Chemical composition diversity of fucoidans isolated from three tropical brown seaweeds (Phaeophyceae) species

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Abstract. Lutfia FN, Isnansetyo A, Susudarti RA, Nursid M. 2020. Chemical composition diversity of fucoidans isolated from three tropical brown seaweeds (Phaeophyceae) species. Biodiversitas 21: 3170-3177. Fucoidan is a polysaccharide with high sulfate content, found in the cell walls matrix of brown seaweed. Its bioactivities vary depending on the algae species and the chemical structure. The aims of this research were to extract, purify, and characterize fucoidan from three Indonesian brown seaweed, Sargassum sp., Turbinaria sp., and Padina sp. The extraction was carried out using acid method followed by precipitation with ethanol and CaCl2, while its purification using DEAE-cellulose ion-exchange chromatography. Characterization was performed by FTIR and 1H-NMR spectroscopic analyses. Chemical components of fucoidan determined were total carbohydrates, sulfate residue, uronic acid residue, and monosaccharide components. The results revealed that the Turbinaria sp. produced highest yield of fucoidan (4.8% dry matter), followed by Sargassum sp. (2.7% dry matter) and Padina sp. (2.6% dry matter). The carbohydrate contents of Sargassum sp., Turbinaria sp. and Padina sp. fucoidan were 64.55±0.12%, 67.43 ± 0.03% and 62.90 ± 0.04% with sulfate residues of 0.013% ± 4.71; 19.47±0.002% and 0.016%±0.81% and uronic acid residues of 25.19±0.03%; 12.69±0.03% and 12.91±0.01%, respectively. Sargassum sp., Turbinaria sp., and Padina sp. fucoidans consist of fucose and some other minor monosaccharides.

Keywords: Characterization, Padina, Sargassum, sulfated carbohydrate, Turbinaria

INTRODUCTION

Approximately 1800 species of brown seaweed (Phaeophyceae) in the world have been identified and some have been exploited as sources of functional foods, pharmaceuticals, nutraceuticals, and cosmetics (Morya et al. 2012). One of the valuable substances is fucoidan due to its multi-bioactivities. Brown seaweed group is the only algal class of Phaeophyceae which contains fucoidan as the cell wall component. Fucoidan is a type of sulfated polysaccharide consisting of sulfated L-fucose with a small portion of xylose, galactose, glucose, mannose, rhamnose and uronic acid (Thinh et al. 2013) Fucoidan have extensive bioactivities, such as antioxidants, anti-inflammatory, anticoagulant, immuno-stimulatory and antitumor (Li et al. 2008; Fernando et al. 2020). The properties of fucoidan bioactivity have been the subject of interest of scientists, and various food and fine chemical industries in recent years which drive increasing demand for fucoidan extracted from brown seaweed. Many articles have discussed and reviewed thoroughly the biological, pharmacological, and pharmaceutical applications of fucoids, which have made it a hot topic in the past few decades. All of these studies tried to investigate the molecular mechanism of fucoidans in relation to their chemical structure and physicochemical properties (Zayed and Ulber 2020).

We have previously reported the anti-cancer activity of fucoidans extracted from three Indonesian Phaeophyceae species (Isnansetyo et al. 2017) and immunostimulating activity of fucoidan from tropical Phaeophyceae species (Isnansetyo et al. 2016; Setyawan et al. 2018; Purbomartono et al. 2019). As the activities highly correspond to Fucoidans from Phaeophyceae have a very complex structure with varying chemical composition within and between species. In addition, other factors affecting the chemical composition of fucoidan are growth environment, age, and collection season (Lee et al. 2006), and also geographical location (Lim et al. 2019). The differences in chemical composition of fucoidans were thought to be associated with their biological activities (Thinh et al. 2013). However, little research on the comparison of chemical characteristics of fucoidan among tropical species Phaeophyceae is found.

Diverse species of Phaeophyceae are distributed around the world, but limited species are exploited commercially for the source of fucoidan. Obviously, a lot more research needs to be done to explore the potential of Phaeophyceae as a possible source of medicinal agents especially fucoidan. In the previous study, we found that three Phaeophyceae species, Sargassum sp., Turbinaria sp. and
Fucoidan characterization

FTIR analysis

The infrared spectra of purified fucoidan in KBr pellets (2 mg fucoidan in 100 mg KBr) were recorded using a FTIR spectrometer (Perkin Elmer) at a wavenumber range of 400 to 4000 cm⁻¹. The IR spectra of purified fucoidan were compared with that of standard fucoidan (Sigma-Aldrich, St. Louis, MO, USA).

