Science Diliman (January-June 2018) 30:1, 45-59

Fucoidan Content in Philippine Brown Seaweeds

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ABSTRACT

This study aims to determine which brown macroalgae in the Philippines has the highest content of partially purified fucoidan. Percent fucoidan content of brown seaweeds *Sargassum* spp., *Padina* sp., *Hydroclathrus* sp., *Turbinaria ornata* J. Agardh, *Hormophyza cuneiformis* PC Silva, and *Dictyota dichotoma* Lamouroux were determined in fifty sites across 14 provinces in Northern Luzon (Cagayan, Ilocos), West Luzon (Pangasinan), the eastern seaboard of Luzon (Quezon Province, Camarines, Sorsogon), Central and Eastern Visayas (Bohol, Cebu, Negros Oriental, Negros Occidental), and Northern Mindanao (Camiguin, Lanao del Norte, Misamis Oriental, Misamis Occidental). Crude and semi-pure fucoidan were extracted through acid hydrolysis and ethanol precipitation using 50 grams of dried and milled seaweed biomass. Extracts were verified using infrared spectroscopy with fucoidan from *Fucus vesiculosus* as standard. *Sargassum* spp. is the most widely distributed source of fucoidan found in all sites. *T. ornata* was found in only 11 sites. Both have significantly higher percent

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ISSN 0115-7809 Print / ISSN 2012-0818 Online

content ($p \geq 0.05$) of fucoidan than other sampled seaweeds. Higher percent content of semi-purified fucoidan were observed in *D. dichotoma* from Bohol (1.53%), *H. cuneiformis* from Cebu (2.17%), *Hydroclathrus* sp. from Pangasinan (2.23%), *Padina* sp. from Quezon Province (3.69%), *Sargassum* spp. from Camiguin (4.30%), and *T. ornata* from Cagayan (7.03%).

Keywords: Brown seaweeds, distribution, fucoidan, fucoidan yield

INTRODUCTION

The Philippines is known for its diverse marine flora, particularly seaweeds. The country contains 966 taxa of macroalgae, with 893 species in 82 families. Brown macroalgae (Order Ochrophyta, Phaeophyceae) are represented in 171 taxa with 153 species in 10 families (Ang et al. 2013). Brown algae contain fucoxanthin, a xanthophyll pigment responsible for its distinguishing brown color. Their cell walls are composed of cellulose, alginic acid, and other algal polysaccharides (Trono 1997).

Fucoidan is a sulfated polysaccharide commonly found in brown seaweeds, as well as in marine invertebrates (Mak et al. 2013). Fucoidan is absent in green algae (Chlorophyceae) and red algae (Rhodophyceae) (Berteau and Molloy 2003). Fucoidans are structurally diverse macromolecules with a backbone of a (1,3)- and (1,4)-linked α(1,4)-bonded α-L-fucopyranose residues. The polysaccharide may be arranged in stretches of alternating α(1,3)- and α(1,4)-bonded L-fucopyranose residues or through (13)-α-fucan (Ale et al. 2011).

Fucoidans have been extensively studied due to their biological activity which includes anti-inflammatory, antioxidant, anticoagulant, antitumor, antiangiogenic, antithrombotic, antiviral, and immunomodulatory properties. It is widely studied because it comes from different inexpensive sources, and has a potential for drug development or as a functional food resource (Li et al. 2008).

Despite the diverse potential applications of fucoidans, information on the fucoidan content of seaweeds in the Philippines remain limited. This information is necessary to guide manufacturers and researchers on which species and sites should be utilized for the maximization of the extraction of the algal polysaccharide. In this study, we determined the fucoidan content of brown seaweeds collected from different provinces all over the Philippines through acid hydrolysis extraction and fractional ethanol precipitation. We further compared which among the species has the highest content of fucoidan.

MATERIALS AND METHODS

Sample collection

Gratuitous permits were issued by the different regional offices of the Bureau of Fisheries and Aquatic Resources (BFAR). Fresh seaweed thalli were collected from the intertidal zones across fifty sites in fourteen provinces in the Philippines (Figure 1, Table 1): Northern Luzon (Cagayan, Ilocos); West Luzon (Pangasinan); the eastern seaboard of Luzon island (Quezon, Camarines, Sorsogon); Central and Eastern Visayas Islands (Bohol, Cebu, Negros Oriental, Negros Occidental); and Northern Mindanao Island (Camiguin, Lanao del Norte, Misamis Oriental, Misamis Occidental). One-time sampling was conducted in all the sites. Not all species were found in

