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FUCOIDAN FROM *Sargassum plagiophyllum* BY MICROWAVE ASSISTED EXTRACTION IN COMPARISON WITH CONVENTIONAL METHODS

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ABSTRACT

Sargassum is a brown seaweed rich in fucoidan which is a sulfated polysaccharide. This study aims to examine the conventional and microwave-assisted extraction (MAE) methods to optimize fucoidan extraction from *Sargassum plagiophyllum* with 0.1 M HCl and water solvents. The extraction of fucoidan was conducted in two different methods, conventional (C) and MAE (M) methods. Microwave-assisted extraction was performed at 450, 600, and 700 W microwave power for five minutes, then the extracted fucoidan was determined for its sulfate and fucose contents, as well as antioxidant activities. In both the conventional (CAwe) and MAE methods (MAfa7), the maximum yields of fucoidan crude extract produced in 0.1 M HCl solvent were 19.10 and 19.20%, respectively. The highest sulfate (MAfa7; 6.14%), and fucose content (MAaf7; 5.95 mg/100g) were found in the HCl solvent using the MAE method. Based on the results, the MAE approach with HCl solvent was a more efficient technology for extracting fucoidan from *S. plagiophyllum* than the conventional method.

Keywords: *Sargassum plagiophyllum*, Fucoidan, Microwave Assisted Extraction (MAE), Conventional Extraction.

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INTRODUCTION

Sargassum sp. is a brown macroalga rich in vitamins, minerals, proteins, polyphenols, dietary fiber, and polysaccharides that has significant potential as nutraceuticals, cosmetics, and pharmaceuticals, with great benefits for health.[1,2] Meanwhile, fucoidan is one of the polysaccharides found in *Sargassum* sp. that has biological activity. L-fucopiranose and other monosaccharides residues including sulfate substituents, glucose, xylose, galactose, mannose, uronic acid, acetyl groups, and protein components are found in fucoidan, a polysaccharide sulfate from *Sargassum*.[3] Microwave-Assisted Extraction (MAE) is currently more commonly employed than the conventional approach for numerous extraction objectives. The microwave approach has the advantage of being both efficient and environmentally beneficial, as it minimizes processing time and the number of solvents used.[4] Extraction optimization, which includes microwave power and frequency, solid: solvent ratio, temperature, and duration is required to obtain higher yields and better-isolated chemicals.[3] In addition, the yield, and composition of fucose-containing sulfated polysaccharides are greatly influenced by the extraction process.[5] Consequently, the extraction is carried out by using various procedures to obtain the best yield. Although considerable investigations have been conducted on the extraction of fucoidan from brown seaweed, studies on the extraction of fucoidan from *Sargassum plagiophyllum* are limited. This study aims to determine the best conditions for extracting fucoidan from *Sargassum plagiophyllum* using conventional and microwave methods and analyze its antioxidant activity. The quality criteria studied include fucoidan yields, as well as sulfate and fucose contents.

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EXPERIMENTAL

Procedure for Conventional Extraction

Fucoidan was extracted using three different methods with HCl 0.1 M (CA) and water extraction (CW): (A) fucoidan pre-extraction with ethanol and extraction was carried out after the alginate had been separated (PE-A-F). A total of 50 grams of *S. plagiophyllum* were pre-extracted for 24 hours at room temperature with 300 ml ethanol, then the sample was heated to 70°C for an hour and filtered by centrifugation at 3000 rpm for 15 minutes. After drying, 200 ml of $CaCl_2$ 2% was added to the residue, and then heated at 70°C for 1 hour before centrifugation at 3000 rpm for 15 minutes. The residue was macerated for 24 hours at room temperature using two different solvents of 350 ml each, then the sample was filtered and fucoidan precipitated (CAaf and CWaf). (B) The pre-extraction technique is the same as in method A, but maceration was carried out with two different solvents before adding $CaCl_2$ 2% (PE-F-A). After centrifugation, the filtrate was added $CaCl_2$ 2% to precipitate alginate. The alginate was separated and the filtrate was added with ethanol to precipitate fucoidan (CAfa and CWfa). (C) This was treatment without pre-extraction and separation of alginate (WPE). 50 g of *S. plagiophyllum* was macerated for 24 hours at room temperature with two different solvents followed by filtration and then precipitation (CAwe and CWwe).

