

Electrophoretic identification of fucoidan in *Fucus vesiculosus* L.

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Summary

Macroalgae have been found to be built of a number of compounds including polysaccharides, proteins, lipids, water and a variety of macro- and microelements. Brown algae contain fucoidan, a fucose-containing sulfonated polysaccharide. Fucoidan from brown algae such as the common bladder wrack (*Fucus vesiculosus* L.) is now widely examined in many countries for its interesting biological and therapeutic properties. Its antiviral, anti-tumor and cardioprotective effects have already been proved. In this study, fucoidan was separated and identified in bladder wrack thalli during extraction in water and hydrochloric acid. Preliminary tests allowed for identifying fucoidan during five-minute extraction. A simple, repeatable analytic procedure was developed using inexpensive apparatus for cellulose acetate membrane electrophoresis.

Key words: brown algae *Fucus vesiculosus* L., fucoidan, cellulose acetate membrane electrophoresis

INTRODUCTION

Some contemporary scientific research on algae aims at finding and analysing biologically active chemical compounds. Polysaccharides, along with proteins, lipids, water and elements are the main chemical compounds that macroalgae are built of. The average content of polysaccharides is 30–50% for brown algae, 30–60% for red algae and 25–50% for green algae [1].

Among chemical compounds now widely examined in many countries for their interesting biological and therapeutic properties there is fucoidan (fucan sulfate), a fucose-containing sulfonated polysaccharide (fig. 1) [2, 3]. The polysaccharide was named “fucoidin” for the first time when it was isolated from marine brown algae in 1913. Nowadays, it is named “fucoidan” according to IUPAC rules, although, some sources it is also called fucan, fucosan or sulfated fucan [2].

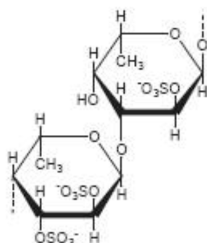


Figure 1. The structure of fucoidan from *Fucus vesiculosus* L. [6].

Fucoidans from several species of brown algae, for example *Fucus vesiculosus* L., are of simple chemical compositions, mainly being composed of fucose and sulfate. But the chemical compositions of most fucoidans are complex. Apart from fucose and sulfate, they also contain other monosaccharides (mannose, galactose, glucose, xylose, etc.) and uronic acids, even acetyl groups and proteins. The composition and biochemical activity of algae, including the composition of fucoidans, depends on the kind of seaweeds and their place of origin [2, 4].

Recently, it has been shown that fucoidans from representatives of the order Fucales (Cyclosporophyceae), namely *Fucus evanescens* C., *Ascophyllum nodosum* L. and *Fucus vesiculosus* L., have a backbone built up of alternating (1→3)- and (14)-linked α -L-fucopyranose residues [5, 6].

Algae have been used for their therapeutic purposes for quite a long time. Systematic studies began in the 1950s and were concentrated on active substances searching. Brown algae *Laminaria* and *Fucus*, because of their fucoidan content are of particular interest. Fucoidans isolated from different species have been extensively studied due to their varied biological properties, including anticoagulant and antithrombotic, antiviral, antitumor and immunomodulatory, anti-inflammatory, blood lipids reducing, antioxidant and anticomplementary effects, activity against hepatopathy, uropathy and renalopathy, gastric protective effects and therapeutic potential in surgery. Compared with other sulfated polysaccharides, fucoidans are widely available in various kinds of cheap sources and that is why they have been increasingly investigated in recent years to develop drugs or functional foods. [2, 3].

In recent years, consumers in Poland have shown growing interest in seaweeds. Unfortunately, this trend is not accompanied by sufficient current scientific research of their composition and the content of biologically and therapeutically active chemical compounds. It is known that, due to low salinity of the Baltic Sea,

the alga flora along the Polish coast is meagre. There are representatives of the brown algae *Polysiphonia*, *Ceramium*, *Furcellaria fastigiata* [7] as well as green alga *Enteromorpha* in the Gulf of Gdańsk area [8]. Thus, the only source of fucoidan available in Poland is the bladder wrack.

Many contemporary analytic methods are successfully used in physicochemical analyses of algae. They include chromatographic and electrophoretic methods such as capillary electrophoresis (CE) [9-11] and micellar electrokinetic capillary chromatography (MECC) [12, 13]. Cellulose acetate membrane electrophoresis (CAME) or cellulose acetate electrophoresis (CAE) have been used relatively rarely in identifying fucoidan [14-16].

