

SIMPLE EXTRACTION AND MOLECULAR WEIGHT CHARACTERIZATION OF FUCOIDAN FROM INDONESIAN *Sargassum* sp.

(Ekstraksi Sederhana dan Karakterisasi Berat Molekul Fukoidan dari *Sargassum* Sp
Asal Indonesia)

Lukman Junaidi

Balai Besar Industri Agro, Jl. Ir. H. Juanda No. 11, Bogor 16122

E-mail : lukmanjunaidi@gmail.com

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ABSTRAK. *Fukoidan merupakan senyawa polisakarida kompleks yang ditemukan pada alga coklat. Senyawa ini memiliki berbagai sifat biologis untuk pencegahan penyakit seperti anti-viral, anti-tumor, anti-adhesive, menginduksi apoptosis, mengatur sistem imun tubuh dan lain-lain. Ada berbagai metode yang digunakan untuk mengekstrak fukoidan dari alga coklat, seperti menggunakan etanol, aseton, HCl dan gelombang mikro. Penelitian ini bertujuan untuk mengekstraksi dan mengkarakterisasi fukoidan dari *Sargassum* sp. asal Indonesia dengan variabel larutan pengekstrak, suhu ekstraksi dan waktu ekstraksi. Larutan ekstraksi yang digunakan adalah air dan HCl. Suhu yang digunakan adalah 50°C, 70°C dan 100°C pada waktu 1, 2 dan 4 jam. Hasil penelitian menunjukkan bahwa rendemen ekstraksi tertinggi diperoleh dari proses ekstraksi menggunakan air dengan suhu ekstraksi 100°C dan waktu ekstraksi 4 jam. Berat molekul fukoidan yang diekstraksi dengan air sebagian besar memiliki berat molekul 57,77 kDa dan 2,71 kDa, sementara fukoidan yang diekstraksi dengan HCl sebagian besar memiliki berat molekul 46,43 kDa dan 3,38 kDa. Proporsi fukoidan dengan berat molekul tinggi pada ekstraksi dengan air adalah 52,24% sedangkan ekstraksi dengan HCL adalah 44,10%.*

Kata kunci: berat molekul, ekstraksi dan karakterisasi, fukoidan, *Sargassum* sp.

ABSTRACT. *Fucoidan is a complex polysaccharide compounds found in brown algae. Fucoidan exhibits various biological properties for disease prevention. There are various methods used to extract fucoidan from brown algae, such as using ethanol, acetone, HCl and microwave. This research aims to extract and characterize fucoidan from *Sargassum* sp. using simple methods with the variables on extracting solutions, temperature and time of extraction. Extraction solution used were water and HCl. Temperature used was 50°C, 70°C and 100°C using 1, 2 and 4 hours. The result showed that the highest extraction yield was obtained from the extraction by water with 100°C and 4 hours of extraction. Fucoidan extracted by water has molecular weight of 57.77 kDa and 2.71 kDa, while fucoidan extracted by HCl has molecular weight of 46.43 kDa and 3.38 kDa. The proportion of high molecular weight fucoidan extracted by water was 52.24% compared with HCl was 44.10%.*

Keywords: extraction and characterization, fucoidan, molecular weight, *Sargassum* sp.

1. INTRODUCTION

The use of natural active ingredients (bioactive) for the purpose of disease prevention is growing recently. In nature there are quite a lot of bioactive compounds that have the properties as an antitumor. One of the marine natural

products have antitumor bioactive compounds is fucoidan that could be extracted from brown algae (brown seaweed).

Fucoidan, a sulfated polyfucose polysaccharide exhibits biological properties, including antithrombotic, antiviral, anti-

adhesive, and anti-tumor activities, and it plays a role in modulating host immune systems, arresting the cell cycle, and inducing apoptosis (Tokita *et. al.*, 2010). Li *et.al.* (2008) summarized that fucoidan could be distinguish based on the molecular weight of fucoidan. Fucoidan divided into two groups: low and high molecular weight. But the difference between the two categories is not mentioned explicitly.