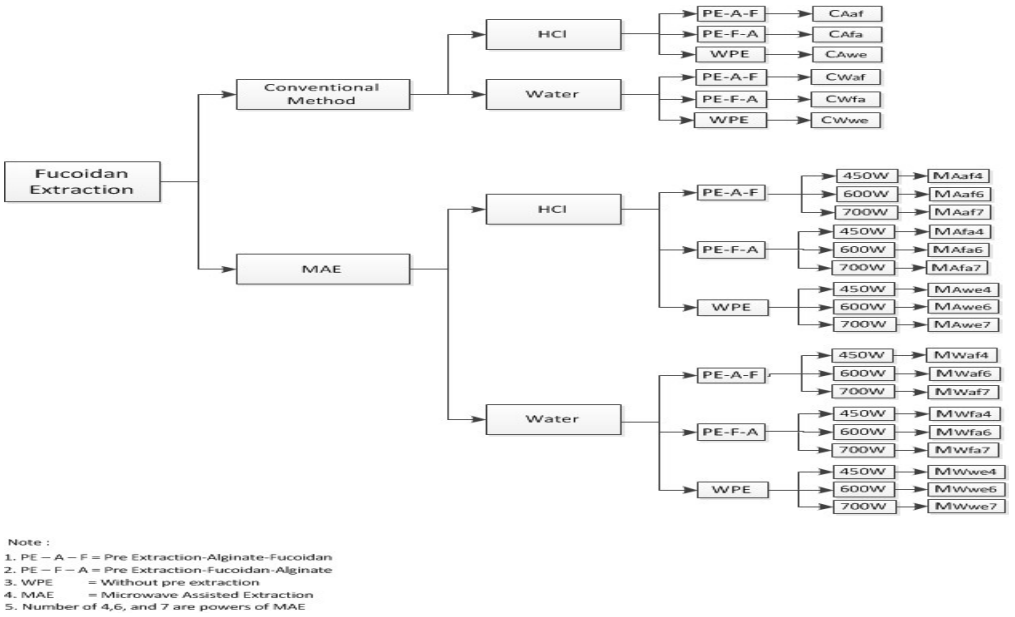

Fig.-1: Fucoidan Extraction Procedure with Conventional Method and MAE

Procedure for Microwave-Assisted Extraction (MAE)

The MAE extraction is nearly identical to the conventional method, except that maceration for 24 hours at room temperature is replaced by using a microwave for 5 minutes at 450W, 600W, and 700W. Figure-1 shows a flow for fucoidan extraction using conventional and microwave methods.

Determination of Total Sugar and Sulfate Content

The total sugar of fucoidan was determined by the phenol-H_2SO_4 method using L-fucose as the standard. Determination of Sulfate Content using the $BaCl_2$-gelation method and Na_2SO_4 was employed as a standard.[6]

Antioxidant Activity

Antioxidant activity was determined by the DPPH protocol.[7] Amount 1.5 mL of 0.1 mM DPPH was mixed with 0.1 ml of the sample, the mixture was held at ambient temperature for 30 minutes before being tested at a wavelength of 517 nm.

RESULTS AND DISCUSSION

Conventional and Microwave-Assisted Extraction

This study used *S. plagiophyllum* and is the first investigation of the occurrence, extraction methods, and antioxidant activity of fucoidan from this indigenous species in Yogyakarta, Indonesia. The physical

properties of the color and form of the fucoidan extract are shown in Fig.-2. The extraction conducted using water solvent produced a lighter color than the HCl solvent in powder form. The color of fucoidan extract obtained with water solvent was slightly brown, indicating the presence of salt and other monomers. In contrast, the color of the extract obtained with HCl was browner, indicating that the fucoidan polymer was more predominant. Pre-extraction with ethanol potentially removes pigments, proteins, mannitol which is an essential reserve carbohydrate, as well as chlorophyll, and specific ions using an ethanolic solution.[6] The yield of fucoidan extract obtained using the conventional HCl and water solvent method ranged from 10.5% - 19.1% and 5.8% - 14.9%, respectively. Meanwhile, the yields from the extraction conducted using the MAE method ranged from 7.20% to 19.2% for HCl solvent and 6.4% to 15.3% for water solvent. The result showed that the conventional method produced almost the same yield compared to MAE. In contrast, the MAE approach was more efficient in terms of extraction time, namely 5 minutes compared to the 24-hour extraction. Based on the results, the conventional approach yielded largely acid extraction than the water, as shown in Table-1. The HCl solution is excellent in extracting fucoidan from the matrix because it allows hydrolysis of the cell wall matrix of polysaccharides, which breaks the connections between polysaccharides and protein in cells. It also converts alginate to alginic acid, which is insoluble in water and can be separated from solid seaweed residue, to produce reasonably pure fucoidan.[8]

a b

Fig.-2: Fucoidan Extracted a) HCl Solvent; b) Water Solvent

Sulfate and Total Sugar Content

The sulfate concentration of extracted fucoidan with HCl solvent ranged from 0.78% - 6.14%, and water solvent from 0.45% - 3.86% as shown in Table-2. These values are higher than the extraction carried out with water but were similar to brown algae (*Ecklonia maxima, Splachnidium rugosum,* and *Laminaria pallida*) where acid extraction had the highest sulfate content when compared to hot water and salt techniques.[9]

