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Research Article

Determination of the Seasonal Yields of Total Fucose and Fucoidan Yields in Brown Seaweeds (Order Fucales) Distributed along the Coast of Urla (izmir, Turkey)

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Abstract

The brown seaweeds have been recognized as a potential source of biologically active Fucoidan, a mucilaginous sulfated polysaccharide consisting mainly of a polymer of α-L-fucose-4-sulfate but with persistent amounts of other sugars, mainly Xylose and Galactose and have been reported to have Anti-Cancer, Anti-Obesity and Anti-Inflammatory effects.

This study was carried out to determine the seasonal yields of total Fucose and Fucoidan from brown seaweed species (Order Fucales) of *Cystoseira barbata, C. compressa* and *Sargassum* vulgare distributed naturally along the coast of Urla (İzmir, Turkey) using a colorimetric method.

Mean fucoidan yields were 6.44±1.44% (dry weight basis) for *Cystoseira barbata*, 3.84±1.14% for *Sargassum vulgare* and 3.04±1.77% for *C. compressa* (n=3). The highest Fucoidan yields in these species were determined in the fall season (P≤0.05).

Keywords: Algae; Colorimetric determination; Cystoseira; Extraction; Sargassum

Introduction

In recent years, great research and commercial attention have been given to extraction and production of high-value biochemical from marine seaweed biomass [1]. The use of seaweed biomass has several advantages, among them (a) available rich resources; (b) feasibility of growing fast in the open ocean; (c) higher photosynthetic efficiency than terrestrial biomass; (d) no dependence on freshwater and little dependence on temperature; and (e) low costs of harvest [2,3]. The chemistry of marine seaweeds and terrestrial biomass sugar polymers are different [1,4]. Seaweeds contain several unique and commercially important Phycocolloids, such as Alginate, Carrageenan and Agar-Agar [1,4]. Seaweeds contain other interesting and Phytochemically active molecules such as, Phycobilin pigments, HUFA and PUFA Fatty acids, Proteins with essential Amino acids, Vitamins and Mineral elements, which are compounds with potential applications in Food, Cosmetic, Pharmaceutical and Medical fields [3,5,6].

Brown Seaweed-derived polysaccharides such as Fucoidan, Laminaran, Alginates and Mannitol have been studied due to their biological effectiveness as Anticoagulant, Antitumor, Antithrombotic, Anti-inflammatory, Contraceptive and Antiviral agent [7-10].

Fucoidans, is a term that covers a family of Fucose-Containing Sulfated Polysaccharides (FCSPs), may constitute up to 25-30% of the seaweed dry weight, depending on the seaweed species and, to a lesser extent, on life history stage and season [11,12].

Fucoidans participate in the building of cell walls and the support of their structure, as well as in releasing of spores and gametes from reproductive organs. Due to the sulfated groups, these polysaccharides can bind positively charged ions, such as K^+, Na^+, Ca^{2+}, Mg^{2+} and participate in ion exchange with the environment, which facilitates adaptation of seaweeds to water salinity fluctuations and to the toxic effects of heavy metals. Fucoidans play a substantial role in morphogenesis of zygotes of Fucoid algae, as they participate in establishing the cell polarity and fixing the cell-division axis, thus determining the direction for the development of the rhizoidal and apical poles of young thalli [13].

Documented biological functionalities of these compounds are the activities against Hepatitis, Herpes and Human Acquired Immune Deficiency (AIDS) Viruses, the Anticoagulant, Anti-inflammatory Heparin and molecules that modify cell proliferation and adhesion and fertilization functions [13-20].

Despite all the above-mentioned benefits, research on the Fucoidan content of Seaweeds in Turkey is limited.

The aim of the current study was to determine the sulfated polysaccharides (Fucoidan) yields of seaweeds of *Cystoseira barbata*, *C. compressa* and *Sargassum vulgare* (Order Fucales) that are distributed on the Turkish coast. An experimental design was implemented to evaluate the Fucoidan content and effect of the seasonal variation on the Fucoidan yield among the species with specific goal to determine the best time of the year to obtain Fucoidan from natural populations of these species.

Materials and Methods

Seaweed samples of *Cystoseira barbata*, *C. compressa* and *Sargassum vulgare* used in this study was collected monthly by hand along the coast of Urla (izmir Bay, Turkey; Figure 1). Approximately

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10 m long study area where seaweed collected from sup-tidal rocky zone located between the port of Urla and Urla Island road. All three types of seaweed samples were collected from the same type of environment on the same dates. The studies species were collected from the dense beds through the year. *Cystoseira* species were identified according to Herbarium of Prof. Dr. Sukran CIRIK and personal communications with him.