Determination of total carbohydrates content

One milligram of purified fucoidan was dissolved in 1 ml of distilled water. Fifty microliters of solution were transferred into a microplate well containing 150 µl of concentrated H₂SO₄ (Merck, KGaA, Darmstadt, Germany) then shaken for 20 minutes, followed by the addition of 30 µl of 5% phenol. The microplate was heated at 90°C for 5 minutes and then left at room temperature for 5 minutes. The absorbance of the solution was then measured at 490 nm. The same experiment was done for a series of standard L-fucose (Nacalai Tesque, Kyoto, Japan) solution (100 to 1000 ppm) to obtain the standard curve (Masuko et al. 2005).

Determination of sulfate residue

Preparation BaCl₂-Gelatin solution

The measurement of sulfate residue in the fucoidan was conducted using BaCl₂-gelatin method (Dodgson et al. 1962) using Na₂SO₄ (Merck KGaA, Darmstadt, Germany) at concentrations of 100-1000 ppm as a standard solution. Gelatin solution was prepared by dissolving 0.25 g gelatin in distilled water (60°C) to a final 50.0 ml of solution, then allowed to cool and stored at 4°C overnight. The BaCl₂-gelatin solution was prepared by dissolving 0.25 g BaCl₂ in gelatin solution to a final volume of 50.0 ml. BaCl₂-gelatin solution was stored at 4°C before use for as long as a week.

Measurement of sulfate residue

One mg of fucoidan was dissolved in 1 ml of 1N HCl and then heated in an oven at 105°C for 4 hours. Ten microliters solution was added into microplate well containing 190 µl of TCA (Merck KGaA, Darmstadt, Germany) 4%. The mixture was then added 50 µl BaCl₂-gelatin solution and shaken for 20 minutes. The absorbance of the solution was measured with a microplate reader (HEALES, MB-580, Shenzhen, China) at 360 nm (Dodgson et al. 1962).

Determination of Uronic Acid Residue

The uronic acid residues were determined by a spectrophotometer (Genesys 10S UV-VIS Spectrophotometer, Thermo Scientific) using glucuronic acid (AppliChem GmbH, Darmstadt, Germany) as standard (Kurita et al. 2008). One milligram of fucoidan sample was dissolved in 1 ml of 1% NaCl then 250 µl of solution was put into a test tube and then added 2 ml of cold concentrated sulfuric acid (Merck KGaA, Darmstadt, Germany). The mixture was heated in a water bath at 100°C for 10 minutes then added 100 µl solution of trimethyl-phenol (0.1 g trimethyl-phenol powder (TCI, Tokyo, Japan) dissolved in of glacial acetic acid (Merck

Materials and Methods

Sample Collection

Three Phaeophyceae species samples were taken at low tide conditions in the intertidal zone of coastal regions of Drini, Gunung Kidul, Yogyakarta, Indonesia in May 2015. The Phaeophyceae species were placed in a cool container and then carried to the lab. The algae samples were rinsed with tap water before air drying to obtain dry biomass. The dried samples were powdered and then stored in plastic bags at 5°C until used. The identification of specimens and characterization of fucoidan were carried out based on previously published methods (Trono et al. 1999; Dhargalkar et al. 2014).

Extraction and isolation of fucoidan

Fifty grams of dried powdered brown seaweed was soaked in 800 ml of 0.1 N HCl (Mallinckrodt, Baker inc., Paris, Kentucky) at room temperature for 24 hours then filtered. The filtrate was neutralized with NaOH then precipitated by the addition of twice the volumes of ethanol and then allowed to stand at 5°C overnight. The mixture was centrifuged at 3,500 rpm for 30 minutes to remove the supernatant. The remaining sediment was dissolved in pH 2 by adding HCl then added with CaCl₂ to a final concentration of 4 M. This mixture was then stored at 5°C overnight before being centrifuged at 3,000 rpm for 15 minutes. The supernatant was collected and added with ethanol, in order to precipitate fucoidan. The mixture was centrifuged at 3,000 rpm for 20 minutes. The crude fucoidan obtained was further purified by DEAE-cellulose column chromatography.

