Fucoidan extracted from brown seaweed *Bifurcaria bifurcata*: seasonal variations on physicochemical features and anti-tumor activity against breast cancer cell line

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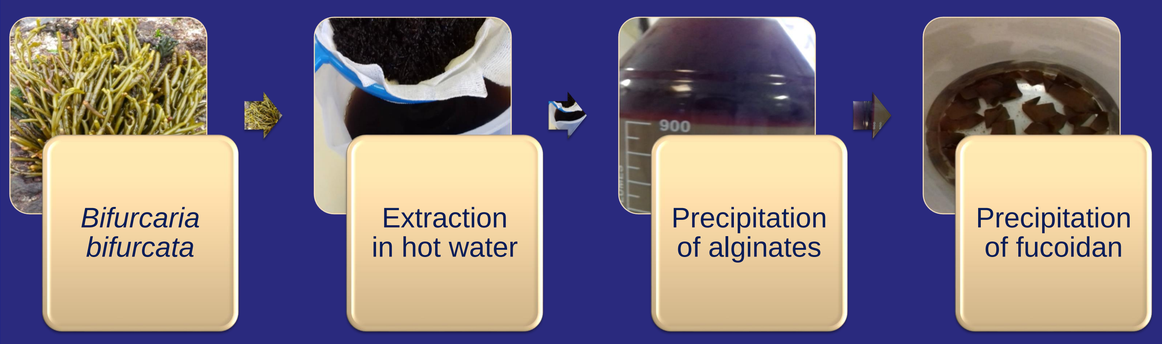
INTRODUCTION

In recent years there has been growing interest in the scientific areas that study the applications of marine-derived materials. Fucoidan is a class of sulfated fucose rich polysaccharides found in brown seaweed. Fucoidan presents a variety of bioactive properties of enormous interest, such as: anti-tumoral [1], antithrombotic, antioxidant, anti-inflammatory, anticoagulant, antiviral and others. The properties of this polysaccharide are of interest for many industrial applications, namely for pharmaceuticals and nutraceuticals or in the design of innovative biomedical applications [2].
Nevertheless, these bioactivities are influences by the chemical structure of the biopolymer, which by its turn depends on the biology of macroalgae (species, maturity / stage of algae, part of algae), geographic, seasonal and environmental conditions (water temperature, nutrients, sunlight), collection and extraction / purification techniques [3].
The seasonal variation of fucoidan is often mentioned in the literature, although there is very little published data on the subject at present and the few references cover only a few months of the year. Considering the change in biomedical properties due to the varying composition of fucoidan, understanding the seasonal variation of the chemical content of fucoidan is very important [3].
The brown alga *Bifurcaria bifurcata* is an edible seaweed scarcly studied and is proposed within the present work as source of sulfated polysaccharide, fucoidan, further assessing batch-to-batch variability (seasonality) regarding the extracts composition, fucoidan chemical properties and biological activity as potential anti-tumor compound.

MATERIALS AND METHODS

1. Extraction of fucoidan



2. Characterization

- FTIR
- ^{1}H NMR spectroscopy
- Micro BCA
- Quantification of sugars
- Analysis of uronic acids
- Determination of sulfates contents

3. Biological Assays

RESULTS AND DISCUSSION

The extraction of fucoidan was possible from *B. bifurcata* through water-based methodology, with yields of:

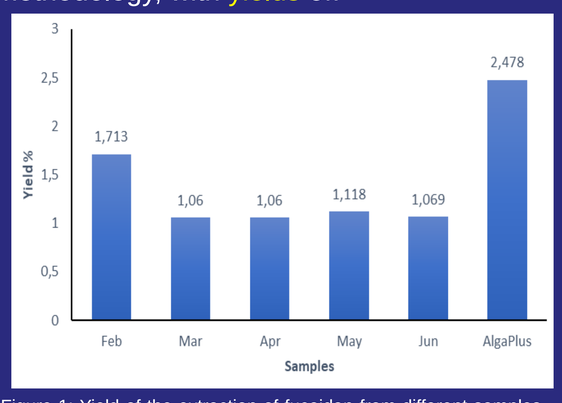


Figure 1: Yield of the extraction of fucoidan from different samples of *B. bifurcata*, collected in consecutive months. Fucoidan extracted from a sample supplied by Alga+ (Ílhavo, Aveiro) was also analysed.

FTIR and NMR results are compatible with the identification of the extracted polysaccharide as fucoidan, showing the presence of fucose and sulfate.