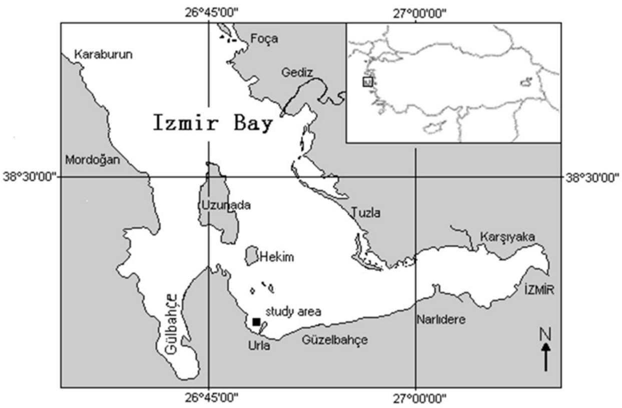

Figure 1: The map of study area.

The collected seaweeds in the lab were cleaned from epiphytes and epizoans washed with distilled water, dried at (60ºC), ground and stored in the freezer until use. Unless stated otherwise, chemicals and solvents used in this study were of Analytical Grade. Extraction and colorimetric determination of Fucose and Fucoidan content of Seaweeds was estimated using the method described by [21], as described below.

Extraction

A 1g sample of seaweed material for each species of *Cystoseira barbata*, *C. compressa* and *Sargassum vulgare* for each sampling was accurately weighed into a 100 ml conical flask. 25 ml 0.2 N HCl was added and the suspension was heated at 70ºC in a temperature-controlled water bath for 1 hour. The suspension was stirred (approximately 200 rpm) or shaken during the extraction. The flask was cooled in tap water and the particles were allowed to settle. The liquid was poured through a filter into a 200 ml conical flask. Subsequent extracts from the same sample were filtered into the same flask. The particles retained on the filter were transferred back into the extraction flask with 25 ml 0.02 N HCl. The flask was heated at 70ºC for 1 hour, cooled in tap water and re-filtered. The combined extracts contain the acid-soluble, Fucose-containing polysaccharides used in the colorimetric procedure for Fucose determination.

Colorimetric determination of fucose

A 1 ml of sample extract was taken into a test tube placed in an ice-water bath and 4.5 ml dilute H_2SO_4 was added carefully and allowed the mixture cool for approximately 1 minute and mixed with a glass rod. The tube was placed in a boiling-water bath for exactly 10 minutes and then cooled in tap water to room temperature (20ºC). 0.1 ml Cysteine solution was added into the tube and mixed with a glass rod and the tube was left at room temperature for 30 minutes.

The absorbance at 396 and 427 nm was read in this solution. The total Fucose, F, in milligrams was calculated from the following formula:

$$F = B \times 0.06 \times V$$

Where,

B is the difference between the two absorbancy readings (396 and 427 nm)

0.06 is a factor converting absorbance to amount of Fucose in miligrams per mililiter and

V is the total volume of the extract in mililiters

To obtain the approximate amount of Fucoidan of the sample, the Fucose content was multiplied by a factor of 2 [21].

Statistical analysis

Fucose and fucoidan yield data were analyzed using the General Linear Model (GLM) with ANOVA procedure using SPSS [22]. All data were expressed as mean ± standard deviation. Means of the fucose and means of the fucoidan yield were compared among the species and seasons in the same species group using Tukey's Multiple comparison test in SPSS [22].

Results and Discussion

Total yield of Fucoidan in dry weight (dw) averaged at 6.44±1.44% in *Cystoseira barbata,* 3.04±1.77% in *C. copressa* and 3.84±1.14% in *Sargassum vulgare* (Table 1). Mean Fucoidan yield of *C. barbata* was significantly greater than that *C. compressa*, however no significant difference was found between the mean Fucoidan yield of *C. barbata* and *S. vulgare* and *C. compressa* and *S. vulgare* (Table 1).

