Fucoidan Content from Brown Seaweed (*Sargassum filipendula*) And Its Potential As Radical Scavenger

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Abstract. One of the health problems arising from the increasing of free radicals in the environment. Therefore, we need an alternative compound that can act as a radical scavenger agent. Fucoidan is one of the sulfated polysaccharides in the cell wall of brown seaweed such as *Sargassum filipendula*. It is composed of L-fucose, sulfate and small amounts of monosaccharides such as galactose, xylose, glucose, and mannose which are thought to have free radical scavenger activity. However, the content and activity of active compounds are influenced by the extraction method, temperature, time and solvent concentration. This study aims to determine the content and antioxidant activity of fucoidan from brown seaweed (*Sargassum filipendula*) on the variation of HCl solvent concentrations (0.01M; 0.03M; and 0.05M) and extraction times (10 minutes; 15 minutes; and 20 minutes) using Ultrasonic Assisted Extraction method. The stages of this research include sample preparation, analysis of raw materials, sample pre-treatment, extraction using Ultrasonic Bath, alginate precipitation, fucoidan precipitation, determination of fucoidan content and antioxidant activity of fucoidan (IC$_{50}$). The results showed that the highest content and antioxidant activity (IC$_{50}$) of fucoidan from *Sargassum filipendula* was at 0.03 M of HCl concentration and 15 minutes of extraction time, respectively at 6.07% and 85.46 ppm. From IC$_{50}$ values, it is known that fucoidan has strong antioxidant activity and potential to be a radical scavenger agent.

1. Introduction
The increasing number of free radical sources in our daily environment can lead to oxidative stress and endemically degenerative diseases. The oxidative stress stimulated by reactive oxygen species (ROS) such as radical hydroxyl, hydrogen peroxide, superoxide anions, and nitric oxide were reacted with biomolecules such as DNA, proteins, lipids and alter the normal cellular functions. This leads to tissue damage, cell death and finally to degenerative disease [1]. For this reason, a bioactive compound is required to counteract the free radicals, one of which is fucoidan from brown seaweed.

Fucoidan is a water-soluble polysaccharide consisting mainly of L-fucose and sulfate groups, in addition to other minor components monosaccharides such as mannose, glucose, xylose, glucuronic acid, etc [2]. Fucoidan can be extracted from brown seaweed, one of them is from *Sargassum filipendula*. Fucoidan is soluble in hot water and acid solvent. Fucoidan has bioactivity such as antitumor, anticoagulant, antioxidant, antivirus, immune activation, and liver protection activities [3], but the bioactivity of...
fucoidan is influenced by the chemical composition and structure of fucoidan. The yield and bioactivity of fucoidan were differed based on the species of seaweed, vicinity, growing conditions, extraction method and analytical methods such as solvent concentration and extraction time [4,5]. Ultrasonic-Assisted Extraction (UAE) is one of the extraction methods that can keep bioactive compounds from being damaged because the temperature and extraction time can be shorter used. The used temperature and extraction time will give different yields and bioactivity of fucoidan. The relationship between solvent concentration and extraction times using UAE on yield and antioxidant activity of fucoidan is still rarely conducted. So, this study aims to determine the content and antioxidant activity of fucoidan from brown seaweed (Sargassum filipendula) on the variation of HCl solvent concentrations and extraction times using the UAE method. From this research, it can be seen the content of fucoidan in S. filipendula and its potential as a radical scavenger.

2. Materials and methods

2.1. Materials and tools
The tools needed in this study were ultrasonic bath (Power Sonic405), analytical scale (Memmert), hot plate stirrer (Fisher Scientific), disk mill machinery, cold centrifuge, autoclave (Tony High-Pressure Steam Sterilizer), Laminar Air Flow Magnehelic, spectrophotometer UV-VIS (Jenway 6305), vortex (Corning LSE), shaker water bath (Memmert), glassware (Erlenmeyer, beaker glass, measuring pipettes, drop pipettes), falcon tube, spatulas and bulbs. The ingredients needed to be Sargassum filipendula from Sumenep-Madura, methanol, chloroform, HCl 0,01 M;0,03 M;0,05 M, CaCl2 1 %, ethanol 96%, acetone, and aquades.