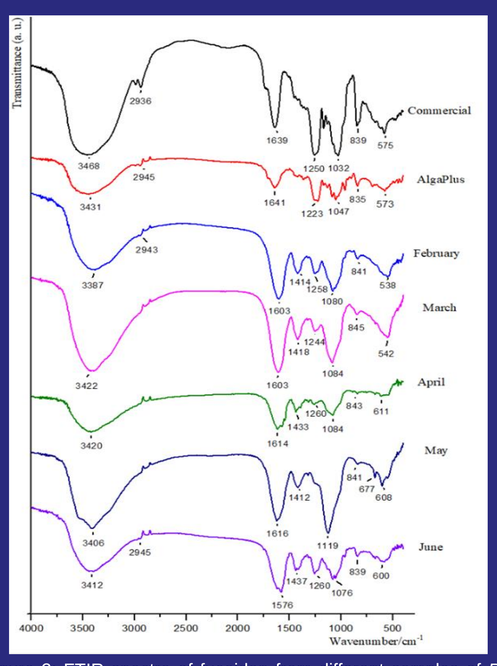


Figure 2: FTIR spectra of fucoidan from different samples of *B. bifurcata*, collected in consecutive months. Fucoidan extracted from a sample supplied by AlgaPlus (Ílhavo, Aveiro) was also analysed, as well as a commercial sample, as reference.

Table 1: ^{1}H NMR chemical shifts detected for the fucoidan extracts obtained from *B. bifurcata* samples collected in consecutive months. Fucoidan extracted from a sample supplied by AlgaPlus (Ílhavo, Aveiro) was also analysed, as well as a commercial sample, as reference.

	February	March	April	May	June	AlgaPlus	Commercial
Anomeric Proton	4.85-5.13	4.85-5.13	4.85-5.13	4.85-5.15	4.85-5.16	4.85-5.16	4.85-5.36
Proton ring	3.67-3.94	3.65-3.97	3.65-3.97	3.67-4.00	3.70-3.91	3.70-4.08	3.76-4.27
Acetyl proton	2.10-2.25	2.10-2.25	2.10-2.25	2.01-2.25	2.10-2.25	2.20-2.25	2.20-2.28
Proton methyl	1.19-1.50	1.19-1.51	1.19-1.51	1.19-1.52	1.19-1.51	1.19-1.50	1.19-1.44

The extracts revealed high levels of fucose and the presence of sulfates, the main features of fucoidan.

Table 2: Monosaccharide content (results in percentage) of fucoidan extracts obtained from *Bifurcaria bifurcata* samples collected in consecutive months. Fucoidan extracted from a sample supplied by Alga+ (Ílhavo, Aveiro) was also analysed, as well as a commercial sample, as reference.

Monosaccharide	February	March	April	May	June	Alga+	Commercial
Fucose	43.06±1.73	36.27±3.75	29.21±1.70	20.55±1.43	57.99±3.09	68.24±1.86	71.40±0.34
Rhamnose	-	-	3.08±0.03	11.24±2.20	1.18±0.07	0.35±0.05	0.08±0.11
Ribose	-	-	3.68±0.26	1.39±1.97	1.49±0.30	0.61±0.04	-
Arabinose	-	-	2.62±0.30	1.06±1.50	0.95±0.02	0.44±0.05	0.30±0.43
Xylose	5.88±0.27	-	5.09±0.05	3.24±0.38	2.78±0.00	2.00±0.07	7.92±0.23
Mannose	7.87±1.43	14.09±4.23	8.70±0.62	3.67±0.11	2.34±0.06	1.83±0.07	1.03±0.12
Galactose	15.53±1.79	11.15±0.29	12.79±0.17	12.79±0.01	13.29±3.97	10.38±0.24	3.22±0.09
Glucose	1.33±0.17	3.64±0.49	3.97±0.19	9.02±0.75	1.78±0.16	0.74±0.70	0.63±0.08
Uronic acid	26.32±5.39	34.56±0.30	30.86±1.90	37.03±8.36	18.21±0.28	15.41±1.84	15.42±1.21

Their sugar composition and the protein contents depend on the month the seaweeds were sampled, suggesting a seasonal variability of fucoidan extracts.

Table 3: Sulfate and protein content (results in percentage) of fucoidan extracts obtained from *B. bifurcata* samples collected in consecutive months. Fucoidan extracted from a sample supplied by Alga+ (Ílhavo, Aveiro) was also analysed, as well as a commercial sample, as reference.

	February	March	April	May	June	Alga+	Commercial
Sulfate	11.43±2.50	4.71±1.72	3.54±1.20	17.50±0.61	12.27±0.29	23.93±1.33	23.64±0.97
Protein	22.43±0.72	13.67±0.62	20.98±0.36	24.88±0.24	13.74±0.29	12.98±0.15	3.39±0.41

The results of the protein content show that the extract was not pure and would require a more efficient purification procedure and / or an improvement in seaweed pre-treatment.