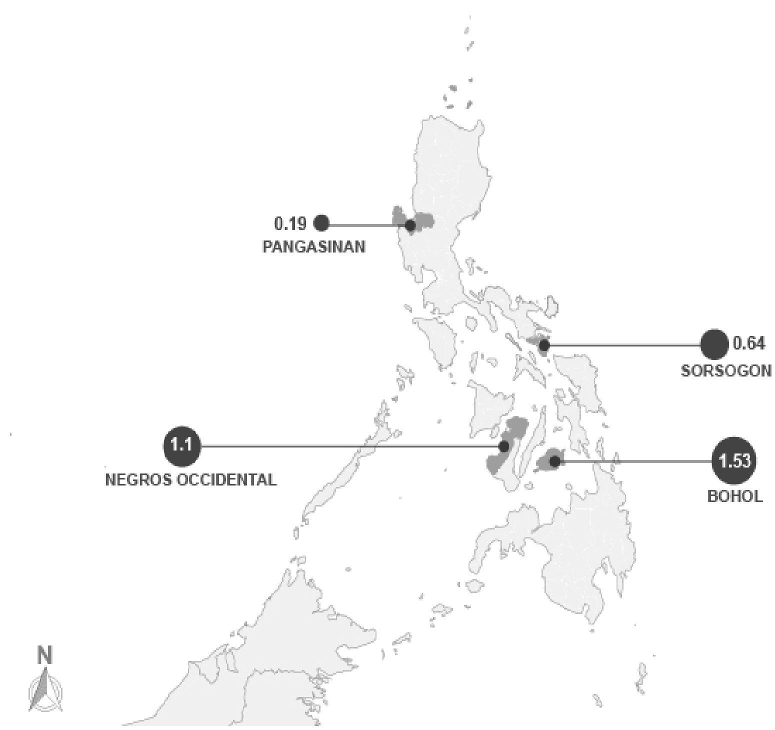

Figure 1. Percent fucoidan content (semi-purified) of Dictyota collected in Pangasinan, Sorsogon, Negros Occidental and Bohol.