2.2 Methods

2.2.1 Sample preparation
The sample was obtained from brown seaweed (Sargassum filipendula) from Sumenep-Madura. The sample was washed from remaining mud and other impurities with water, dried by drying without being exposed to the sun for 3-7 days, crushed using a grinder up to 1 mm in size, stored in plastic and put into a jar until it was ready to use.

2.2.2 Raw material analysis
Before sample pre-treatment, the sample was analyzed of raw materials (including the content of carbohydrates, proteins, lipids, moisture, ash, and crude fibers). The content of carbohydrates was determined spectrophotometrically at 485 nm, by the phenol-sulfuric method [6]. The content of protein was determined by Lowry’s method [7]. The content of lipids was extracted according to [8], determined gravimetrically after solvent (chloroform) evaporation. The moisture content was determined by the oven method at 105°C, ash content at 550°C overnight and crude fibers (successive hydrolysis with 100°C 0.05 N H2SO4 and 0.05 N NaOH for 30 minutes each) [9].

2.2.3 Sample pre-treatment
This process was used to relieve pigment and lipid. 1 g of sample was mixed with 20 mL of chloroform: methanol (2:1) (v/v) in Erlenmeyer, homogenized with stirrer magnetic about 20 minutes in room temperature, centrifuged about 20 minutes (2500 rpm) and dried in room temperature (+- 25°C).

2.2.4 Extraction of samples using Ultrasonic Bath
1 g of each sample was mixed with 20 mL of 0.01 M, 0.03 M, and 0.05 M HCl. The volume ratio of the sample and HCl was 1:20, then left for 10 minutes. Then extracted each sample with Ultrasonic Bath (the instrument was set in 350 W of energy, 40 kHz of frequency, and 70°C of water temperature) for 10, 15 and 20 minutes. After the extraction process then cooled the samples. Then the sample was filtered with filter cloth or filter paper, so it was obtained filtrate I and residue I.

2.2.5 Alginate precipitation
The filtrate I of each sample was added with 1% CaCl₂ (1:1 (v/v), stored at 4°C for 8 hours. Then centrifuged for 15 minutes in 4°C (8000 rpm). The sample was filtered with filter paper, so it was obtained residue II (alginate) and filtrate II. The obtained filtrate was used for further processing.

2.2.6 Fucoidan precipitation
The obtained filtrate II was added with 96% ethanol (1:2) (v/v) and stored in the refrigerator at 4°C for 8 hours. After 8 hours, then the filtrate was centrifuged for 15 minutes in 4°C (8.000 rpm) and filtered with filter paper, so it was obtained filtrate III and residue III (crude fucoidan). Residue III was washed with 96% ethanol and acetone and dried at room temperature (±25 °C) to a constant weight and stored to analyze the content of fucoidan and antioxidant activity.

2.2.7 Content of fucoidan extract
The content of fucoidan extract was determined by weighing the obtained crude fucoidan to a constant weight, then put in the formula:

\[
\text{Content of fucoidan} (\%) = \frac{\text{weight of fucoidan (g)}}{\text{weight of dry sample (g)}} \times 100\% 
\]  

(1)

2.2.8 Antioxidant activity of fucoidan (IC₅₀)
5 mg of samples fucoidan was dissolved in 5 mL of aquabidest (1000 ppm of stock solution). Then the stock solution was made to 3 mL concentration series of 5; 10; 15; 20 and 25 ppm. Each sample then added with 1 ml 0.2 mM DPPH, incubated in dark room for 30 minutes and the absorbance of each sample was measured using a UV-Vis spectrophotometer at λ 517 nm [10]. Calculation of antioxidant activity is presented by the following formula:

\[
\text{Antioxidant Activity} (\%) = \frac{\text{Abs of blank} - \text{Abs of sample}}{\text{Abs of blank}} \times 100\% 
\]  

(2)