Fucoidan was found in the intracellular of brown algae (Tokita *et.al.*, 2010). So it is required specific measures such as the use of temperature and solvent extractors for extracting fucoidan. There are several methods that can be used to extract brown algae. Ponce *et. al.* (2003) conducted research to extract fucoidan from *Adenocystis utricularis*. The extraction was conducted with 80% aq ethanol under mechanical stirring at room temperature and then at 70°C (each for 24 hours). The final residue was recovered by centrifugation and then split into three equal fractions that were separately extracted with 800 ml of water, 2% CaCl₂, and HCl (diluted to pH 2). Each extraction was carried out for 7 hours at room temperature. The residues were centrifuged off and re-extracted exhaustively with the same solvents at 70°C, up to the point where only small amounts of sugars were

detected in the extract. The extracts were concentrated at reduced pressure, dialyzed and recovered by freeze-drying.

Rioux *et. al.* (2007) conducted extraction of fucoidan using selective solvents with a constant mechanical stirring between 455 ± 5 rpm. Temperature was controlled using a water bath. First, ethanol 85% was used to extract pigments and proteins. The solvent was separated from residual seaweeds by vacuum filtration. Residual seaweeds were treated with CaCl₂ 2% at 70°C in order to precipitate alginates as well as to extract laminaran and fucoidan from the mixture and then, centrifuged. Fucoidan was extracted from the residual seaweeds with HCl 0.01M, pH 2, at 70°C (3 x 3 hours) and then centrifuged.

Rodriguez-Jasso *et. al.* (2011) was conducted research on extraction of sulfated polysaccharides (fucoidan) from brown seaweed *Fucus vesiculosus* by micro wave assisted extraction (MAE). They concluded that MAE at 120 psi, 1 minute, using 1 g algae/25 ml water was the best condition for the fucoidan recovery and found l-Fucose was the main constituent of this polysaccharide, which also contained xylose and galactose. Several method for extraction of fucoidan from brown algae is presented in Table 1.

Table 1. Several method for extraction of fucoidan from brown algae from literatures

Year of Publish	Inventor/ researcher	Kind of Brown Algae	Methods of Extraction	Percentage of Fucoidan
2003	Ponce <i>et. al.</i>	<i>Adenocystis utricularis</i>	Solvent extraction with 80% aq ethanol at room temperature and then at 70°C (each for 24 hours). The final residue was recovered by centrifugation and then split into three equal fractions that were separately extracted with water, CaCl ₂ 2%, and HCl (pH 2). Each extraction was carried out for 7 h at room temperature.	2.1 – 10.8%
2007	Rioux <i>et. al.</i>	<i>Ascophyllum nodosum</i> ; <i>Fucus vesiculosus</i> ; <i>Saccharina longicruris</i>	Solvent Extration with ethanol 85%, CaCl ₂ (aq), T = 70°C, HCl (aq) 0.01M, T = 70°C	1.1% - 1.4%
2011	Rodriguez-Jasso <i>et. al.</i>	<i>Fucus vesiculosus</i>	Microwave extraction method (applied pressure: 120 psi for 1 minute)	18.22%
2011	Ale <i>et. al.</i>	<i>Sargassum sp</i>	Single-step extraction using HCl	7%
2012	Suresh <i>et. al.</i>	<i>Sargassum plagiophyllum</i>	Sequential extraction, using acetone; acidic water extraction with pH 3 at 60°C for 4 hours; acidic water extraction with pH 11 at 100°C, neutral extraction at 80°C for 24 hours	2 - 26%

The reason to choose water or HCl as an extraction solution is based on the simplicity of the extraction process for conducted by small scale industry. Using water as an extractor also has advantage as become more environmentally friendlier. Ale (2012) stated that typical extraction of Fucose-Containing Sulfated Polysaccharides (FCSPs) from brown seaweed involves a harsh processing condition and several purification steps, that could influence the chemical nature of sulfated polysaccharide. Fewer steps extraction approach minimizes the structural alteration of algae sulfated polysaccharides and, thereby, maintains the natural bioactive characteristics of FCSPs.