Table 1. Sampling sites and GPS coordinates

Genera	Site	Sample Collection		
		Date	Latitude	Longitude
Sargassum	Patar, Bolinao, Pangasinan	17-19 May 2010	16°19'59.11"N	119°47'13.95"E
	Patar, Bolinao, Pangasinan	17-19 May 2010	16°19'59.11"N	119°47'13.95"E
	Trensiera, Bolinao, Pangasinan	17-19 May 2010	16°26' 24.84"N	119°56' 45.87"E
	Lucero, Bolinao, Pangasinan	17-19 May 2010	16°24'10.50"N	119°54'22.91"E
	Villa Manzano Norte, Alabat, Quezon	25-30 Nov 2011	14°1'43.17"N	122°5'17.56"E
	Sabang, Alabat, Quezon	25-30 Nov 2011	14°3'28.52"N	122°9'33.78"E
	Gonzaga, Cagayan	25-29 July 2010	18°17'16.30"N	121°59'24.89"E
	Sta. Ana, Cagayan	25-29 July 2010	18°28'49.06"N	122°8'26.09"E
	Sta. Ana, Cagayan	25-29 July 2010	18°28'49.06"N	122°8'26.09"E
	Blue Lagoon, Ilocos Norte	25-29 July 2010	18°37'22.49"N	120°51'34.16"E
	Burgos, Ilocos Norte	25-29 July 2010	16°2'44.93"N	119°45'13.97"E
	Burgos, Ilocos Norte	25-29 July 2010	16°2'44.93"N	119°45'13.97"E
	Burgos, Ilocos Norte	25-29 July 2010	16°2'44.93"N	119°45'13.97"E
	Lioes, Ilocos Norte	25-29 July 2010	18°0' 59.68"N	120°29' 11.37"E
	Pangil, Ilocos Norte	25-29 July 2010	18°0'24.19"N	120°29'20.19"E
	Bgy Nailon, Bogo City, Cebu	10-16 Aug 2010	11°2'52.02"N	124°2'28.96"E
	Bgy Nailon, Bogo City, Cebu	10-16 Aug 2010	11°2'52.02"N	124°2'28.96"E
	Alcoy, Cebu	10-16 Aug 2010	9°40'25.97"N	123°30'18.65"E
	Alcoy, Cebu	10-16 Aug 2010	9°40'25.97"N	123°30'18.65"E
	Maribago, Mactan, Cebu	10-16 Aug 2010	10°17'8.17"N	124°0'26.30"E
	Catmon, Cebu	10-16 Aug 2010	10°43'20.72"N	124°1'4.35"E
	Dalaguete, Cebu	10-16 Aug 2010	9°45'54.64"N	123°32'10.47"E
	Dalaguete, Cebu	10-16 Aug 2010	9°45'54.64"N	123°32'10.47"E
	Bgy. Paypay, Daanbantayan, Cebu	10-16 Aug 2010	11°13'20.67"N	123°58'56.78"E
	Ubojan East, Garcia-Hernandez, Bohol	10-16 Aug 2010	9°36'32.40"N	124°18'7.14"E
	Larapan, Jagna, Bohol	10-16 Aug 2010	9°38'55.90"N	124°22'7.31"E
	Pamilacan Island, Baclayon, Bohol	10-16 Aug 2010	9°29'25.84"N	123°54'59.53"E
	Pamilacan Island, Baclayon, Bohol	10-16 Aug 2010	9°29'25.84"N	123°54'59.53"E
	Sitio Basdio, Loon, Bohol	10-16 Aug 2010	9°48' 2.22"N	123°47' 20.61"E
	Punta-Cruz, Maribojoc, Bohol	10-16 Aug 2010	9°43'56.69"N	123°47'54.99"E
	Sitio Daorong, Bgy. Danao, Panglao, Bohol	10-16 Aug 2010	9°32'41.70"N	123°46'1.16"E
	Bgy. Lawis, Panggangan Island, Calape, Bohol	10-16 Aug 2010	9°54'13.80"N	123°50'27.84"E
	Bgy. Lawis, Panggangan Island, Calape, Bohol	10-16 Aug 2010	9°53' 40.86" N	123°50' 29.37"E
	Pungtod Island, Panglao, Bohol	10-16 Aug 2010	9°40'41.37"N	123°51'0.69"E
	Sitio Hoyohoy, Bgy. Tawala, Panglao, Bohol	10-16 Aug 2010	9°33'23.51"N	123°48'32.91"E
	Sto. Domingo, (Bicol)	8-13 Nov 2010	13°23'42.40"N	123°11'29.20"E
	Sto. Domingo, (Bicol)	8-13 Nov 2010	13°23'42.40"N	123°11'29.20"E
	Pasacao, (Bicol)	8-13 Nov 2010	13°30'30.59"N	123°0'27.70"E
	Bulusan, Sorsogon	8-13 Nov 2010	12°44'52.69"N	124°8'33.59"E
	Bulusan, Sorsogon	8-13 Nov 2010	12°44'52.69"N	124°8'33.59"E
	Sangay, Sorsogon	8-13 Nov 2010	13°36'22.02"N	123°32'51.26"E
	Matnog, Sorsogon	8-13 Nov 2010	12°35'59.45"N	124°6'24.24"E
	Pinagtigasan, Sorsogon	8-13 Nov 2010	14°9'56.41"N	122°58'34.42"E
	Poblacion, Oroqueta City, Misamis Occidental	18-24 Oct 2010	8°28'55.05"N	123°49'37.89"E
	Poblacion, Oroqueta City, Misamis Occidental	18-24 Oct 2010	8°28'55.05"N	123°49'37.89"E
	Talisayan, Poblacion, Misamis Oriental	18-24 Oct 2010	9°0'53.98"N	124°52'15.25"E
	Cantaan, Guinsilaban, Camiguin	18-24 Oct 2010	9°5'36.28"N	124°47'29.84"E
	Tagcatong, Carmen, Misamis Oriental	18-24 Oct 2010	9°5'36.28"N	124°47'29.84"E
	Tagcatong, Carmen, Misamis Oriental	18-24 Oct 2010	8°31'8.56"N	124°37'48.51"E
	Gingoog, Misamis Oriental	18-24 Oct 2010	8°49'57.16"N	125°5'49.66"E
	Kauswagan, Lanao del Norte	18-24 Oct 2010	8°12'3.60"N	124°4'57.57"E
	Liangan, Maigo, Misamis Oriental	18-24 Oct 2010	8°9'36.72"N	123°56'20.24"E
	Bgy. Roque, Mantigue Island, Camiguin	18-24 Oct 2010	9°10'14.48"N	124°49'26.41"E
	San Jose, Negros Occidental	17-20 Sept 2010	9°25'10.65"N	123°14'36.85"E
	Sipalay City, Negros Occidental	17-20 Sept 2010	9°44'47.75"N	122°23'50.14"E
	Dumaguete, Negros Oriental	17-20 Sept 2010	9°18'17.53"N	123°18'40.40"E

Table 1. Sampling sites and GPS coordinates (Cont.)