Purification of fucoidan

Crude fucoidan was applied to DEAE-cellulose (Sigma-Aldrich, Co., Spruce St, Louis, USA) ion-exchange chromatography and eluted with distilled water followed by a linear gradient of 0.5 to 3 M NaCl. Fractions of 20 ml were collected and total carbohydrate content was determined by phenol-sulfuric acid method according (Masuko et al. 2005) using L-fucose (Nacalai Tesque, Kyoto, Japan) as a reference. Carbohydrate-containing fractions were treated with ethanol and the mixture was centrifuged at 3,000 rpm for 15 minutes to separate the supernatant. The purified fucoidan was dissolved in distilled water, dialyzed (12,300 Da molecular weight cut-off) (Sigma) for 48 hours before freeze-drying. Finally, the purified fucoidan was stored at 5°C for further experiments.
KGaA, Darmstadt, Germany) until 100.0 ml of solution). The solution was vortexed and allowed to stand for 10 minutes and then the absorbance was measured at 450 nm.

**Determination of Monosaccharides**

The content of monosaccharides in fucoidan was determined after acid hydrolysis. Five milligrams of fucoidan standard (Sigma-Aldrich Co., Spruce St. Louis, USA, Product of Australia) was dissolved in 1 ml HPLC grade water then treated with 1 ml of 4 M trifluoroacetic acid. The solution was heated for 2 hours at 100°C then filtered by 0.45 μm pore size filter (Merck KGaA, Darmstadt, Germany). The filtrate was neutralized with 0.1M NaOH and then evaporated with liquid nitrogen until the remaining volume of 0.5 ml. The content of monosaccharides of the fucoidan hydrolyzate was analyzed by High-Performance Liquid Chromatography (HPLC) (Shimadzu, SIL-10AD) using a refractive index detector (RID) equipped with a ligand-exchange column for Carbohydrate (Agilent Hi-Plex H, 7.7 × 100 mm) eluted with 0.005 M H₂SO₄ at a flow rate of 0.7 ml/min. A sample of 20 µl was injected with separation time of 30 minutes. The standard monosaccharides used were fucose (Nacalai Tesque, Kyoto, Japan), glucose (Merck KGaA, Darmstadt, Germany), galactose (Merck KGaA, Darmstadt, Germany), rhamnose (Cica Reagen, Kanto Chemical, Tokyo, Japan), mannose (Merck KGaA, Darmstadt, Germany) and xylose (Merck KGaA, Darmstadt, Germany).

**RESULTS AND DISCUSSION**

**Extraction and Purification of Fucoidan**

In this study, three species of Phaeophyceae were extracted by using acid method with different yields of fucoidan. *Turbinaria* sp. produced highest yields of fucoidan (4.8%) followed by *Sargassum* sp. (2.7%), and *Padina* sp. (2.6%). The yields of fucoidan are various among the species of Phaeophyceae. For instance, the yields of fucoidan are 3.9% for *Undaria pinnatifida* (Kim et al. 2007), 2.2% for *Saccarina latissima* (Bilan et al. 2010), and 1.25% for *Ascophyllum nodosum* (Jiang et al. 2010).

Each species of Phaeophyceae has physiological and biochemical mechanisms to produce different metabolites. Number of metabolites produced is also directly influenced by the nutrients contents in the environment that also affect the yield of fucoidan in brown seaweed. Besides the species, other factors that affect the yield of fucoidan are time of sampling, the age of the sample and extraction methods (Lee et al. 2006)

Fucoidans isolated from *Sargassum* sp., *Turbinaria* sp., and *Padina* sp. were confirmed with a standard fucoidan based on their FTIR spectra (Figure 1). The overall FTIR spectra of those fucoidans exhibit similar absorption pattern (Isnansetyo et al. 2016; Isnansetyo et al. 2017). The notable difference is the appearance of absorption band at 1733 cm⁻¹ in the FTIR spectrum of standard fucoidan indicated the presence of carbonyl group.

**1H-NMR Analysis**

The ¹H-NMR spectra of fucoidan samples (10 mg in 600 µl of D₂O) were recorded by NMR JEOL ECS 400 MHz and then compared with that of standard fucoidan.

