Characterization of Fucoidan Extracted from Binuangeun's Brown Seaweeds

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***Abstract*—** The objective of this research is to characterize of fucoidan extracted from 3 species of brown seaweeds from the same habitat. Those seaweeds are *Sargassum sp, Tubinaria sp* and *Padina sp*. Raw material were obtained from fresh seaweed then macerated in methanol: chloroform : water (4:2:1) for 12 hours (1 night), filtered and rinsed with acetone and then was left to dry in room temperature. The each of, brown seaweed was extracted with dilute HCl in room temperature while mixing. Fucoidan and alginate were separated with addition $CaCl_2$ 4M. The quality of fucoidan extract were characterized for its total sugar, functional group using FTIR, monomer content, total ash, and total sulphate in ester form. Results showed that yields highest *Sargassum sp of 4.02%, Tubinaria sp 2.68%, Padina sp of 2.01%,* respectively. All three brown seaweeds contained fucose as the primary sugar component followed by galactose, mannose and xylose.

***Keywords*—** Fucoidan, *Sargassum sp, Tubinaria sp*, Brown Seaweeds

I. Introduction

Indonesia there is various species of brown seaweed. Brown seaweed that has been widely known are from the genus *Sargassum sp, Turbinaria sp, Padina Sp. Sargassum sp* has hundreds of species. In the brown seaweeds are the main polysaccharides alginate compound, laminaran and fucoidan while, fucoidan is a sulfated polysaccharide compound bound sulfate that is only found in brown seaweed and sea cucumbers (Li Bo *et al*., 2008). Fucoidan is one of these compounds, which are heterogeneous sulphated polysaccharides commonly found in marine invertebrates and brown seaweed (Skriptsova, Shevchenko, Zvyagintseva, & Imbs, 2009).Their composition varies between species of brown seaweed, but usually consists of _-1,3-linked and _-1,4-linked l-fucose residues, and sulphate as major constituents along with small amounts of uronic acid and other sugars such as galactose, mannose, xylose, glucose, etc. (Bilan *et al*., 2002; Lee, & Lee, 2010).The structural characteristics of fucoidan are likely to be dependent on the extraction technique (Ponce, Pujol, & Damonte, 2003), species of seaweed, season of harvest (Honya, Mori, Anzai, Araki, & Nishizawa, 1999), geographic location (Rioux, Turgeon, & Beaulieu, 2007), and algal maturity (Zvyagintseva *et al*., 2003).

The content of fucoidan in brown seaweed is influenced by several factors, among others are the method of extraction, growth, season, and species, these characteristics affect the bioactivity of fucoidan. Binuangeun (Banten) is one of the producers of brown seaweed that has been widely studied in Indonesia (Yunizal *et al*, 2006). However, during this study brown seaweed is still on the macro compounds in the brown seaweed. This research will focus on the characteristic of compounds in three different genus of brown seaweed of genus *Sargassum sp, Turbinaria sp, Padina Sp*, respectively. The objective of this study is to investigate the characteristics of fucoidan extracted from these brown seaweeds genus of in the same growth area.

II. Materials Dan Methods

A. Materials

Raw material used in the research are obtained from brown seaweed Binuangeuns. Chemical reagents $CaCl_2$ from Merck, ethanol $CHCl_3$, methanol, HCl, H_2SO_4 p.a. Fucoidan standard is purchased from sigma Aldrich and its extracted from spesies *Fucus vesiculosis*.

III. Methods

Fucoidan Extraction

The fucoidan was extracted from fresh brown seaweeds that were form foreign substances the fresh seaweeds were maceration in MeOH-$CHCl_3$- H_2O with ratio 4:2:1 for 12 hours then the wet biomass was washed with aceton and was dried outside . Dry seaweed used was powdered into 60 mesh size. Seaweed powder soaked with dilute HCl pH 4 with a ratio of 1: 10 for 6 hours while stirring, then filtered through 350 mesh nylon. The filtrate was collected and added $CaCl_2$ 4M then was incubated for 30 minutes. The mixture was filtered, the filtrate was collected and until be concentration become 2M $CaCl_2$ solution and then was centrifuged at 10,000 rpm for 15 minutes, then added $CaCl_2$ 3 M. Then centrifuged at a speed of 10,000 rpm for 15 minutes. Filtrate collected and added ethanol at a ratio of 1: 2, then left. The precipitate was collected separately by centrifugation with a speed of 10,000 rpm for 15 minutes. The precipitate was collected and dissolved with aquabidest. Then last was dried using freez

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dryer to obtain fucoidan extracts. All yields are calculated on the weight of seaweed flour converted in to percent.