MATERIALS AND METHODS

The following materials and reagents were used in the examination: fucoidan from *Fucus vesiculosus* L. (CAS Nr 9072-19-9, Sigma-Aldrich, Poland), *Fucus* algae packed and distributed by the Zakład Konfekcjonowania Ziół FLOS, Poland.

Dried alga *Fucus vesiculosus* L. was dipped in an aqueous formaldehyde solution (3.7%, 100ml) and kept in a closed flask at 30°C overnight in order to remove lipophilic compounds. The suspension was stirred and extracted at 80°C with 0.15M HCl (100 ml) or distilled water (100 ml) for 5, 15 or 30 minutes, or with CaCl₂ (2%) for 2 h. The filtrate was concentrated under reduced pressure to about half of its original volume. Then 20 ml of 10% CaCl₂ was added to the aqueous and acid extracts. The precipitate formed was separated by centrifugation. For the electrophoretic analysis, reference fucoidan solutions were prepared: A – 1%, B – 0.5%, C – 0.25%, D – 0.125%. The samples (2 µl nonprecipitated extract) and references were subjected to electrophoresis on a strip of cellulose acetate film (CA-SYS-MINI Cellulose Acetate Systems) in 0.2 M calcium acetate (pH=7.5) at 3 mA, max. 240 V for 2.5 h. The stripes were stained with 0.5% toluidine blue in 3% acetic acid solution and then rinsed in distilled water and air-dried. The results are shown in figures 2–4.

RESULTS AND DISCUSSION

Cellulose acetate electrophoresis (CAE) or cellulose acetate membrane electrophoresis (CAME) is a widely used diagnostic method of medical analytics; however, it has only been used several times in order to identify fucoidan [14-16].

Typically, electrophoretic clinical tests in blood plasma are performed using a barbital buffer for serum protein electrophoresis (pH=8.6). Analysing the image of electrophoretic separation of plasma proteins is crucial for diagnosing as it allows for preliminary evaluation of changes in the composition of patient's protein fractions. Shifts in the percentage composition of the plasma protein fractions

accompany inflammatory conditions. They are also a simple sign of an ongoing inflammatory process or an activity of the immunological system. Content differences between the fractions of albumin, α_1 - and α_2 -globulins as well as β - and γ -globulins may suggest myeloma, nephrosis, haemorrhages or liver diseases.

In this study, CAE was adopted to identify fucoidan in *Fucus vesiculosus* L. Dried alga was dipped in an aqueous formaldehyde solution in order to remove lipophilic compounds. The defatted alga was then extracted with 0.1 M HCl to prevent extraction of alginates. The filtrate was concentrated under reduced pressure to about half of its original volume. Subsequently, 10% CaCl_2 was added to the acid extracts and the precipitate formed was separated by centrifugation. The samples (2 μl nonprecipitated extract) were subjected to electrophoresis on a strip of cellulose acetate film. The developed method of defatting, extracting and concentrating preparations leads to obtaining analytic samples that can be analysed using CAE. The results are shown in figure 2.

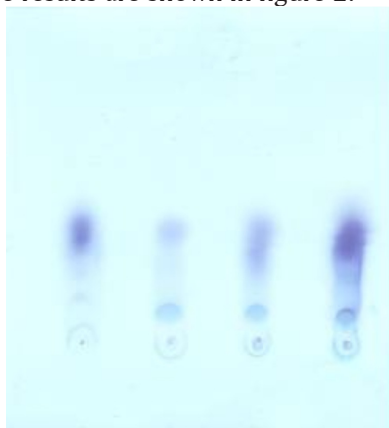


Figure 2. Cellulose acetate membrane electrophoresis of fucoidan from acid extract (0.1 M HCl). From left to right: C – 0.25% reference fucoidan solution, 5-min extraction, 15-min extraction, 30-min extraction.

As a result of the electrophoretic analysis, satisfactory preliminary separation of samples was achieved. The successively growing times of bladder wrack extraction (5, 15 and 30 min) are manifested by increasingly intensive bands from fucoidan. The electropherogram also reveals traces of bands that stayed near the starting position. The position and presence of these bands can be explained by insufficient separation of alginates, possibly accompanied by their partial hydrolysis into guluronic acid (G) residues and mannuronic acid (M) residues. At the present stage of these preliminary examinations, the samples were not separated earlier or fractionated in preparatory columns, as suggested by the authors of similar fucoidan identifications [15].

Fucoidan in samples of bladder wrack can be also identified after earlier extraction at 80C in distilled water. The results of the comparative analysis are shown in figure 3.