![Figure 1. The Overlay FTIR spectra of Sargassum sp. (red), Turbinaria sp. (green), Padina sp. (blue) fucoidans and a commercial fucoidan as standard (black)](image-url)
Purification results by using DEAE-cellulose ion-exchange chromatography revealed that the purification process yielded brown powder high purity fucoidan. This result was confirmed by HPLC analyses indicating Sargassum fucoidan was eluted as a single peak at 6.845; sp., Turbinaria sp., Padina sp. fucoidans, and also a commercial 6.772; 6.742 and 6.676 minutes of retention times, respectively (Isnansetyo et al. 2017). The fucoidan of each species of brown seaweed was then characterized for their total carbohydrate content, sulfate and uronic acid residues, and monosaccharides components compared to standard fucoidan.

Characterization of fucoidan

Total carbohydrate content

The total carbohydrate contents of Sargassum sp., Turbinaria sp., Padina sp. fucoidans were higher than that of a commercial fucoidan as shown in Table 1. The highest carbohydrate content (67.42%) was obtained from Turbinaria sp., followed by Sargassum sp. (64.55%) and Padina sp. (62.90%).

Fucoidan in Phaeophyceae is heterogeneous, and composed of various units of carbohydrates and non-carbohydrates (sulfate and acetyl) (Cumashi et al. 2007). Previous studies reported that the carbohydrate content in fucoidan of some Phaeophyceae species varies, for example, 52.34% in sporophylls of Undaria pinnatifida (Kim et al. 2007), 69.0% in Cladosiphon okamuranus (Tako et al. 2000) and 49.2% in Fucus evanescens (Men’shova et al. 2013).

Sulfate residue

Fucoidans are polysaccharides having ester sulfate groups as one of the main constituents. The concentrations of sulfate residues of fucoidan extracted from three species of Phaeophyceae and standard fucoidan varied (Table 2) and the highest concentrations were found in the fucoidan from Turbinaria sp. (19.47%), Fucoidan of Padina sp. and Sargassum sp. contained 8.82% and 4.71% sulfate residue, respectively, and were lower than that in the standard fucoidan (14.4%).

The main skeleton of fucoidan consists of a straight-chain or sometimes branched fucose, in which the sulfate group is attached to the C-2, C-3 or C-4 of fucose. Sulfate group is essential for the bioactivity of fucoidan (Isnansetyo et al. 2017). Sulfate content in fucoidan from Phaeophyceae species varies greatly. This is due to the different biochemical and physiological mechanisms in the absorption of nutrients in the environment. Based on the sulfate content, fucoidan from Turbinaria sp. is expected to be more active than the other two species studied as well as the fucoidan standard, as it contains higher sulfate content. Fucoidan from Turbinaria sp. obtained from Tamilnadu, India contains 38 ± 0.42% of residual sulfate (Marudhupandi et al. 2014). The differences in growth environment and sample age greatly affect the content of sulfate in fucoidan (Lee et al. 2006).

Uronic Acid Residue

Uronic acids are sugars in which the terminal carbon’s hydroxyl group has been oxidized to a carboxylic acid. Uronic acid is found in fucoidan with different concentrations that might be influenced by species of algae. Uronic acid content in fucoidan of three Phaeophyceae species studied ranged from 12.69 to 25.19% (Table 3). The results showed the highest uronic acid content (25.19 ± 0.03) was obtained in fucoidan from Sargassum sp. The lower contents were found in fucoidan from Turbinaria sp. (12.69 ± 0.03), Padina sp. (12.91 ± 0.01), and standard fucoidan (14.31 ± 0.01). Several studies reported that the uronic acid content varied in fucoidan. For instance, fucoidan from Undaria pinnatifida and Saccharina latissima contain uronic acid of 26.2% (Kim et al. 2007) and 23.3% (Bilan et al. 2010), respectively.

Monosaccharides component

The content of specific monosaccharides of fucoidan was determined using HPLC after TFA hydrolysis. Standard monosaccharides (fucose, rhamnose, xylose, mannose, galactose, and glucose) individually appear at retention times of 9.760; 11.585; 10.726; 10.346; 10.358; 10.298; and 9.760 minutes, respectively (Table 4). Glucuronic acid appears at 8.491 minutes. The retention times of xylose, mannose, galactose are very close, so they appeared as a single peak at 10.342 minutes when analyzed together (Figure 2). This raises a problem to detect the existence of these three monosaccharides independently in the fucoidan. Chromatogram of each sample of hydrolyzed fucoidan is shown in Figure 3.

