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EFFECT OF CaCl$_2$ CONCENTRATION ON THE PHYSICOCHEMICAL AND STRUCTURAL CHARACTERISTICS OF CRUDE FUCOIDAN OF *Sargassum* sp.

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Abstract

Fucoidan is a sulfated polysaccharide derived from brown seaweed. Fucoidan exhibits various biological activities and potential therapeutic applications. The extraction method and conditions determine the yield and characteristics of the fucoidan products. This study aimed to evaluate the physicochemical characteristics and structure of crude fucoidan extracted using various concentrations of CaCl$_2$. Crude fucoidan was extracted using CaCl$_2$ solutions with concentrations of 2, 3, and 4 M. Parameters analyzed included yield, moisture, ash, pH, color, and functional groups using Fourier Transform Infrared Spectroscopy (FTIR). The results showed that extraction using 2 M CaCl$_2$ produced the highest yield (8%) and moisture (13.3%). Extraction using 4 M CaCl$_2$ showed the highest absorption intensity for sulfate and hydroxyl groups, as indicated by the FTIR spectrum. The ash content did not differ significantly between the treatments and ranged from 0.50–0.57%. The pH of the extract was in the neutral range, namely 7.01–7.11. Color analysis showed that increasing the CaCl$_2$ concentration produced a greenish-brown color. Extraction using 3 M CaCl$_2$ resulted in the highest brightness (L*=25.31) and lowest redness (a*=−0.56). FTIR analysis confirmed the presence of hydroxyl, carbonyl, sulfate ester, and glycosidic bonds as characteristic features of the fucoidan structure. This study concluded that the concentration of CaCl$_2$ affects the yield and structural characteristics of crude fucoidan. The selection of CaCl$_2$ concentration needs to be adjusted to the utilization objectives, both to obtain high yields and to improve the characteristics of sulfate groups that have the potential to support bioactivity.

Keywords: brown seaweed, color, infrared spectroscopy, sulfated polysaccharide, yield

Pengaruh Konsentrasi CaCl$_2$ terhadap Karakteristik Fisikokimia dan Struktural Fukoidan Kasar *Sargassum* sp.

Abstrak

Fukoidan merupakan polisakarida sulfat yang berasal dari rumput laut cokelat. Fukoidan memiliki berbagai aktivitas biologis serta potensi aplikasi terapi. Metode dan kondisi ekstraksi menentukan rendemen dan karakteristik fukoidan yang dihasilkan. Penelitian ini bertujuan untuk mengevaluasi karakteristik fisikokimia dan struktur fukoidan kasar hasil ekstraksi menggunakan CaCl$_2$ pada konsentrasi berbeda. Fukoidan kasar diekstraksi menggunakan larutan CaCl$_2$ dengan konsentrasi 2, 3, dan 4 M. Parameter yang dianalisis meliputi rendemen, kadar air, kadar abu, pH, warna, dan gugus fungsi menggunakan Fourier Transform Infrared Spectroscopy (FTIR). Hasil penelitian menunjukkan bahwa ekstraksi menggunakan CaCl$_2$ 2 M menghasilkan rendemen tertinggi sebesar 8% dan kadar air tertinggi sebesar 13,3%. Ekstraksi menggunakan CaCl$_2$ 4 M menunjukkan intensitas serapan tertinggi pada gugus sulfat dan hidroksil

berdasarkan spektrum FTIR. Kadar abu tidak berbeda nyata antar perlakuan dan berkisar antara 0,50–0,57%. Nilai pH ekstrak berada pada kisaran netral, yaitu 7,01–7,11. Analisis warna menunjukkan bahwa peningkatan konsentrasi CaCl₂ menghasilkan warna cokelat kehijauan. Ekstraksi menggunakan CaCl₂ 3 M menghasilkan tingkat kecerahan tertinggi (L*=25,31) dan tingkat kemerahan terendah (a*=−0,56). Analisis FTIR mengonfirmasi keberadaan gugus hidroksil, karbonil, ester sulfat, dan ikatan glikosidik sebagai ciri khas struktur fukoidan. Penelitian ini menyimpulkan bahwa konsentrasi CaCl₂ memengaruhi rendemen dan karakteristik struktur fukoidan kasar. Pemilihan konsentrasi CaCl₂ perlu disesuaikan dengan tujuan pemanfaatan, baik untuk memperoleh rendemen tinggi maupun untuk meningkatkan karakteristik gugus sulfat yang berpotensi mendukung aktivitas bioaktif pada industri farmasi dan kosmetik.