Determination of chemical composition

Fucose content of fucoidan was determined according to Dubois methods using phenol-H_2SO_4 reagent and L-fucose (Sigma) as the standard (Dubois, 1956). The sulphate content was quantified using the BaCl2-gelatin method using K2SO4 (BDH Limited) as the standard using hydrolyzed fucoidan. Fucoidan was hydrolized (15 mg) in 3 M HCl for 17 h at 100 ◦C (Dodgson & Price, 1962). Uronic acid and protein content of fucoidan were determined using the carbazole-sulphuric acid borate reaction using d-glucuronic acid (Sigma) as the standard (Bitter & Muir, 1962), respectively. All yields were calculated from the dried weight of fucoidan and converted into a percentage. Absorbance measurements were recorded in triplicate using an Ultrospec 2100 UV/visible spectrophotometer.

Hydrolysis of fucoidan

Solution of fucoidans (5 mg) in 3 N TFA (1000 ml) was heated at 121 ◦C using autoclave for 1 h, and sample was netralized with 4M NaOH. Monosaccharide composition was determined by HPLC using a column of H^+ Plex with size (30 x 4mm) eluted with dilute sulfuric acid, the flow rate 0,6mL / min using refractive index detector. Rhamnose, mannose, fucose, galactose, xylose, and glucose were used as standards for HPLC

IR spectra of polysaccharides sulphates recorded with Perkin–Elmer 577 (tablet KBr) dan Specord M-80.

IV. Results And Discussion

The yield of fucoidan extracts of each species of brown seaweed were: *Sargassum sp (*4.02%), *Turbinaria sp* (2.68%), and *Padina sp* (2.01%). The highest content of fucoidan extract was obtained from *Sargassum sp*. Based on the physical form of the brown seaweed third leaf *Sargassum sp* more than *Turbinaria*, whereas in the *Padina sp* just a sheet with no stem. High content of fucoidan does not determine the fucoidan high bioactivity. Based on the literature, the molecular weight and the content of sulfate affect bioactivity of fucoidan.

Chemical compositon

The yields and composition chemical of extraction of crude brown seaweeds are presented in Table 1.

Table 1
Chemical Composition From Crude Brown Seaweeds, Respectively

Brown seaweeds	Yields (%)	Fucose (%)	SO_4^{2-} (ppm)
Sargassum sp	4.02	54.99 ± 0.09	80 ± 0.02
Turbinaria sp	2.68	17.01± 0.04	60 ± 0.01
Padina sp	2.01	28.88 ± 0.08	70 ± 0.01

Depend on result of chemical composition that highest fucose obtained from *Sargassum sp*, *Padina sp and Turbinaria sp*, respectively.The same obtained for sulphated content. Source available of brown seaweed in Binuangeun, *Sargassum sp*,is abundance. Different parts of the seaweed have also been shown to contain varying amounts of fucoidan. The structural characteristics of fucoidan are likely to be dependent on the extraction technique, species of seaweed, season of harvest, , geographic location (Rioux, Turgeon, & Beaulieu, 2007), and algal maturity (Mak, 2013).

Monosaccaride Composition Of Crude Fucoidans

The highest content fucose obtained from *Sargassum sp* and the lowest from *Turbinaria sp*. Based on the physical form *Turbinaria sp* show that short and not have leaves this allows content fucose be slightly. In different *to Sargassum* sp while dominant leaf. According to Mak *et al*, 2013 that the content of the sporofil fukosa more than in blade, sporofil be in a position close to the leaves. The each of species did show the chemical composition of fucoidan content of varying this is because the process of biosynthesis different. The same term is obtained from the content of sulfated. The highest content sulphated obtained from *Sargassum sp* and the lowest from *Turbinaria sp*.