Table 1. Total carbohydrate content of fucoidans from three Phaeophyceae species and a commercial fucoidan

<table>
<thead>
<tr>
<th>Brown seaweed species</th>
<th>Total carbohydrate of fucoidan (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sargassum sp.</td>
<td>64.55 ± 0.12</td>
</tr>
<tr>
<td>Turbinaria sp.</td>
<td>67.42 ± 0.03</td>
</tr>
<tr>
<td>Padina sp.</td>
<td>62.90 ± 0.04</td>
</tr>
<tr>
<td>A commercial fucoidan (standard)</td>
<td>62.42 ± 0.05</td>
</tr>
</tbody>
</table>

Table 2. Sulfate residue of fucoidans from three Phaeophyceae species and a commercial fucoidan

<table>
<thead>
<tr>
<th>Brown seaweed species</th>
<th>Sulfate content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sargassum sp.</td>
<td>4.71 ± 0.013</td>
</tr>
<tr>
<td>Turbinaria sp.</td>
<td>19.47 ± 0.002</td>
</tr>
<tr>
<td>Padina sp.</td>
<td>8.82 ± 0.016</td>
</tr>
<tr>
<td>A commercial fucoidan (standard)</td>
<td>14.4 ± 0.015</td>
</tr>
</tbody>
</table>

Table 3. Uronic acid residue of fucoidans from three Phaeophyceae species and a commercial fucoidan

<table>
<thead>
<tr>
<th>Brown seaweed species</th>
<th>Uronic acid residue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sargassum sp.</td>
<td>25.19 ± 0.03</td>
</tr>
<tr>
<td>Turbinaria sp.</td>
<td>12.69 ± 0.03</td>
</tr>
<tr>
<td>Padina sp.</td>
<td>12.91 ± 0.01</td>
</tr>
<tr>
<td>A commercial fucoidan (standard)</td>
<td>14.31 ± 0.01</td>
</tr>
</tbody>
</table>
Table 4. Retention time (min) of monosaccharides of fucoidan from three Phaeophyceae species and a commercial fucoidan

<table>
<thead>
<tr>
<th>Retention time of fucoidan samples</th>
<th>Retention time of standard monosaccharides</th>
<th>Monosaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sargassum sp.</td>
<td>Turbinaria sp.</td>
<td>Padina sp.</td>
</tr>
<tr>
<td>11.598</td>
<td>11.593</td>
<td>11.614</td>
</tr>
</tbody>
</table>

Figure 2. HPLC chromatogram of fucose, rhamnose, xylose, mannose, galactose, glucose and glucuronic acid mixture as standard monosaccharides

Figure 3. HPLC chromatograms of hydrolyzed Sargassum sp. (A), Turbinaria sp. (B), Padina sp. (C) fucoidans, and a commercial fucoidan (D). 1. Fucose, 2. Mannose/xylose/galactose, 3. Glucose

FTIR analysis
The FTIR spectra are particularly informative with broad strong bands around 3435 cm⁻¹ attributed to O-H stretching of hydrogen-bonded hydroxyl groups (Na et al. 2010) common to all fucoidan. The weak band around 2935 cm⁻¹ represents C-H stretching vibration of pyranose ring (Marudhupandi et al. 2014). Two bands around 1630 cm⁻¹ and 1420 cm⁻¹ characteristic for asymmetrical and symmetrical stretching vibration of carboxylate (RCOO⁻), respectively indicate the existence of uronic acid residue (Na et al. 2010). The existence of sulfate ester group as a characteristic component of fucoidan was represented by strong bands around 1255 cm⁻¹ attributed to S=O stretching vibration. Absorption band around 1035 cm⁻¹ indicating C-
O and C-C bonds of pyranose ring and glycoside C-O-C bond (Marudhupandi et al. 2014), whereas in the region of 800 cm\(^{-1}\) shows the bond C-O-S (Synytsya et al. 2010). The vibrational band at 1733 cm\(^{-1}\) in the FTIR spectrum of standard fucoidan indicates the presence of C=O group. This confirmed the standard fucoidan contains acetylated sugar. In contrast, the fucoidans obtained from three algae species in the present study did not contain acetylated sugars. In fact, only a small fraction of such fucoidans are present in brown seaweed, such as Undaria pinnatifida (Synytsya et al. 2010) and Costaricostata (Ermakova et al. 2011). The largest components of fucoidan, in general, are L-fucose and sulfate (Senthilkumar et al. 2013). The FTIR spectral data of fucoidan from three species of Phaeophyceae and a commercial fucoidan is shown in Table 5.