3. Result and Discussion
3.1 Raw Material Analysis
Table 1 indicates the chemical composition of carbohydrates, proteins, lipids, moisture, ash, and crude fibers in *S. filipendula*. Based on the result, the highest chemical composition of *S. filipendula* was represented in the content of carbohydrates. Carbohydrates in seaweed contain many interesting molecules of polysaccharides such as alginate, agar, carrageenans, fucoidan, and laminaran [11]. High levels of carbohydrates in brown seaweed can affect the yield of fucoidan extracts. It is because fucoidan is one of the sulfate polysaccharides found in brown seaweed. Based on [12] the content of carbohydrates in *S. polycystum* was 38.76%, while the content of carbohydrates in *S. filipendula* (the result of this study) was 48.10%. It means that the content of carbohydrates in *S. filipendula* has higher than that is in *S. polycystum*. This shows that *S. filipendula* has the potential to have more fucoidan yields compared with other types of *Sargassum sp* such as *S. polycystum*. Chemical compositions of brown seaweeds were differed based on the species of seaweed, vicinity, growing conditions, extraction method and analytical methods [4,5].

| Chemical Composition | Contents  | References |
|----------------------|-----------|------------|
| Carbohydrates        | 48.10%    | 38.76%*    |
| Proteins             | 4.38%     | 4.70%*     |
| Lipids               | 0.07%     | 2.92%**    |
| Moisture             | 15.03%    | 9.95%***   |
| Ash                  | 32.42%    | 44.29%**** |
| Crude Fibers         | 42.55%    | 34.10%***  |

Note: *[12], **[13], ***[14], ****[15]*
3.2 Content of fucoidan extract
Table 2 indicates the highest content of fucoidan extract (6.07%) is in the treatment of 0.03 M of HCl concentration and 15 minutes of extraction time using the Ultrasound-Assisted Extraction method. The concentration of solvent and extraction time influences the content of fucoidan. The content of fucoidan increases with the increasing of solvent concentration but decreases at a concentration of 0.05M HCl. The content of fucoidan also increases with extraction time, but decrease in 20 minutes of extraction time. Based on kinds of literature, most polysaccharides can be released in the early of ultrasonic processing time, and parts of the polysaccharides can be depolymerized at high ultrasonic strength (frequency) and longer time [16, 17]. Besides, excessively high concentrations of acid solvents can break the bonding of the cell wall matrix followed by acid penetration into the intercellular tissue which influences to partial degradation and fucose fucoidan damage. The use of acid solvent at higher concentrations can produce fucoidan with lower yields, due to depolymerization of the polysaccharide chain [18]. Based on [12], the yield of fucoidan from *S. polycystum* was 4.51%. It shows that the content of fucoidan in *S. filipendula* has higher than that is in *S. polycystum*. These differences indicate that the type of species determines the amount of fucoidan yield.

| Treatment | HCl Concentration | Extraction Time | Content of Fucoidan Extract (%) |
|-----------|-------------------|-----------------|---------------------------------|
|           | 0.01 M            | 10 minutes      | 5.52 ± 0.04<sup>d</sup>         |
|           |                   | 15 minutes      | 5.41 ± 0.02<sup>d</sup>         |
|           |                   | 20 minutes      | 5.19 ± 0.06<sup>e</sup>         |
|           | 0.03 M            | 10 minutes      | 5.57 ± 0.06<sup>f</sup>         |
|           |                   | 15 minutes      | 6.07 ± 0.07<sup>h</sup>         |
|           |                   | 20 minutes      | 4.86 ± 0.09<sup>b</sup>         |
|           | 0.05 M            | 10 minutes      | 5.49 ± 0.08<sup>e</sup>         |
|           |                   | 15 minutes      | 5.64 ± 0.04<sup>g</sup>         |
|           |                   | 20 minutes      | 4.54 ± 0.06<sup>e</sup>         |

3.3 IC<sub>50</sub> value of antioxidant activity
Table 3 indicates the greatest IC<sub>50</sub> value of 85.46 ppm linear with the content of fucoidan, which is in the treatment of 0.03 M of HCl concentration and 15 minutes of extraction time. The concentration of solvent and extraction time influences the antioxidant activity of fucoidan. The antioxidant activity of fucoidan increases with the increasing of solvent concentration but decreases at a concentration of 0.05M HCl. The antioxidant activity of fucoidan also increases with extraction time, but decrease in 20 minutes of extraction time. Higher solvent concentration and extraction time influence the degradation of polysaccharide, so it contributes to the lowering of the antioxidant activity of the compound.

