

# Depigmentation and Characterization of Fucoidan from Brown Seaweed *Sargassum binderi* Sonder

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**Abstract.** Fucoidan has many uses in the field of pharmacology, therefore it is necessary to improve the quality of fucoidan by increasing its purity. The objective of this study was to remove brown pigment from seaweed and observe the effect of the result to the activity of isolated fucoidan. In this study, the pigment was removed by organic solvents in the maceration step. The pigment removal using ethanol was found to give a better result than that of the solvent mixture (methanol: chloroform: water) from previous study, indicated by the appearance of fucoidan color. The result showed fucoidan has a better color, total carbohydrate was 89.23% and total sulphate 18.74%.

Keywords: Characterization, Depigmentation, Fucoidan, Organic solvent

## 1. Introduction

Fucoidan is a polysaccharide composed of L-fucose with sulfate ester, found in brown seaweeds, *Sargassum binderi* Sonder. For over decades, research regarding fucoidan has developed rapidly, since its massive number of biological activities, such as anticoagulant, antiviral, antiinflammation, and antitumor were published [3]. Based on the previous study [8] [9], isolated fucoidan had blackish brown color. Meanwhile, the standard color of fucoidan is white yellowish to light brown. That blackish brown color indicates the presence of brown seaweed pigments, such as fucoxanthin, chlorophyll a and c, beta carotene, and violaxanthin, trapped in fucoidan during the isolation [6] [17]. Therefore, in this study, the pigment will be removed by edible organic solvents in the maceration step, which is ethanol. In earlier experiment, the maceration step had been done using organic solvents, such as methanol, chloroform, petroleum benzene [5] [10] [11] [15]. It is believed that edible solvent can give a better quality of fucoidan, safe for consumption, and environmentally friendly.

Depigmentation of brown seaweed *Sargassum binderi* Sonder using maceration method by edible organic solvent, ethanol, has been done in this research. The isolated fucoidan was characterized by FTIR, and the total carbohydrate, and total sulfate were also analyzed. The objective of this research is to discover whether ethanol can be used for depigmentation of brown seaweed and to see if the quality of treated fucoidan can be improved.

## 2. Material and Method

### 2.1. Material

The raw material used in this work was brown seaweed *Sargassum binderi* Sonder which was collected from Lampung, Indonesia. Chemical reagents used were mostly analytical grades and some technical grades.



## 2.2 Depigmentation of Brown Seaweed *Sargassum binderi* Sonder

Sample (50 g) was chopped, then macerated in Ethanol (95%) with the ratio of 1: 8 (w/v), with variations of time 24 h, 72 h, 120 h, and 168 h, respectively at room temperature.

## 2.3 Isolation of Fucoïdan from Brown Seaweed *Sargassum binderi* Sonder

Brown seaweed (50 g) was soaked with 0.1 N HCl (1:10) (w/v) and stirred for 6 h at room temperature. The mixture was filtered using 500 mesh planktonet, the filtrate was collected and neutralized with NaOH (3 M). CaCl<sub>2</sub> (2%) was added to the filtrate and stirred. It was left at room temperature for 4 hours. The mixture was centrifuged (5000 rpm) for 15 min at 5° C. The residue was removed and then ethanol (1:2) was added to the filtrate. The mixture was allowed to stand for 24 hours. The precipitate was separated by centrifugation (5000 rpm) for 15 min at 5° C. The precipitate was dissolved in water, and the solution was dialyzed in 0.5 M NaCl and aquabidest to obtain extract fucoïdan (F) (Sinurat 2011).

## 2.4 Characterization of Fucoïdan from Brown Seaweed *Sargassum binderi* Sonder

### 2.4.1 Observation of Color Changes

Determination of fucoïdan color was based on visual observation of the initial treatment of brown seaweed maceration against the standard fucoïdan.

### 2.4.2 Identification of Functional Groups of Fucoïdan Result of Isolation by FTIR

Sample (2 mg) was added with KBr to gain a mass of 200 mg. The mixture was grinded to homogeneous and fine powder then pressed to form a KBr-sample pellet and analyzed it with FTIR spectrophotometer. The FTIR used is *Perkin Elmer precisely Spectrum One FT-IR Spectrometer*.