Table 5. FTIR Spectral data of fucoidans from three Phaeophyceae species and a commercial fucoidan (Pretsch et al. 2009)

<table>
<thead>
<tr>
<th></th>
<th>Sargassum sp. (cm(^{-1}))</th>
<th>Turbinaria sp. (cm(^{-1}))</th>
<th>Padina sp. (cm(^{-1}))</th>
<th>A commercial fucoidan (cm(^{-1}))</th>
<th>General spectra (cm(^{-1}))</th>
<th>Functional groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3,432</td>
<td>3,435</td>
<td>3,421</td>
<td>3,467</td>
<td>3,200-3,500</td>
<td>O-H</td>
</tr>
<tr>
<td></td>
<td>2,938</td>
<td>2,942</td>
<td>2,935</td>
<td>2,939</td>
<td>2,700-3,000</td>
<td>C-H</td>
</tr>
<tr>
<td></td>
<td>1,611</td>
<td>1,639</td>
<td>1,630</td>
<td>1,639</td>
<td>1,550-1,610</td>
<td>asym. - COO⁻</td>
</tr>
<tr>
<td></td>
<td>1,419</td>
<td>1,420</td>
<td>1,420</td>
<td>1,417</td>
<td>1,300-1,420</td>
<td>sym. - COO⁻</td>
</tr>
<tr>
<td></td>
<td>1,256</td>
<td>1,255</td>
<td>1,255</td>
<td>1,258</td>
<td>1,000-1,300</td>
<td>S=O</td>
</tr>
<tr>
<td></td>
<td>1,035</td>
<td>1,051</td>
<td>1,036</td>
<td>1,030</td>
<td></td>
<td>C-O, C-C</td>
</tr>
<tr>
<td></td>
<td>817</td>
<td>835</td>
<td>800</td>
<td>844</td>
<td>700-850</td>
<td>C-O-S</td>
</tr>
</tbody>
</table>

Figure 4. \(^{1}\)H-NMR spectra of Sargassum sp. (A), Turbinaria sp. (B), Padina sp. (C) fucoidans and a commercial fucoidan (D)
1H-NMR Analysis

The 1H-NMR spectra of Sargassum sp., Turbinaria sp., Padina sp. fucoidans, and a commercial fucoidan showed in Figure 4. The existence of fucose is clearly confirmed by the appearance of a signal at 1.1-1.5 ppm. In the 1H-NMR spectrum of standard fucoidan, this signal appears as a doublet and as assigned was the resonance of the methyl protons (C-6) coupled to the neighboring methine proton (C-5) (Tako et al. 2000). Signal at 3-5 ppm was assigned as the resonances of hydroxyl groups of the monosaccharides of fucoidans. The anomeric protons of sugars were represented by the downfield signals at 4.9 to 5.6 ppm (Marudhupandi et al. 2014). The signal at 2.8 ppm in the 1H-NMR of fucoidans from studied brown seaweed species indicated the presence of amino sugars, wherein one hydroxy group of the monosaccharide is replaced by the amino group. This signal does not seem to be not found in the standard fucoidan spectrum. The amino sugars have been found in fucoidan from V. vesiculosus (Nishino et al. 1994) and Saccharina japonica (Saravana et al. 2016). The signal at 2.1 ppm in the spectrum of standard fucoidan supported the existence of the acetyl group (Tako et al. 2000).

In conclusion, extraction of brown seaweed species, Sargassum sp., Turbinaria sp., and Padina sp. resulted in the isolation of pure fucoidan with a yield of 2.7%, 4.8% and 2.6% of the dry matter, respectively. This study confirmed the variation of the chemical characteristics of the fucoidans present in the three brown seaweed species from Indonesia.

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