions by observing absorbance between 480-490 nm with a UV-Vis spectrophotometer. [12]

Sulforhodamine B (SRB) assay: This assay was used to determine the cytotoxicity of FCSP extracts towards LNCap, PC-3, MCF-7, CaCo-2, LU-1. Cells were placed into 96 well plates (106 cells/mL), six dilutions of extracts were tested in triplicate, incubated at 37°C for 72 hrs. [11]

Bioassay-Guided Optimizationof the method : Method optimization for the extraction of Shirakiku Nishime (SM) was considered based on the SRB assay results. SM was decolored and lyophilized overnight. The variables were: 0.1M and 0.2M of HCl, extraction temperatures at 30 ºC, 60 ºC, 85 ºC, and extraction times of 1hr, 3hr, and 5hr. This amounted to 18 different parameter combinations.

Discussions and Conclusion

Preliminary study support the hypothesis that Hawaiian marine algae are promising sources of fucoidan. Methods 2 and 3 yielded crude fucoidan from *Padina* sp. as determined via ^{1}H NMR spectra in comparison to a fucoidan standard. The FRAP assay determined antioxidant activity of method 2 crude FCSPs with a standardized positive control fucoidan. The initial negative result of FCSPs might be due to lower concentrations of crude fucoidan. DEAE-ion exchange column was used to purify crude fucoidan from *Padina* sp. All samples were evaluated for cytotoxicity in five different cancer cell lines. Only Shirakiku Nishime extracted fucoidan (method 4) demonstrated cytotoxic effects against CaCo-2 and LU-1 cancer cell lines [9,10]. The findings indicated the potentiality of Shirakiku Nishime as an valuable source of anticancer capabilities, and the selective inhibition of the growth of two cancer cell lines.

Acknowledgements

The authors thank HiSREP and Dr. Susan Jarvi for the funding support, and are grateful to Dr. Maria Haws, Pacific Aquaculture & Coastal Resources Center, for assisting the collection of *Padina* sp., and Dean John Pezzuto and Dr. Kenneth Morris for providing the research facility and support for this study).

INBRE Acknowledgement Statement

"This project was supported by grants from the National Institute of General Medical Sciences, National Institutes of Health, award number: P20GM 103466. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health."

References:

1. Kim W.J. (2007). Purification and Anticoagulant Activity of Fucoidan from Korean *Undaria pinnatifida* Sporophyll. *Algae*. 22(3):247-252.
2. Anastyuk S.D. (2014). Rapid Mass Spectrometric Analysis of a Novel Fucoidan, Extracted from the Brown Alga *Coccophora langsdorfii*. *The Scientific World Journal.* 2014, Article ID 972450 9 pages. – ISSN 1611-2156.
3. Mavlonov G.T. (2011). Low Molecular Fucoidan and its macromolecular complex with Bee Venom Melittin. *Advances in Bioscience and Biotechnology*. 2: 298-303.
4. Xing R. (2013). Extraction and Separation of Fucoidan from *Laminaria japonica* with Chitosan as Extractant. *BioMed Research International*. 2013, Article ID 193689, 4 pages.
5. Mak W. (2014). Anti-proliferation Potential and content of Fucoidan extracted from sporophyll of New Zealand *Undaria pinnatifida*. *Frontiers in Nutrition*. 1:9.
6. Kaushik A. (2012). FRAP (Ferric reducing ability of plasma) assay and effect of *Diplazium esculentum* (Retz) Sw. (a green vegetable of North India) on Central Nervous System. *Indian Jour. of Nat. Prod. And Resources.* 3(2): 228-231.
7. Kelman D. (2012). Antioxidant Activity of Hawaiian Marine Algae. *Mar. Drugs*. 10: 403-416.
8. Cho M.L. (2011). Relationship between Oversulfation and Conformation of Low and High Molecular Weight Fucoidans and Evaluation of Their *in Vitro* Anti-cancer Activity. *Molecules*. 16:291-297.
9. Lee H. (2012). Fucoidan from Seaweed *Fucus vesiculosus* Inhibits Migration and Invasion of Human Lung Cancer Cell via PI3K-Akt-mTOR Pathways. *PLOS| ONE*. 7(11):1-10
10. Ikeguchi H. (2011). Fucoidan reduces the toxicities of chemotherapy for patients with unresectable advanced or recurrent colorectal cancer. *Oncology Letters*. 2: 319-322.
11. Yao, G. (2009). Bioactive Sulfated Sesterterpene Alkaloids and Sesterterpene Sulfates from the Marine Sponge *Fasciospongia* sp. *J. Nat. Prod*. 72: 319-323
12. Skriptsova, A. V. (2009). Monthly changes in the content and monosaccharide composition of fucoidan from *Undaria pinnatifida* (Laminariales, Phaeophyta). *J Appl Phycol.* 22: 79-86.