Genera	Site	Sample Collection		
		Date	Latitude	Longitude
	Hinobaan 2, (Negros)	17-20 Sept 2010	9°32'23.05"N	122°30'56.19"E
	San Juan, (Negros)	17-20 Sept 2010	10°36'32.86"N	122°54'36.25"E
	Bais City, (Negros)	17-20 Sept 2010	9°34'44.33"N	123°10'19.55"E
	Hinobaan 1, (Negros)	17-20 Sept 2010	9°35'57.65"N	122°27'50.81"E
	Sta. Catalina, (Negros)	17-20 Sept 2010	9°19'40.42"N	122°51'54.28"E
	Lazi, (Negros)	17-20 Sept 2010	9°19'21.00"N	123°19'33.01"E
	Maria 2, (Negros)	17-20 Sept 2010	10°43'52.81"N	122°56'2.08"E
Turbinaria	Trensiera, Bolinao, Pangasinan	17-19 May 2010	16°26' 24.84"N	119°56' 45.87"E
	Villa Manzano Norte, Alabat, Quezon	25-30 Nov 2011	14°1'43.17"N	122°5'17.56"E
	Gonzaga, Cagayan	25-29 July 2010	18°17'16.30"N	121°59'24.89"E
	Blue Lagoon, Ilocos Norte	25-29 July 2010	18°37'22.49"N	120°51'34.16"E
	Maribago, Mactan, Cebu	10-16 Aug 2010	10°17'8.17"N	124°0'26.30"E
	Punta-Cruz, Maribojoc, Bohol	10-16 Aug 2010	9°44' 4.20" N	123°47' 26.04" E
	Sitio Hoyohoy, Bgy. Tawala, Panglao, Bohol	10-16 Aug 2010	9°32' 57.91"N	123°46' 52.84"E
	Sangay, Sorsogon	8-13 Nov 2010	13°36'22.02"N	123°32'51.26"E
	Talisayan, Poblacion, Misamis Oriental	18-24 Oct 2010	9°0'53.98"N	124°52'15.25"E
	Bgy. Balite, Sagay, Camiguin	18-24 Oct 2010	9°6'14.35"N	124°42'48.10"E
	Bgy. Roque, Mantigue Island, Camiguin	18-24 Oct 2010	9°10' 16.99"N	124°49' 22.38"E
	Sipalay City, Negros Occidental	17-20 Sept 2010	9°44'47.75"N	122°23'50.14"E
	Hinobaan 2, (Negros)	17-20 Sept 2010	9°32'23.05"N	122°30'56.19"E
	San Juan, (Negros)	17-20 Sept 2010	10°36'32.86"N	122°54'36.25"E
Padina	Patar, Bolinao, Pangasinan	17-19 May 2010	16°19'59.11"N	119°47'13.95"E
	Perez, Alabat, Quezon	25-30 Nov 2011	14°10'48.33"N	121°55'27.49"E
	Gonzaga, Cagayan	25-29 July 2010	18°17'16.30"N	121°59'24.89"E
	Sta. Ana, Cagayan	25-29 July 2010	18°28'49.06"N	122°8'26.09"E
	Burgos, Ilocos Norte	25-29 July 2010	16° 2'44.93"N	119°45'13.97"E
	Alcoy, Cebu	10-16 Aug 2010	9°40'25.97"N	123°30'18.65"E
	Maribago, Mactan, Cebu	10-16 Aug 2010	10°17'8.17"N	124°0'26.30"E
	Bgy. Paypay, Daanbantayan, Cebu	10-16 Aug 2010	11°13'20.67"N	123°58'56.78"E
	Pamilacan Island, Baclayon, Bohol	10-16 Aug 2010	9°29'25.84"N	123°54'59.53"E
	Sitio Basdio, Loon, Bohol	10-16 Aug 2010	9°47'51.87"N	123°47'1.22"E
	Sto. Domingo, (Bicol)	8-13 Nov 2010	13°23'42.40"N	123°11'29.20"E
	Bulusan, Sorsogon	8-13 Nov 2010	12°44'52.69"N	124°8'33.59"E
	Sipalay City, Negros Occidental	17-20 Sept 2010	9°44'47.75"N	122°23'50.14"E
	Dumaguete, Negros Oriental	17-20 Sept 2010	9°18'17.53"N	123°18'40.40"E
	Caliling, (Negros)	17-20 Sept 2010	9°59'32.31"N	122°28'14.94"E
	Hinobaan 1, (Negros)	17-20 Sept 2010	9°35'57.65"N	122°27'50.81"E
Hormophyza	Patar, Bolinao, Pangasinan	17-19 May 2010	16°19'59.11"N	119°47'13.95"E
	Trensiera, Bolinao, Pangasinan	17-19 May 2010	16°26' 24.84"N	119°56'45.87"E
	Lucero, Bolinao, Pangasinan	17-19 May 2010	16°24'10.50"N	119°54'22.91"E
	Maribago, Mactan, Cebu	10-16 Aug 2010	10°17'8.17"N	124°0'26.30"E
	Dalaguete, Cebu	10-16 Aug 2010	9°45'54.64"N	123°32'10.47"E
	Sto. Domingo, (Bicol)	8-13 Nov 2010	13°23'42.40"N	123°11'29.20"E
	Bulusan, Sorsogon	8-13 Nov 2010	12°44'52.69"N	124° 8'33.59"E
	Talisayan, Poblacion, Misamis Oriental	18-24 Oct 2010	9°0'53.98"N	124°52'15.25"E
	Hinobaan 2, (Negros)	17-20 Sept 2010	9°32'23.05"N	122°30'56.19"E
	San Juan, (Negros)	17-20 Sept 2010	10°36'32.86"N	122°54'36.25"E
Hydroclathrus	Patar, Bolinao, Pangasinan	17-19 May 2010	16°19'59.11"N	119°47'13.95"E
	Patar, Bolinao, Pangasinan	17-19 May 2010	16°19'59.11"N	119°47'13.95"E
Dictyota	Lucero, Bolinao, Pangasinan	17-19 May 2010	16°24'10.50"N	119°54'22.91"E
	Ubojan East, Garcia-Hernandez, Bohol	10-16 Aug 2010	9°36'32.40"N	124°18'7.14"E
	Pamilacan Island, Baclayon, Bohol	10-16 Aug 2010	9°29'25.84"N	123°54'59.53"E
	Bulusan, Sorsogon	8-13 Nov 2010	12°44'52.69"N	124°8'33.59"E
	Hinobaan 2, (Negros)	17-20 Sept 2010	9°32'23.05"N	122°30'56.19"E
	San Juan, (Negros)	17-20 Sept 2010	10°36'32.86"N	122°54'36.25"E
	Maria, (Negros)	17-20 Sept 2010	10°20'58.91"N	122°50'54.02"E

