

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

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Marine origin polysaccharides are among the most abundant compounds in the oceans, playing structural, energetic and other roles in several organisms. Although still poorly explored, in the recent decade sulfated polysaccharides are receiving significant attention, with diverse chemical properties and biological activities being disclosed. Seaweeds have been of economic importance for centuries, with certain cultures using various types of algae aiming treatment for various diseases, now being reinvigorated by their use as raw-materials for the production of those highly valuable biopolymers. In particular, the brown alga *Bifurcaria bifurcata* is an edible seaweed scarcely studied and is proposed with the present work as source of sulfated polysaccharide, fuoidan, further assessing batch-to-batch variability (seasonality). Accordingly, *B. bifurcata* seaweed was collected monthly on the Aver-o-Mar beach and fuoidan was extracted following a water-based methodology, with yields varying between 1.06% and 2.48%. FTIR and NMR analyses of the obtained polysaccharide extracts showed results compatible with the identification of the extracted polysaccharide as fuoidan, namely demonstrating the presence of fucose and sulfate groups, which was confirmed also by the analysis of monosaccharides and quantification of sulfate contents, which revealed fucose as the dominant sugar (20.55-71.40%) in all extracts, as well as the presence of sulfate groups (3.54-23.93%). Nevertheless, It was also possible to observe variability on the physicochemical properties of fuoidan with the seaweed sampling month. The determination of protein contents disclosed still significative values (3.39-24.88%), suggesting the need of additional purification procedure and / or an improvement in seaweed pre-treatment. In addition, the assessment of the biological performance of the obtained extracts as antitumoral agents was studied by evaluating the eventual cytotoxic effect exerted over a breast cancer cell line. Extracts exhibited different behavior, with only some seeming to exhibit anti-tumor activity, demonstrating the seasonal variation on the production of this brown seaweed polysaccharide. This knowledge is pivotal when considering industrial applications of this polysaccharide, namely to identify optimal harvest times and ensure consistent product composition, enabling the establishment of *B. bifurcata* seaweed for the extraction of fuoidan for further biotechnological application.