### 2.4.3 Determination of Total Carbohydrates

Total carbohydrate was analyzed using the phenol-sulfate colorimetric method. L-fucose as a standard was diluted and concentrated into 20, 40, 80, 160, 320, and 640 µg/mL. Meanwhile, fucoïdan sample (200 µL) was put into a reaction tube containing phenol 5% (0.5 mL) and H<sub>2</sub>SO<sub>4</sub> 18 M (2.5 mL). Each tube was shaken and incubated for 30 min. The absorbance was measured on a spectrophotometer at a wavelength of 480 nm [14].

### 2.4.4 Determination of Total Sulfate

Total sulfate on polysaccharides was done using BaCl<sub>2</sub>-gelatin method (Dodgson 1961).

Gelatin (2 g) was diluted in hot water (60-70°C, 400 mL) and the resulting semi-gelatinous solution was left overnight at 4° C. BaCl<sub>2</sub> (2 g) dissolved in 400 mL semi-gelatinous solution to made 0,5% BaCl<sub>2</sub>-gelatine and it was left for 2-3 hours before use. Fucoïdan (2 mg) was dissolved in aquadest (2 mL). TCA 3% (30 mL) and BaCl<sub>2</sub>-gelatine 0.5% (10 mL) was added and stirred. As standard, we used Na<sub>2</sub>SO<sub>4</sub> in 10, 20, 30, 40, and 50 µg/mL concentration. The solution was allowed to stand for 15 min and the absorbance was measured at  $\lambda = 360$  nm.


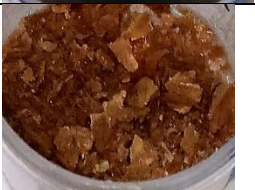
## 3. Results and Discussion

### 3.1. Characterization of Fucoïdan

#### 3.1.1. Observation of Color Changes

The result of maceration (**Table 1**) shows that maceration in ethanol resulted in fucoïdan with light brown color. This result clearly indicates that ethanol was able to dissolve the pigments better than the mixture of methanol:chloroform:water. The solubility of pigments such as chlorophyll and fucoxanthin in ethanol is probably due to hydrogen bonding interaction. This interaction may also apply to the pigments in methanol or water. However, the presence of chloroform in the mixture may partially block this hydrogen bonding, resulting a darker fucoïdan.

**Table 1.** Characteristics of Fucoidan from: a). Methanol: Chloroform: Water (4:2:1); b). Ethanol

Fucoidan	Color Changes	Total sulfate ( $\text{SO}_4^{2-}$ )	Total Carbohydrate
Methanol : Chloroform : Water (4:2:1) <sup>a)</sup>		18,63 %	82,79 %
Ethanol <sup>b)</sup>		18,74 %	89,23 %