the same locations. The type of seaweeds collected varied among sampling sites. Samples were washed with distilled water to remove salt and epiphytes, air dried, and milled for extraction. Voucher specimens were dried, identified, labeled, and kept at the GT Velasquez Phycological Herbarium. Study collection sites were limited by geopolitical and accessibility considerations.

Extraction of fucoidan

Milled samples were used for extraction following the methods presented by Ale et al. (2011) with slight modifications. Fifty grams of dried and milled seaweed thalli were briefly acid-treated using dilute hydrochloric acid, heated, allowed to cool, and centrifuged. Residues were discarded afterwards. The solution was neutralized to pH 7.0 using sodium hydroxide pellets, forming brown precipitate of crude fucoidan. Partial purification was performed by fractional precipitation using 30% and 60% ethanol, in order to remove alginate contamination and to precipitate semi-pure fucoidan. Semi-pure fucoidan extracts were subjected to infrared spectroscopy to verify signature peaks of functional groups of fucoidan from *Fucus vesiculosus*. Using FT-IR spectrophotometer, dry fucoidan was mounted to the attenuated total reflectance (ATR) accessory sample holder and scanned from 400 cm^{-1} to 4000 cm^{-1}. Fucoidan from *F. vesiculosus* (Sigma Aldrich: F5631) was used as standard for structure elucidation.

Data analyses

Percent fucoidan content of the sampled seaweeds from different sites were pooled within respective provinces. Percent fucoidan content was calculated using the weight of the semi-purified fucoidan (in grams) divided by the dried and milled biomass (50 g) of seaweeds. The resulting value was then multiplied by 100. The average value of the percent fucoidan content from its corresponding seaweed sources were plotted on the Philippine map through QGIS, an open-source geographic information software. There were differences in the occurrence of seaweeds per sampling site. Statistical analyses on the percent content were conducted via one-way ANOVA and Tukey's Multiple Comparison Test.