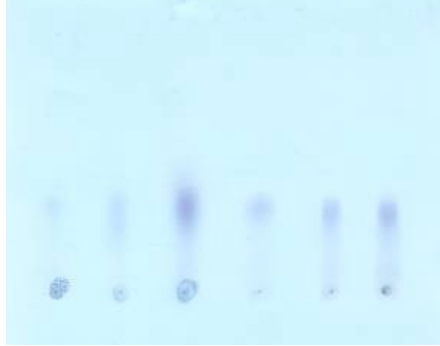


Figure 3. Cellulose acetate membrane electrophoresis of fucoidan from acid (0.1M HCl) and water extraction. From left to right: 5-minute extraction in acid; 15-min extraction in acid; 30-min extraction in acid; 5-min extraction in water; 15-min extraction in water; 30-min extraction in water.

After the analyse of figure 3, it can be said that preliminary identification of fucoidan is possible for samples extracted in any time, ranging from 5 to 30 minutes, irrespective of the extraction environment, in acid or water. The intensity of fucoidan bands depends on the volume of sample after evaporating and on the efficiency of preliminary separation of fucoidan from alginates. The clearer bands in the starting position, produced by the alginates or the alginic acid in the samples of the extracted bladder wrack, are more visible for extraction in hydrochloric acid, as they are in figure 2.

In many recently published studies, cardioprotective diet supplementation is emphasized [17-18], especially during chemotherapy, using alga products that contain fucoidan. Following these studies, electrophoretic identification of fucoidan was conducted in the samples of the extracted bladder wrack. Extraction in a solution of calcium chloride (2% CaCl_2 , two-hour extraction, 80°C) was compared with five-minute extraction in 0.1 M HCl and in water at 80°C .

After the analyse of the results of the examination in electropherogram (fig. 4.), it can be carefully estimated than 250ml standard bladder-wrack tea contains up to about 600 mg fucoidan (the values were calculated in relation to fucoidan concentration in the reference solution C).

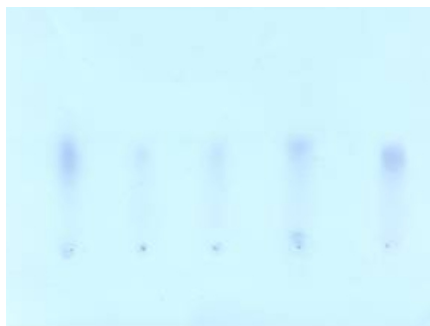


Figure 4. Cellulose acetate membrane electrophoresis of fucoidan. From left to right: C- 0.25% reference fucoidan solution, extraction in 2% CaCl_2 (bands 2 and 3); 5-min extraction in acid; 5-min extraction in water.

CONCLUSIONS

The preliminary results of electrophoretic examination of fucoidan in bladder wrack *Fucus vesiculosus* L., as presented in this study, are very promising. The preliminary tests allowed for separating and identifying fucoidan during five-minute extraction in water or 0.1 M hydrochloric acid. The advantage of this study is developing a simple, repeatable analytic procedure using modern but inexpensive apparatus for cellulose acetate membrane electrophoresis. The study will be continued in order to develop a method of quantitative determination of fucoidan content in samples so that this value can be added to the standard information about the composition of commercially available products.

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WYKORZYSTANIE ELEKTROFOREZY DO IDENTYFIKACJI FUKOIDYNY W *FUCUS VESICULOSUS* L.

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Streszczenie

W makroalgach stwierdzono obecność szeregu związków chemicznych, w tym polisacharydów, białek, lipidów, wody i pierwiastków zaliczanych do makro- i mikroelementów. W brunatnicach występuje fukoidyna, zawierający fukozę – polisacharyd sulfonowany. Fukoidyna z brunatnic, np. z morskoczynu (*Fucus vesiculosus* L.) należy do obecnie intensywnie badanych na świecie związków chemicznych interesujących z punktu widzenia biologicznego i terapeutycznego. Fukoidyna ma udowodnione działanie przeciwwirusowe, przeciwnowotworowe, a także nasercowe – kardioochronne. W niniejszych badaniach dokonano wydzielenia i identyfikacji fukoidyny w wyniku ekstrakcji plech morskoczynu pęcherzykowatego w wodzie i kwasie solnym. Wstępne próby pozwoliły na identyfikację fukoidyny podczas pięciominutowej ekstrakcji. Opracowano prostą, powtarzalną procedurę analityczną z zastosowaniem taniej aparatury do elektroforezy na membranach z octanu celulozy.

Słowa kluczowe: brunatnice *Fucus vesiculosus* L., fukoidan, elektroforeza na membranach w octanie celulozy