<sup>a</sup> Ref. [7]<sup>b</sup> Data of this study

### 3.1.2. Total Carbohydrate and Total Sulphate

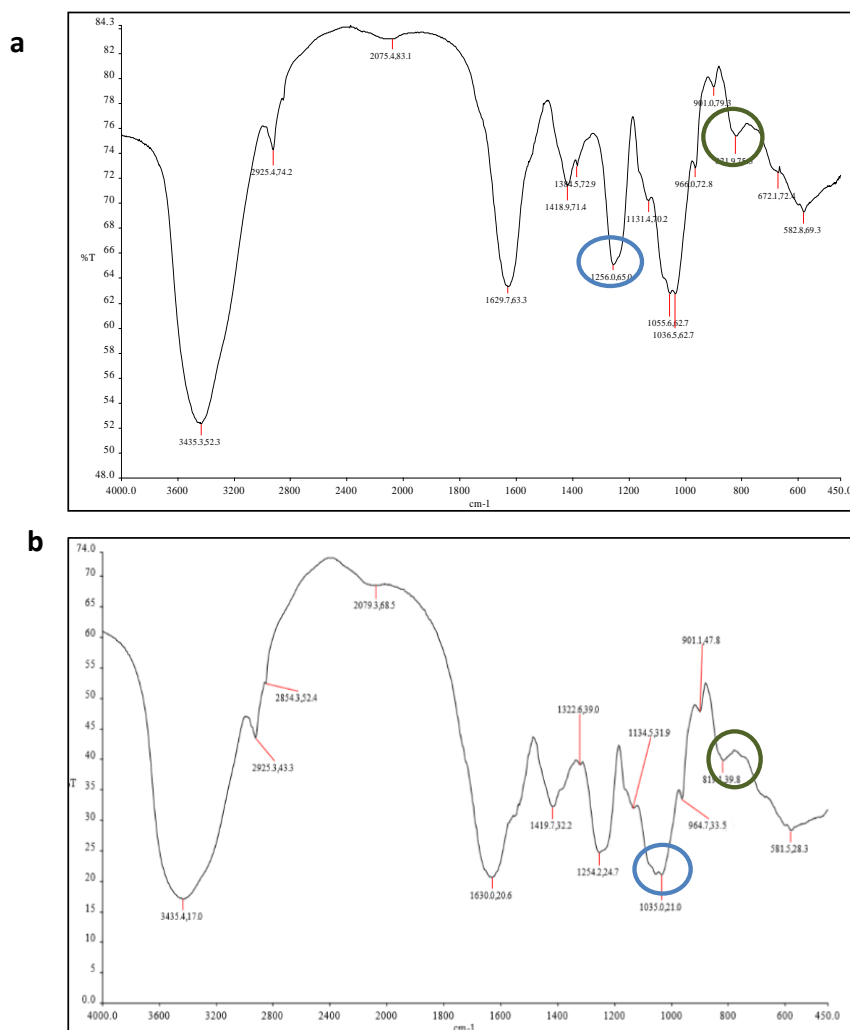
Fucoidan, which is a sulfated hetero-polysaccharide, shows variation in total carbohydrate and total sulfate content, depending on pre-treatment, isolation method, and how it is stored. Carbohydrate and sulfate in fucoidan were measured to determine the component and purity of fucoidan. Sulfate content has been shown to have a role for the activity of fucoidan. The higher sulfate content, the more active fucoidan. Related to sulfate activity, in the previous study, fucoidan without depigmentation has been proved to have good activity against breast cancer cell T47D [8].

Both solvent that used as pigment remover produced total carbohydrates and total sulfates around the common percentage, where the total carbohydrate around 35-60% [4] [9] [13] and total sulfate around 15-30 [2] [7] [12]. Based on Table 1, maceration with ethanol proved to give higher total carbohydrate and sulphate, 89.23% and 18.74%, respectively.

### 3.2. Identification of Functional Groups of Fucoidan Result of Isolation by FTIR

The FTIR spectrum of the sample shows some characteristics of fucoidan. Wave number at 1260-1250  $\text{cm}^{-1}$  is the characteristic sulfate ester functional groups; Absorption at 3600-3400  $\text{cm}^{-1}$  indicates the presence of -OH (hydroxyl); around 850  $\text{cm}^{-1}$  to 820  $\text{cm}^{-1}$  indicating position of sulfate, axial or equatorial; and in 1650-1600  $\text{cm}^{-1}$  indicating carbonyl functional groups from uronic acid [16].

Peaks at fingerprint area confirm the results above. Bending vibration occurred at 1260-1250  $\text{cm}^{-1}$  with medium intensity indicating the presence of sulfate ester. Then, the wavenumber of 825-819  $\text{cm}^{-1}$  indicates the location of the sulfate bonded at the equatorial position. The results indicated that the samples were confirmed as fucoidan (Figure 1).



**Figure 1.** FTIR result: (a) Maceration with ethanol; (b) Maceration with methanol: chloroform: water

#### 4. Conclusion

Macerated-pigment-removal with ethanol from brown seaweed, *Sargassum binderi* Sonder indicated lighter brown color as result. Based on the characterization of fucoidan, we can conclude that pigment removal gave fucoidan better appearance, higher total carbohydrate and total sulphate. Further research about the activity of fucoidan to support characteristics result is needed.

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