Fucoidans extracted by different methods may also have different structures (Ponce *et. al.*, 2003). The common structures of fucoidans extracted from brown algae is presented in Figure 1. Brown algae are found in abundance in Indonesian sea-waters. Based on the result of various researchs as mentioned above it was concluded that brown algae have a potential source for producing of fucoidan. Fucoidan exhibits various biological properties that give advantage to the disease prevention. To investigate the potency for producing fucoidan, this research was conducted with the aims to obtain optimum methods of extracting fucoidan from brown algae (*Sargassum* sp). *Sargassum* sp that collected from Banten area is shown in Figure 2.

A. *Ascophyllum nodosum*/*Fucus vesiculosus*/*Fucus evanescens*

B. *Ecklonia kurome*

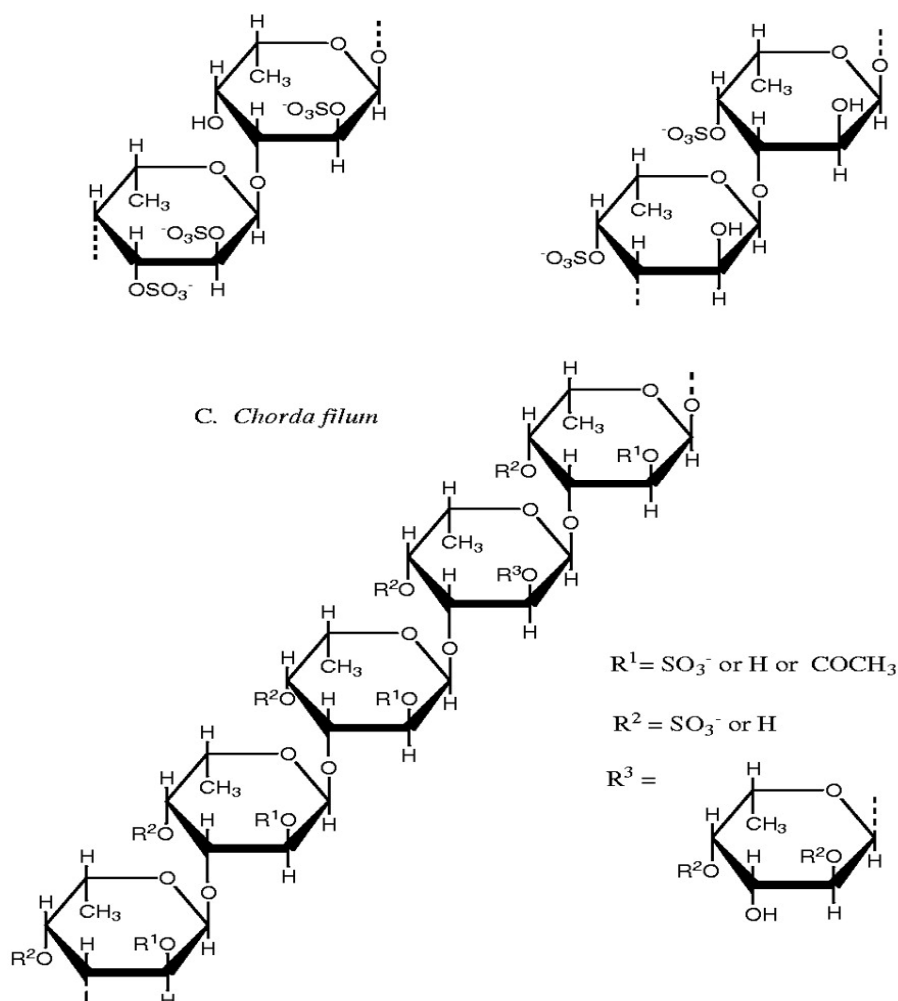


Figure 1. Common structures in fucoidans from brown algae (Berteau and Mulloy, 2003)