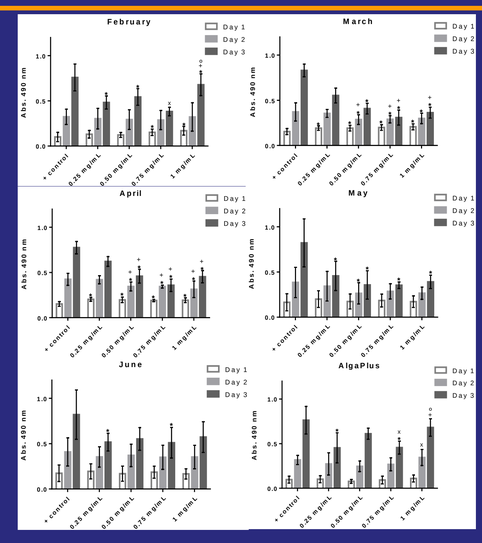


Figure 3: Metabolic activity of mouse fibroblast cell line (L929) cultured during up to 3 days in the presence of fucoidan extracts at different concentrations. Fucoidan extracts were obtained from *B. bifurcata* samples collected in consecutive months and extracted from a sample supplied by Alga+ (Ílhavo, Aveiro) was also analysed. Data were considered statistically different if $p < 0.05$. * indicates signi ficant differences when compared to CTR; +, when compared to 0.25 mg/mL; x, when compared to 0.5 mg/mL; º, when compared to 0.75 mg/mL.

The biological performance as cytotoxic agent against a breast cancer cell line demonstrated that the extracts did not have the same behavior, with only some seeming to exhibit anti-tumor activity.

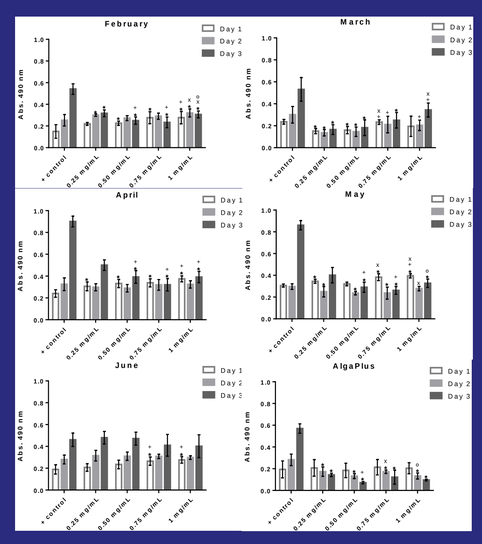


Figure 4: Metabolic activity of human breast cancer cell line (MDA-MB-231) cultured during up to 3 days in the presence of fucoidan extracts at different concentrations. Fucoidan extracts were obtained from *B. bifurcata* samples collected in consecutive months and extracted from a sample supplied by Alga+ (Ílhavo, Aveiro) was also analysed.. Data were considered statistically different if $p < 0.05$. * indicates signi ficant differences when compared to CTR; +, when compared to 0.25 mg/mL; x, when compared to 0.5 mg/mL; º, when compared to 0.75 mg/mL.

CONCLUSIONS

Fucoidan was successfully extracted from the brown algae *Bifurcaria bifurcata.*
Both the extraction yield and the physico-chemical properties of the produced fucoidan extract are dependent on the seaweed sample, most probably of seasonality.
Cytotoxic behavior of the obtained fucoidan extracts was not similar, confirming the batch-to-batch variability observed on chemical characterization: **each extract is one of a kind**!

References:
[1] C. Oliveira et al., "The Key Role of Sulfation and Branching on Fucoidan Antitumor Activity", Macromolecular Bioscience, 17 (2017), 1600340.
[2] Liu, J. *et al.*, 2015 A review of bioactive plant polysaccharides: Biological activities, functionalization, and biomedical applications. Bioactive Carbohydrates and Dietary Fibre,5, 31-61
[3] Fletcher, H, R. et al., 2017. The seasonal variation of fucoidan within three species of Brown macroalgae. Algal Research, 22, 79-86

Acknowledgments: This work was partially funded by European Regional Development Fund (ERDF), through European Union Transborder Cooperation Programme Interreg España-Portugal 2014-2020 (POCTEP), under the scope of project 0302_CVMAR_I_1_P, and through COMPETE2020/PT2020, under the scope of Mobilizer Project ValorMar (ref. POCI-01-0247-FEDER-024517).