RESULTS

The diversity and occurrence of brown seaweeds varied significantly among sampling sites. Samples were grouped and pooled up to genus level. Of the major groups, *Sargassum* was consistently present and collected in all of the 14 provinces, *Turbinaria* in 11 provinces, *Padina* in 10 provinces, *Hormophysa* in six provinces, *Dictyota* in four provinces, and *Hydroclathrus* in only one province (Figures 1-6). Crude fucoidan content varied from 10.23% to 24.55% (*Sargassum* spp., *Turbinaria ornata*), whereas partially-purified fucoidan yield were at 1.89% to 7.03% (*Padina* sp., *T. ornata*) based on dry weight. *Sargassum* samples from Camiguin had the highest fucoidan content (4.3%) among the provinces while samples from Pangasinan had the lowest content (1.89%). *Turbinaria* from the northern Philippine provinces of Cagayan and Ilocos had the highest content (7.03% and 6.85%, respectively),

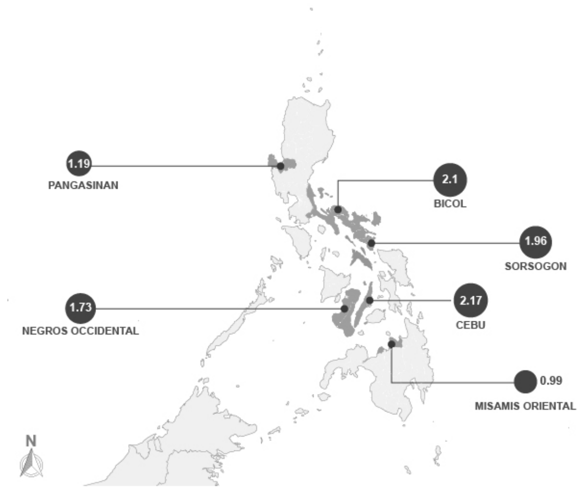

Figure 2. Percent fucoidan content (semi-purified) of the brown seaweed *Hormophysa* collected in six provinces in the Philippines.

Figure 3. Percent fucoidan content (semi-purified) of *Hydroclathrus*. *Hydroclathrus* was only observed in Pangasinan.

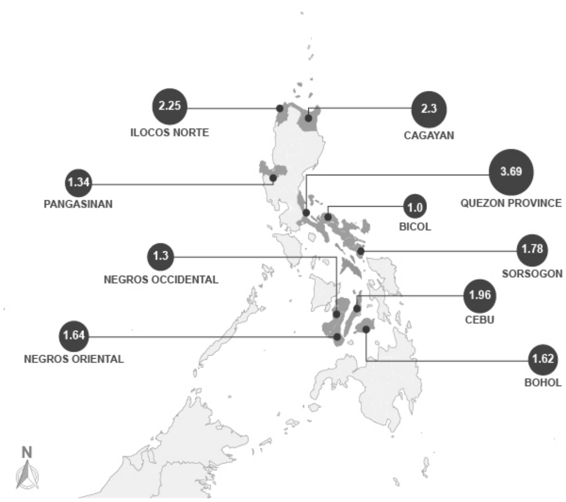

Figure 4. Percent fucoidan content (semi-purified) of *Padina* collected in 10 provinces in the Philippines.

Figure 5. Percent fucoidan content (semi-purified) of *Sargassum*. Samples were collected in 14 provinces in the Philippines. Highest fucoidan yield was observed in Camiguin.

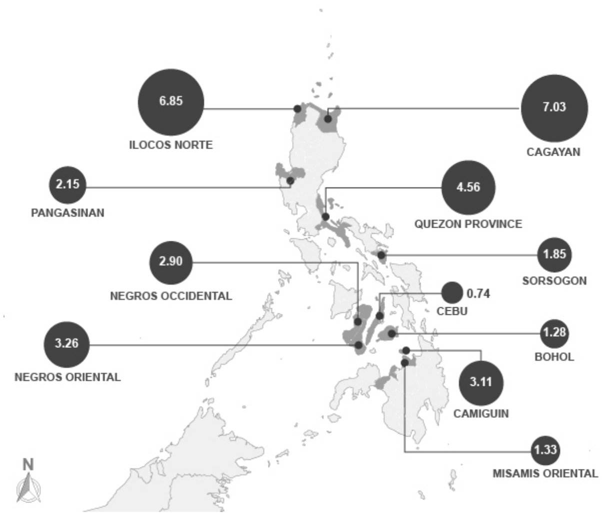

Figure 6. Percent fucoidan content (semi-purified) of *Turbinaria* collected in 11 provinces in the Philippines. Samples from Cagayan obtained the highest fucoidan yield.

while those obtained in Cebu had the lowest fucoidan content at 0.74%. *Padina* from the Quezon Province had the highest content at 3.69%, while the Negros Occidental samples had the lowest content at 1.3%. *Hormophysa* from Cebu had the highest content at 2.17%, while the Misamis Oriental samples obtained the lowest content at 0.99%. *Dictyota* from Bohol had the highest content at 1.53%, while the Pangasinan samples had the lowest content at 0.19%. *Hydroclathrus* from Pangasinan averaged at 2.23% fucoidan content.