Table-1: The Average Yields of Fucoidan Extracted With HCl and Water Utilizing Conventional Technique and MAE

No	Sample	Yield (%)	Sample	Yield (%)
1	MAaf4	8.54	MWaf4	11.10
2	MAaf6	9.90	MWaf6	11.30
3	MAaf7	11.80	MWaf7	6.90
4	MAfa4	10.60	MWfa4	15.30
5	MAfa6	12.90	MWfa6	13.20
6	MAfa7	19.20	MWfa7	7.20
7	CAaf	10.50	CWaf	5.80
8	CAfa	15.00	CWfa	6.60
9	CAwe	19.10	CWwe	14.90
10	MAwe4	7.20	MWwe4	6.40
11	MAwe6	8.86	MWwe6	6.90
12	MAwe7	10.40	MWwe7	7.80

To evaluate the total sugar concentration in carbohydrates, the Dubois test (phenol-sulfuric acid) is utilized. A simple acid-catalyzed condensation process underpins the reaction mechanism. Sulfuric acid dehydrates sugar to furfural derivatives, which condense with phenol to provide colorful molecules with a 490 nm absorption wavelength.[10] The fucose level in HCl solvent which ranged from 0.69 mg/100g – to 5.95 mg/100g was greater than in water solvent with 0.13 mg/100g – 2.89 mg/100g, as shown in Table-2.

Table-2: The Sulfate and Total Sugar Content of Extract Fucoidan from *S. plagiophyllum.*

No	Sample	Sulfate (%)	Fucose (mg/100g)	Sample	Sulfate (%)	Fucose (mg/100g)
1	MAaf4	2.15	4.66	MWaf4	3.86	0.15
2	MAaf6	2.60	2.33	MWaf6	2.75	1.07
3	MAaf7	3.11	5.95	MWaf7	2.15	1.42
4	MAfa4	0.78	0.84	MWfa4	0.45	1.10
5	MAfa6	2.15	0.79	MWfa6	2.35	0.29
6	MAfa7	6.14	0.69	MWfa7	3.96	0.08
7	CAaf	3.01	1.70	CWaf	1.69	2.89
8	CAfa	3.96	3.54	CWfa	2.85	1.54
9	CAwe	1.79	0.93	CWwe	1.14	1.52
10	MAwe4	2.70	1.03	MWwe4	2.60	0.13
11	MAwe6	1.44	1.86	MWwe6	1.19	1.91
12	MAwe7	3.41	4.66	MWwe7	1.64	0.15

Antioxidant Activities of Extracted Fucoidan

Table-3 shows that extracts of MAfa4, Mwfa4, and CAwe have a very strong antioxidant capacity with IC_{50} (µg/mL) of 6.25, 0.19, and 1.12, respectively. Meanwhile, the comparative ascorbic acid had an IC_{50} of 37.11µg/mL. Antioxidant activity is extremely strong if the IC_{50} value is less than10 µg/mL, and strong when the value is between 10 and 50 (µg/mL).[11] Based on the results, only two extracts namely MWfa4 and CWwe demonstrated strong antioxidant activity, while the other had moderate antioxidant activity, indicating that water solvent was ineffective in extracting fucoidan from *S. plagiophyllum*. In addition, compared to the HCl solvent, the yield, sulfate content, and fucose content using water solvent were all lower. The conventional method for samples CAwe yielded antioxidant activity and the yield was larger than the others, possibly because the extraction was still combined with alginate and the other components. Furthermore, MWfa4 extracted with MAE 450 W for 5 minutes (water solvent) exhibited lower fucose and sulfate concentration but stronger antioxidant activity. This demonstrates that antioxidant activity is determined by a variety of factors rather than a single factor.

Table-3: Antioxidant Activity

No	Sample	IC_{50} (µg/mL)	Sample	IC_{50} (µgmL)
1	MAaf4	21.87	MWaf4	76.44
2	MAaf6	44.26	MWaf6	39.16
3	MAaf7	33.67	MWaf7	49.87
4	MAfa4	6.25	MWfa4	0.19
5	MAfa6	31.46	MWfa6	54.35
6	MAfa7	39.25	MWfa7	55.39
7	CAaf	54.82	CWaf	55.39
8	CAfa	56.77	CWfa	74.02
9	CAwe	1.12	CWwe	32.21
10	MAwe4	91.53	MWwe4	80.72
11	MAwe6	123.58	MWwe6	58.69
12	MAwe7	41.34	MWwe7	65.20
	Ascorbic acid	37.11		

CONCLUSION

The MAE approach with HCl solvent is more effective compared to the conventional method, based on the results of the antioxidant activity, as well as the sulfate and fucose content. Fucoidan from *S. plagiophyllum* has much potential as a natural antioxidant.

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