Figure 2. Brown algae *Sargassum sp* from Banten area

2. METHODS

The raw material used in this study was brown algae *Sargassum sp* from Banten area. *Sargassum sp* was chosen because it could be found in abundance in Indonesia sea water area (Yulianto, 2002), and it has high fucoidan content (Hennequart, 2004). The chemical used in the extraction of fucoidan was HCl and ethanol 70%. The brown algae *Sargassum sp*. was collected in August 2009 at Banten area.

The equipments used in this study consisted of a set of glassware for extraction process (boiling flask, flask, erlenmeyer, and spiral cooler), scale, plastic ware, oven, and liquid chromatography apparatus equipped with a refractive index detector with G 5000 PW column for the determination of fucoidan molecular weight.

Extraction

The extraction of fucoidan was performed using variation of treatments as follows: (1) variations in extractor solution: water and HCl, (2) variations in temperature of extraction: 50°C, 70°C, and 100°C, and (3) variation in time of extraction: 1, 2, and 4 hours.

Sargassum sp was air dried and milled in to powder prior to extraction by water or HCl solution to produce fucoidan crude extract. The fucoidan crude extract was then centrifuged at a speed of 3000 rpm for 10 minutes. Sediment separated and the liquid part (filtrate) was added ethanol 70% as with the same amount of

the liquid part until a precipitate is formed. Centrifugation was then performed once again to separate sediment from the liquid. The final precipitate was separated and dried, and analysed for fucoidan content.

Analytical Methods

Determination of relative molecular weight of fucoidan in samples taken in this study is a modification of the method used by Holtkamp (Holtkamp, 2009). The type of column and oven temperature used by Holtkamp is not the same as those used in this study, so it is necessary to modify the method used by Holtkamp. Determination of relative molecular weight of fucoidan performed through Gel Permeation Chromatography using a refractive index detector. For determination of the molecular weight markers is used dextran sulfate (10 kDa, 40 kDa, and 70 kDa). Conditions of analysis were as follows: mobile phase 0.01 M LiNO₃ solution set at a flow rate of 1 ml/minutes, elution time of 20 minutes and the amount of sample injected is 20 µl. Fucoidan extraction yield (% Fuc), were calculated according to Equation (1).

$$\% \text{ Fuc} = W_{\text{MOH}} / W_A \times 100 \dots\dots\dots (1)$$

where W_{MOH} is the dry mass weight obtained after extraction; W_A is the alga weight used in this experiment (Rodriguez-Jasso *et al*, 2011).

3. RESULT AND DISCUSSION

Molecular Weight Characterization of Fucoidan

Characterization of fucoidan was based on the molecular weight (MW) analysis. The importance of characterization of fucoidan is because the biological effects of fucoidan depended on the molecular weight, sulfate content, and sugar constituents (Chevolot *et. al.*, 1999). They suggested that the large difference in molecular weight may also be caused by the difference of the extraction conditions because the fucoidan is not so stable against heat or acid. Cumashi *et. al.*

(2007) mentioned that differences in the MW of various fucoidan preparations influence their bioactivity.

Determination of the fucoidan MW is conducted by Gel Permeation

Chromatography, using standar 10 kDa, 40 kDa and 70 kDa. The chromatograph of standar used for Molecular Weight analysis is presented in Figure 3.