Fucoidan percent content among the genus were also compared. There was no significant difference between the contents of *Sargassum,* and *Hormophysa, Hydroclathrus, Padina*, and *Turbinaria* ($p \leq 0.05$). However, the percent content of *Sargassum* compared to *Dictyota* was significantly higher ($p \geq 0.05$). Percent content from *Turbinaria* was significantly higher compared to *Dictyota*, *Hormophysa,* and *Padina* ($p \leq 0.05$). There was no significant difference between the yields of *Sargassum* and *Turbinaria* ($p \leq 0.05$). Additionally, there was no significant difference between the fucoidan contents of *Padina* and *Hormophysa, Hydroclathrus* and *Dictyota*, and *Hormophysa* against *Hydroclathrus* and *Dictyota* (Figure 7).

Representative data on the semi-purified Fucoidan from *Sargassum*, *Turbinaria,* and *Padina* showed peaks similar to the standard fucoidan from *F. vesiculosus* (Figure 8). There were broad bands at 3321–3415 cm^{-1} and small peaks at 2941-2945 cm^{-1}, indicating signature vibrations of OH groups and CH of pyranoid rings, and C6 of fucose and galactose, respectively (Kim et al. 2010). A peak at 1732 cm^{-1} indicates the O-acetyl group (Chandia and Matsuhiro 2008; Kim et al. 2010; Synytsya et al. 2010). Sulfate stretch was observed at 1241–1242 cm^{-1}, which are peaks unique to ester sulfates. Finally, centered peaks were observed between 830–842cm^{-1}, corresponding to C-O-S with sulfate at equatorial and/or axial positions (Bilan et al. 2004; Kim et al. 2010).

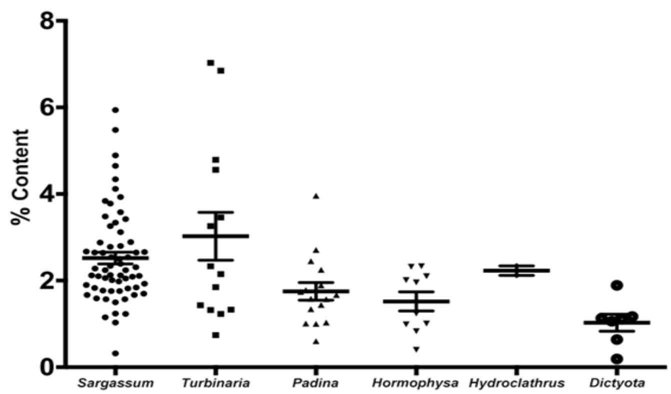

Figure 7. Percent fucoidan content of brown seaweeds in the Philippines. Comparison of the fucoidan yield of samples gathered from different provinces in the country from 2010-2011. Significant differences between the following comparisons were observed at 95% confidence interval: *Sargassum* and *Dictyota*, *Turbinaria* and *Padina*, *Turbinaria* and *Hormophysa,* and *Turbinaria* and *Dictyota*.

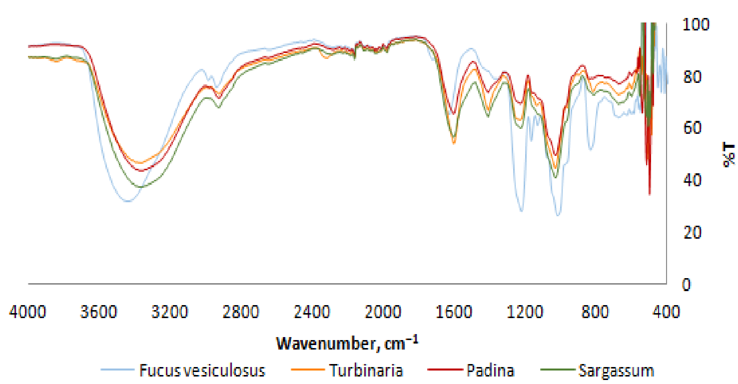

Figure 8. IR spectra of semi-purified fucoidan from different brown seaweed species and *F. vesiculosus*.