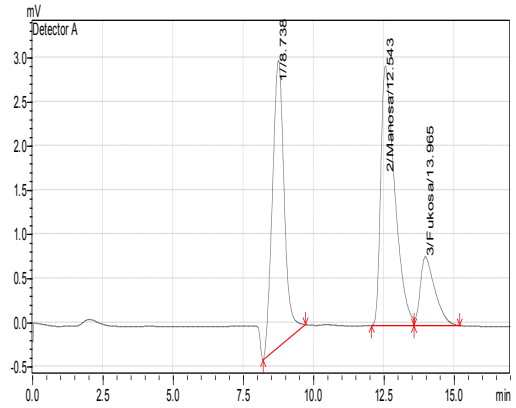

Fig.1A. *Sargassum sp*

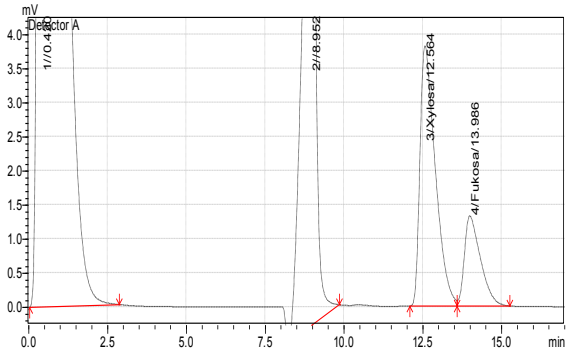

Fig.1B. *Turbinaria deccurens*

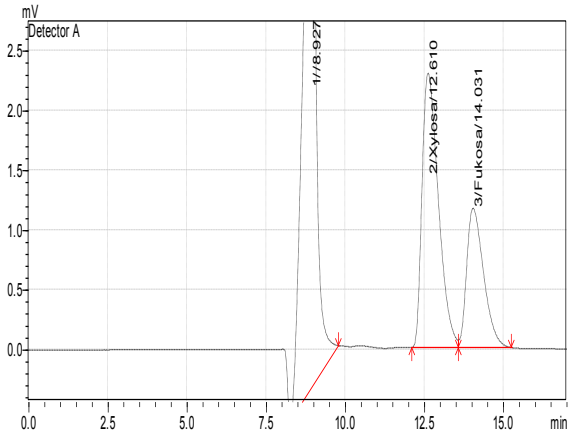

Fig.1C. *Padina australis*

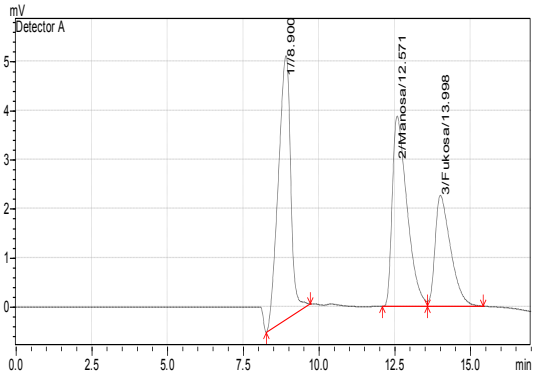

Fig.1D. *Fucus vesiculosis*

Constituent monomers fucoidan of from *Sargassum* sp consist fucose and mannose. *Turbinaria monomer* consist fucose and xylose. *Padina sp* consist fucose and xylose whereas commercial fucoidan is composed of fucose and mannose. There is possibility that exists minor amounts of other type of monomers exist based on low absorption peaks in the graph.

Fourier transforms infra-red (FT-IR):

The FT-IR spectrum of crude fucoidan from *Sargassum sp* contained at 3397 cm^{-1} (OH stretching), 1675 cm^{-1} (COO– stretching) characteristic band of alginate (Fig. 2A). While crude fucoidan from *Turbinaria sp* (Fig. 2B) has it contains at 3397 cm^{-1} (OH stretching), 2133 cm^{-1} (CH stretching), 1624 cm^{-1} (COO– stretching) Pereira *et al*., 2003). The absence of spectrum at 1200 cm^{-1} showed no ester sulphate, this is probably due to the sample is not pure or still a lot of impurities. Crude fucoidan from P*adina sp* (Fig. 2C) has characteristic band at 3397 cm^{-1} (OH stretching), 2133 cm^{-1} (CH stretching), 1624 cm^{-1} (COO– stretching) and 1190 cm^{-1} the letter band is estimated sulphated ester. Those there brown seaweeds should different spectrum, while the spectrum from P*adina sp* clearly showed a sulphated ester band. The different based on result from composition chemical, show that sulfate content highest from *Sargassum sp.* Based on this result to need purification extract fucoidan to more than clearly

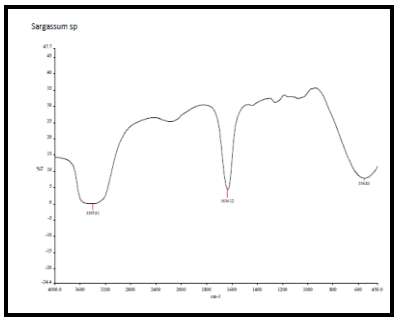

Fig.2A. *Sargassum sp*

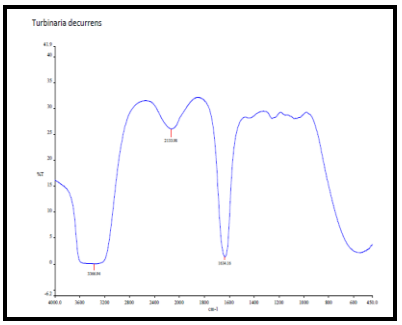

Fig.2B. *Turbinaria deccurens*

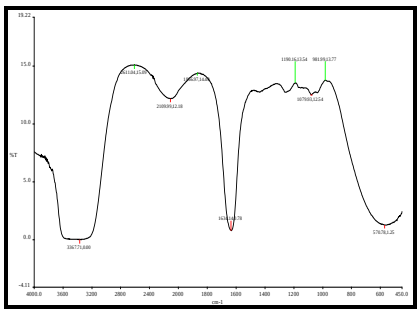

Fig 2C *Padina australis*

V. Conclusion

The highest fucoidan extract yields was obtained from *Sargassum sp* (4.02%) and the lowest from *Padina sp* (2.01%). Each fucoidan has different monomer composition constituents. The highest fucose composition was obtained from *Sargassum sp.* This was due to the physical appearance of brown seaweed with which was dominated by leaf structure. From the FT-IR data, sulphated ester absorption form *Padina sp* more clear than the others.

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