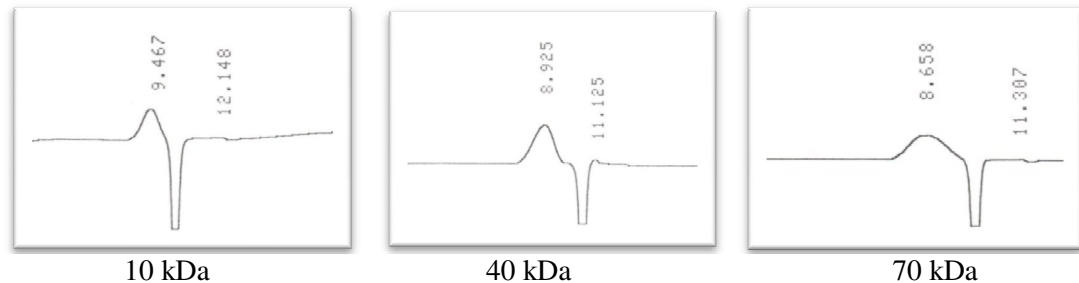


Figure 3. Chromatograph of standar used for Fucoidan MW analysis

Fucoidan MW is calculate by preparing the curve (graph) that show the relation between the MW standar substances with retention times (t_R). Based on the chromatograph result, the equation to estimate the MW of fucoidan was formulated, as shown in Figure 4.

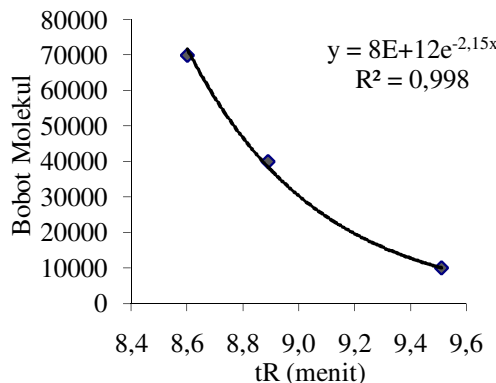


Figure 4. Correlation between t_R and Fucoidan MW

The result of chromatograph of fucoidan extracted from *Sargassum* sp is presented in Figure 5.

From the graphs on Figure 5, it could be seen that there are several peaks area that correspond to the MW of fucoidan extract, compare to the standar used in the Gel Permeation Chromatography analysis. For example, peak area 8.74 (in the chromatograph of fucoidan extracted by water) indicated the MW of fucoidan was 57.7 kDa and represent 29.9% of the total fucoidan

extracted. While peak area 8.81 (in the chromatograph of fucoidan extracted by HCl) indicated the MW of fucoidan was 46.43 kDa and represent 26.88% of the total fucoidan extracted. The complete result of fucoidan MW analysis is presented in Table 2.

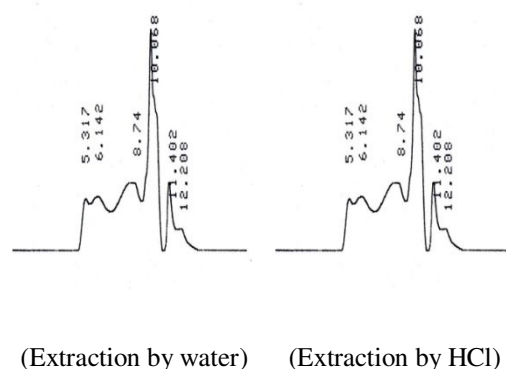


Figure 5. Chromatograph of fucoidan MW analysis

From Table 2 it could be seen that fucoidan extracted by water mostly produced fucoidan with MW 57.77 kDa and 2.71 kDa, while fucoidan extracted by water mostly produced fucoidan with MW 46.43 and 3.38 kDa.

Fucoidan extracted by water give the higher proportion of high molecular weight (HMW) fucoidan compare to fucoidan extracted by HCl. There were 52.24% HMW fucoidan in the product extracted by water compare to only 44.10% HMW fucoidan in the product extracted by HCl.

On the other hand, proportion of low molecular weight (LMW) fucoidan is lower in the product extracted by water compare to fucoidan extracted by HCl. There are 47.75% LMW fucoidan in the product extracted by water compare to 55.90% LMW fucoidan in the product extracted by HCl. From this data it could be assumed the fucoidan extracted by HCl tend to produce a LMW fucoidan compare to fucoidan extracted by water.