The IC<sub>50</sub> value is defined as the concentration of the sample to inhibit oxidation up to 50% or the concentration of the test sample to capture 50% of DPPH free radicals. According to [19], smaller IC<sub>50</sub> value indicates stronger antioxidant ability. Based on [12], the IC<sub>50</sub> of crude fucoidan in *S. polycystum* using the DPPH method is 759.60 ppm. It shows that the antioxidant activity of crude fucoidan in *S. filipendula* has higher than that is in *S. polycystum*. These differences indicate that the type of species not only determines the amount of fucoidan but also determines the antioxidant activity. According to [20], the IC<sub>50</sub> value of <50 ppm exhibits (very strong antioxidant), 50-100 ppm (strong antioxidant), 101-250 ppm (moderate antioxidant), 250-500 ppm (weak antioxidant), and >500 ppm (inactive antioxidant). It means that the antioxidant activity of fucoidan in *S. filipendula* is classified into strong antioxidants and has the potential to be a radical scavenger agent.

| Treatment | HCl Concentration | Extraction Time | Antioxidant Activity (IC<sub>50</sub>) |
|-----------|-------------------|-----------------|---------------------------------------|
|           | 0.01 M            | 10 minutes      | 85.2 ± 0.04<sup><small>f</small></sup> |
|           |                   | 15 minutes      | 85.4 ± 0.04<sup><small>i</small></sup> |
|           |                   | 20 minutes      | 85.5 ± 0.04<sup><small>j</small></sup> |
|           | 0.03 M            | 10 minutes      | 85.6 ± 0.04<sup><small>h</small></sup> |
|           |                   | 15 minutes      | 85.7 ± 0.04<sup><small>h</small></sup> |
|           |                   | 20 minutes      | 85.8 ± 0.04<sup><small>h</small></sup> |
|           | 0.05 M            | 10 minutes      | 85.9 ± 0.04<sup><small>h</small></sup> |
|           |                   | 15 minutes      | 86.0 ± 0.04<sup><small>h</small></sup> |
|           |                   | 20 minutes      | 86.1 ± 0.04<sup><small>h</small></sup> |
Treatment Antioxidant Activity (IC\textsubscript{50})
\begin{tabular}{lll}
 & HCl Concentration & Extraction Time (ppm) \\
 & & 10 minutes & 15 minutes & 20 minutes \\
0.01 M & 10 minutes & 90.37 ± 5.79\textsuperscript{ab} & 88.54 ± 1.61\textsuperscript{ab} & 91.48 ± 4.23\textsuperscript{ab} \\
 & 15 minutes & 88.54 ± 1.61\textsuperscript{ab} & 87.24 ± 7.61\textsuperscript{b} & 89.66 ± 7.92\textsuperscript{b} \\
 & 20 minutes & 91.48 ± 4.23\textsuperscript{ab} & 89.66 ± 7.92\textsuperscript{b} & 101.46 ± 8.86\textsuperscript{a} \\
0.03 M & 10 minutes & 87.24 ± 7.61\textsuperscript{b} & 89.66 ± 7.92\textsuperscript{b} & 94.13 ± 5.35\textsuperscript{a} \\
 & 15 minutes & 85.46 ± 4.17\textsuperscript{b} & 92.65 ± 3.29\textsuperscript{a} & 92.65 ± 3.29\textsuperscript{a} \\
 & 20 minutes & 89.66 ± 7.92\textsuperscript{b} & 92.65 ± 3.29\textsuperscript{a} & 101.46 ± 8.86\textsuperscript{a} \\
0.05 M & 10 minutes & 90.37 ± 5.79\textsuperscript{ab} & 88.54 ± 1.61\textsuperscript{ab} & 91.48 ± 4.23\textsuperscript{ab} \\
 & 15 minutes & 91.48 ± 4.23\textsuperscript{ab} & 87.24 ± 7.61\textsuperscript{b} & 89.66 ± 7.92\textsuperscript{b} \\
 & 20 minutes & 94.13 ± 5.35\textsuperscript{a} & 92.65 ± 3.29\textsuperscript{a} & 101.46 ± 8.86\textsuperscript{a} \\
\end{tabular}

4. Conclusion

Based on the results of the study, the highest content and antioxidant activity (IC\textsubscript{50}) of fucoidan from \textit{Sargassum filipendula} were at 0.03 M of HCl concentration and 15 minutes of extraction time, respectively at 6.07\% and 85.46 ppm. From IC\textsubscript{50} values, it is known that fucoidan has strong antioxidant activity and potential to be a radical scavenger agent.

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