DISCUSSION

In this study, we sampled six genera of brown macroalgae from more than 50 sites within 14 provinces in the Philippines. Some seaweeds were at minimal distribution, if not absent, in sampling sites; hence, it was not feasible to extract sufficient fucoidan for analyses. All samples were not collected in the same sites at the same time during the year. Seasonal differences and varying distribution patterns could account for the absence or presence of certain species in the sampling sites. For instance, *Dictyota* is known to be widely distributed in the Luzon and Visayas regions (Trono 1997), but it was not found during certain collection periods in sampling sites in Mindanao. On the other hand, *Sargassum* was collected in all provinces because it is widely distributed and grows during wet and dry seasons. Trono (1997) detailed the distribution and seasonal variation of brown seaweeds in the Philippines. Thus, the differences in their distribution affect the comparison of fucoidan content among the provinces. As a consequence of this irregularity, we pooled the data collected in each genus or species per provinces. This gives us an estimate of how much content can be obtained in seaweeds from representative sites per province.

At present, there is no standardized purification procedure for fucoidans. Classical methods of extracting fucoidan involve a multi-step aqueous extraction using an acid which is usually hydrochloric acid (Ale et al. 2012). Fucoidan extracted using HCl is similar to the fucoidan supplied by Sigma-Aldrich. It is important to note that the characteristics of fucoidan are dependent on the extraction technique. Different extraction methods and purification treatments of fucoidans have resulted to varied compositional results and structural suggestions for fucoidan and other polysaccharides (Ale and Meyer 2013).

Among the seaweeds sampled, *Dictyota* had the lowest fucoidan content while *Sargassum* and *Turbinaria* had the highest. It is expected that Dictyota will have the lowest fucoidan content because of its fleshy and soft fronds. Fucoidan yield and monosaccharide composition are also affected by plant age or maturity (Skriptsova et al. 2010). Aside from differences in fucoidan content, seaweeds are also reported to exhibit a relatively large variation in composition and structural properties, even those belonging to the same order or family (Ale et al. 2011), resulting to an array of varied intensities of bioactivities. The amount and composition of algal metabolites are influenced by complex exogenous factors and endogenous biological and biochemical processes. Mature and reproductive stages of the macrophyte reportedly produce significant amounts of fucoidan compared to young thalli (Zvyagintseva et al. 2003; Skriptsova et al. 2010). Fucoidan content of reproductive tissues of five macroalgal species were 1.3-1.5 times higher compared to their sterile counterparts. Fucoidan generally accumulates in the reproductive structures of brown seaweeds, whose reproduction cycle also affect the changes in fucoidan monosaccharide composition (Skriptsova et al. 2012).

Geographical location, seaweed species, and seasonal variations may also influence the differences in polysaccharide composition and their chemical structure. In a study conducted by Sinurat et al. (2016), *Sargassum polycystum* from three different sampling sites in Indonesia exhibited differences in their fucoidan and ash contents.

The maturation cycle of the seaweed also influences the changes in fucoidan content. This cycle is primarily influenced by the changes in season. In temperate countries, the increase in water temperature influences the growth and maturity of the seaweeds. This was observed in the brown seaweed *Undaria pinnatifida* collected from September-October 2011 in three different mussel farms in New Zealand, wherein seaweed samples exhibited low fucoidan content on July, an increase in the content on September, and a drastic drop on October 2011. Aside from the changes in the fucoidan content, uronic, sulphate, fucose, and protein contents of the seaweeds were also affected by the month of harvest. The study also suggested that there were variations in the crude fucoidan content and composition between the two different sampling sites (Mak et al. 2013).

Sargassum and *Turbinaria* are potential sources of fucoidan because of their greater fucoidan content and wide distribution in the Philippines. For future studies, it is recommended that the relationships between the fucoidan content of the seaweeds, and the seasonal variations and the reproductive cycles should be investigated. It is also recommended to fine scale the sampling sites, and to focus on locations where there is a high chance of collecting the same type of species in certain months. For example, the preliminary results of this study suggest that the provinces of Camiguin, Negros Oriental, and Pangasinan are possible locations for establishing multiple collection sites for *Sargassum*. For Turbinaria, possible sampling sites can be established in Cagayan, Ilocos Norte, and Quezon Province. By establishing multiple sites in these provinces, researchers and investors can be assisted in deciding which areas should be prioritized for collection and which months the seaweeds should be collected. It is recommended to further study the life cycle and physiology of these fucoidan-yielding species. It is also important to develop culture techniques for local brown seaweeds to prevent the overharvesting of these species in the wild and to create a steady supply of brown seaweeds in the market. For instance, culture techniques for brown seaweeds *Undaria* and *Laminaria* were already developed (Tseng and Fei 1986). Possible investors and farmers may culture the brown macroalgal species in the sites identified.

ACKNOWLEDGEMENTS

This study was funded by the Bureau of Agricultural Research of the Department of Agriculture, Republic of the Philippines. We thank Ms. Hannah Faith Dormido for making the maps presented in this study. This is contribution no. 452 of the Marine Science Institute, University of the Philippines.

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