Table 2. The result of analysis of fucoidan MW and the percentage in the product extracted

Fucoidan Extraction by water	
Molecular Weight (kDa)	Proportion (%)
97185.69	6.44
16926.49	15.89
57.77	29.91
2.71	36.28
0.16	7.46
0.03	4.01
Fucoidan Extraction by HCl	
Molecular Weight (kDa)	Proportion (%)
97185.69	2.19
10934.73	15.03
46.43	26.88
3.38	41.83
0.13	8.26
0.09	5.83

Nishino *et. al.* (1991) mentioned that HMW Fucoidan (*i.e.* 27 and 58 kDa) showed higher anti-coagulant activity than LMW Fucoidan (*i.e.* ≤ 10 kDa). Chandia and Matsuhira (2008) concluded that HMW fucoidan is more suitable as anti-coagulant, and Li *et. al.* (2008) stated that HMW fucoidan is more suitable as anti-tumor and immuno-modulatory activity, anti-coagulant and anti-thrombotic. While Zhao *et. al.* (2005) reported that LMW fucoidan is more suitable as antioxidant, and Li *et. al.* (1999) stated that LMW fucoidan could be used for reducing blood lipids. Song *et. al.* (2012) mentioned that LMW Fucoidan obtained by acid hydrolysis of HMW fucoidan showed more favorable bioactivity.

Based on the information mentioned

above, it could be assumed that the fucoidan extracted by water (in this research) is more suitable as antitumor and immuno-modulatory activity, anti-coagulant and anti-thrombotic activity. On the other hand the fucoidan extracted by HCl is more suitable as an anti-oxidant and reducing blood lipids agent.

Fucoidan Extraction

Fucoidan was extracted from *Sargassum sp.* using water or HCl as an extractor solution. The temperature of fucoidan extraction was set for 50, 70, and 100°C and the time of extraction was chosen 1, 2, and 4 hours. The result of extraction is presented in Table 3.

Table 3. The result of fucoidan extraction as a function of solvent extractors, duration of extraction time, and extraction temperature

Duration of extraction (hours)	Extraction temperature (°C)	Yield (%) by type of solvent extractors	
		Water	HCl 0,1 N
4	100	4.1	3.3
2	100	2.95	2.7
1	100	2.35	2.30
	70	2.01	1.30
	50	1.38	1.04

Influence of the type of extractor solution to the yield of extraction

From Table 3 it could be evaluated the influence of the type of extractor solution to the yield of extraction. Based on data in Table 3, it could be seen that the highest extraction yield was 4.1% resulted by water extraction, while extraction by HCl only produced maximum 3.3% yield. The yield produced by water extraction was higher compare to the yield produced by HCl extraction, in all of the temperature extraction and duration of extraction.

In general, extraction by HCl solution will give the lower yield when compared to the extraction by water. *Sargassum sp* contain not only fucoidan, but it also includes other compounds such as alginate (Fitton, 2003). The impurities in the product extracted by water was

higher compare to product extracted by HCl.

Influence of the temperature of extraction to the yield of extraction

The trend of extraction temperature influence (with the duration of extraction constant 1 hour) is presented in Figure 6. The figure shows the increasing of extraction yield, for both of the solvents used, is in line with the increasing of temperature of extraction when the duration of extraction was constant 1 hour.

From Figure 6 it could be seen that for the lower extraction temperature (50°C) the yield of extraction by water was higher than the the yield of extraction by HCl. The maximum difference between the yield of extraction by water and by HCl will achieve at the extraction temperature 70°C. However if the extraction temperature increasing to 100°C, the yield of extraction by water is the same with the yield of extraction by HCl.