Seaweed Species	Mean*±SD	Minimum	Maximum
Cystoseira barbata	$6.44^{a}\pm1.44$	4.14	8.56
Cystoseira compressa	$3.04^{b}\pm1.77$	2.08	4.51
Sargassum vulgare	$3.84^{ab}\pm1.14$	2.26	6.13

Table 1: Comparison of annual fucoidan yield (% dry basis, with mean and Standard deviation) of *Cystoseira barbata*, *C. compressa* and *Sargassum vulg* are (N=12).

* = Means with common superscript letters are not significantly different (P≤ 0.05); (a, b): comparison between seaweed species.

The fucoidan yields of seaweed species recorded here are similar to that the values in Chotigeat et al. [23], Larsen [21] and Usov et al. [24]. Chotigeat et al. [23] reported a fucoidan content of 2.74±1.18% in dw in *Sargassum polycystum*, which compared favorably with *Pelvetica canaliculata.* Larsen (1978) reported a Fucoidan content of 6-8% in dw in *Ascophyllum nodusum,* 9-11% in *Fucus* species, 5-20% in *Laminaria sp*., and 20% in dw in *Pelvetia canaliculate.* Usov et al. (2001) reported the fucoidan content of between 0.4 and 20.4% in dw in 25 different brown seaweed species and 7.7% in *Fucus evanescens* (Order Fucales).

Mean Fucoidan yield in *Cystoseira barbata* was highest in spring and fall and lowest in winter (Table 2). Similarly, mean fucoidan yield in *C. compressa* was highest in spring and fall and lowest in winter (Table 2). In *Sargassum vulgare,* Mean Fucoidan yield was highest in fall and summer and lowest in winter (Table 2).

Species	Seasons*	Mean*±SD
C. barbata	Winter^{b*}	4.79^{b}±1.59
	Spring^{a}	6.73^{a}±1.53
	Summer^{ab}	6.22^{ab}±1.61
	Fall^{a}	8.10^{a}±1.60
C. compressa	Winter^{b}	2.11^{b}±1.02
	Spring^{a}	3.58^{a}±1.85
	Summer^{ab}	2.94^{ab}±1.37
	Fall^{a}	3.55^{a}±1.49
S. vulgare	Winter^{b}	2.78^{b}±1.21
	Spring^{ab}	3.59^{ab}±1.19
	Summer^{ab}	3.96^{ab}±1.10
	Fall^{a}	5.32^{a}±1.24

Table 2: Comparison of seasonal Fucoidan yield (% dry basis, with mean and Standard deviation) of *Cystoseira barbata*, *C. compressa* and *Sargassum vulgare* (N=3).

*=Means with common superscript letters are not significantly different (P≤0.05); (a, b): comparison between seaweed species.

Chotigeat et al. [23] have also reported a seasonal variation in Fucoidan content in the seaweeds they examined, with the highest yields of Fucoidan found in fall.

Addition to species and the season, the extraction method also is an important factor that has great effect on the Fucoidan yields of the sea weeds showed by Wang and Cheng [25]. In the study of Wang and Cheng it was found that the fucoidan content extracted using method I was 13.13% (of dry weight), 24.00% and 22.67% from *Sargassum glaucescens*, *Sargassum horneri* and *Laminaria japonica*, respectively. By contrast, the fucoidan content extracted using method II was 4.20%, 4.80% and 4.64% from *S. glaucescens*, *S. horneri* and *L. japonica*, respectively. The sample sex-tracted using method II were lower in total sugar content but higher in sulfated content than those extracted using method I. Using method II yielded the highest fucose content in *S. Glaucescens* (20.89%) and *Hizikia fusiformis* (15.89%).

Conclusion

In this paper, the extraction, colorimetric determination of Fucose and Fucoidan yields from *Cystoseira barbata, C. compressa* and *Sargassum vulgare* seaweed distributed along the coast of Urla (izmir, Turkey) has been successfully demonstrated. Results indicate that annual mean content of Fucoidan was significantly different among the three seaweed species. For Fucoidan extraction, *C. barbata*was better source than *C. compressa* and *S. vulgare* and the best period to harvest *C. barbata* was fall seasons (September, October and November).

Addition to the species and seasonal conditions, the extraction method has also a significant effect on the yields of Fucoidan. Considering of sulfate content of Fucoidans, it is supposed that the functional activity of fucoidans between the three species will be similar because sulfate is well-known factor of Fucoidan's biological function. So, each species should be considered a good source for Fucoidan that has the activities against Hepatitis, Herpes and Human Immunodeficiency (AIDS) Viruses, Anticoagulant heparin inflammation, Cell proliferation and adhesion.

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