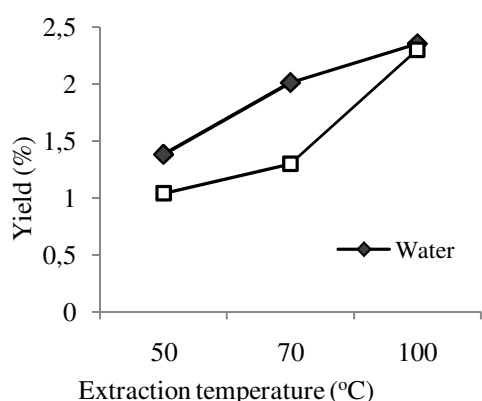


Figure 6. The trend of influence temperature of extraction to yield of fucoidan extraction

From Figure 6, it could be seen for the extraction by water, the increasing rate of yield of extraction is higher when the temperature was increased from 50°C to 70°C, compare to the increasing of temperature from 70°C to 100°C, provided the duration of extraction is kept constant 1 hour. In contrary, for the extraction by HCl, the increasing rate of yield of extraction is higher when the temperature was increased from 70 °C to 100 °C,

compare to the increasing of temperature from 50°C to 70°C.

In general it could be assumed that the use of higher temperatures will resulted in the higher of the extraction yield. The higher temperature of extraction will facilitate the rupture of the cell wall so that the substances contained in it will be easier to come out.

Influence of duration of extraction to the yield of extraction

The trend of influence of extraction duration to the yield of fucoidan extraction is presented in Figure 7. The extraction was conducted using water or HCl at the extraction temperature constant at 100°C. The duration of extraction was chosen 1, 2 and 4 hours. The figure shows the increasing of yield is in line with the increasing of extraction time.

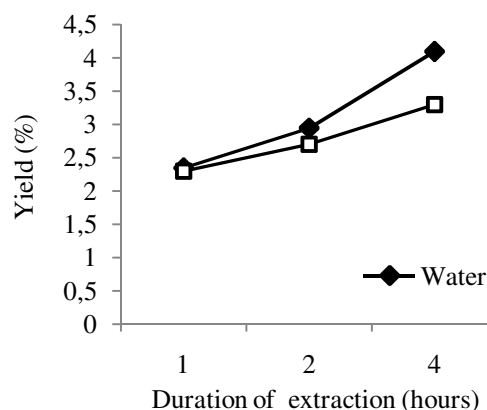


Figure 7. The trend of influence of extraction duration to the yield of fucoidan extraction

From the Figure 7, it could be seen that the yield of extraction, either by water or by HCl, is the same on 1 hour extraction time. When the time of extraction was doubled from 1 hour to 2 hours, the yield of extraction increase from 2.35% to 2.95% (extraction by water) and 2.3% to 2.7% (extraction by HCl). The increasing of the yield of extraction will be more significant if the extraction time was doubled from 2 hours to 4 hours. The slope of increasing of the extraction yield is sharper when fucoidan extracted by water compare to the slope when fucoidan

extracted by HCl. So it could be assumed that the influence of extraction time is more significant to the fucoidan extraction by water than fucoidan extraction by HCl. As a general it could be concluded that for both solvent used, the longer the extraction time will resulted in the higher the extraction yield of extraction.

4. CONCLUSION

The yield of fucoidan extraction process is influenced by the type of solvent extractors, duration of extraction and extraction temperature. The yield of extraction by water produced the higher result compare to the extraction by HCl. The higher the temperature of extraction and the longer the extraction time, for both of the solvent used, resulted in the higher of the extraction yield. The highest yield of the extraction obtained from the extraction by water with a temperature of 100°C and extraction time 4 hours.

Fucoidan extracted by water mostly has molecular weight of 57.77 kDa and 2.71 kDa, while fucoidan extracted by HCl mostly has molecular weight of 46.43 kDa and 3.38 kDa. Fucoidan extracted by water tend to produce a higher proportion of HMW fucoidan compare to fucoidan extracted by HCl. The proportion of HMW fucoidan in the product extracted by water was 52.24% compare to 44.10% in the product extracted by HCl.

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