Kata kunci: polisakarida sulfat, rendemen, rumput laut cokelat, spektroskopi inframerah, warna

INTRODUCTION

Sargassum sp. is a brown seaweed that belongs to the Sargassaceae family. This marine macroalga has a branched thallus and blade-like leaves and comprises approximately 400 species worldwide, primarily found in shallow marine meadows (Rushdi *et al.*, 2020; Joung *et al.*, 2016). *Sargassum* sp. contains several bioactive compounds, such as fucoidan, alginate, and polyphenols, which exhibit a wide array of pharmacological activities (Yende *et al.*, 2014; Rushdi *et al.,* 2020; Lee *et al.*, 2022; Manteu *et al.*, 2021). In Indonesia, 12 species of *Sargassum* sp. have been studied, including *S. duplicatum, S. hystrix, S. echinocarpum, S. binderi Sonder, S. gracilinum, S. crassifolium, S. obtusifolium, S. policystum, S. vulgare,* and *S. polyceratium.* Furthermore, bioactive compounds found in Sargassum, such as fucoidan, have shown potential in cosmeceutical applications, particularly in skincare, owing to their antioxidant, anti-inflammatory, and moisturizing properties (Fernando *et al.*, 2018; Lee *et al.*, 2022; Kang *et al.*, 2023).

Fucoidan, a sulfated polysaccharide derived mainly from brown seaweeds, has garnered significant interest because of its diverse biological activities and potential therapeutic applications (Saeed *et al.*, 2021; George & Shrivastav, 2023; Jeong *et al.,* 2024). It contains substantial percentages of l-fucose and sulfate groups, which contribute to its unique bioactive properties as an antioxidant, anticancer agent, and immunomodulator that can be utilized as raw materials in the food, pharmaceutical, and cosmetic industries (Mabate *et al.*, 2021; Lin *et al.*, 2020; Dörschmann *et al.*, 2019).

The development of appropriate extraction and purification technologies is essential for optimizing the content of bioactive compounds, as various extraction methods can influence their structure and bioactivity. Another challenge is the high cost associated with raw materials and the extraction process, given that the availability and price of seaweed raw materials can fluctuate. Several studies have reported varying yields and purities of fucoidan, contingent upon extraction conditions such as temperature, solvent type, and concentration. A study on the extraction of fucoidan from *Ecklonia radiata* indicated that conventional extraction conditions using hydrochloric acid at 60°C can achieve high yields up to a certain temporal threshold, beyond which the yield plateaus and the purity diminishes due to the co-extraction of contaminants such as laminarin and a reduction in the molecular weight of fucoidan (Lorbeer *et al.*, 2014). The solvent and extraction techniques significantly affected yield and purity. Ultrasonic extraction using deep eutectic solvents was used to extract polysaccharides from *Sargassum horneri*. This method was green, fast, and effective, outperforming conventional hot water extraction in terms of protein and calcium carbonate removal. The optimized extraction conditions involve a specific molar ratio of components, moisture content, solid-liquid ratio, and temperature, which demonstrate the critical role of each variable in achieving a high yield and purity (Nie *et al.*, 2020; Oliyaei & Moosavi-Nasab, 2021). Enzyme-assisted extraction has been shown to be a promising method because it can enhance the yield of fucoidan and preserve its structure.

For instance, celluclast-assisted extraction from *Undaria pinnatifida* resulted in a high yield and sulfate-rich fucoidan composition, which exhibited potent antioxidant activities (Oh *et al.*, 2020). The purification of fucoidan from crude extracts involves several advanced techniques that aim to enhance the quality and yield of the purified fucoidan, such as ion exchange chromatography (Sanniyasi *et al.*, 2019), a combination of hydrothermal-assisted extraction followed by a three-step purification process (Rajauria *et al.*, 2023), and ultrasound-assisted depolymerization, which resulted in a reduction in the antioxidant activity of the extract compared to its crude counterpart (Ummat *et al.*, 2024). Additionally, the extraction technique for fucoidan requires complex stages and expensive solvents and chemicals, such as calcium chloride (CaCl$_2$).

The use of CaCl$_2$ as a solvent for extracting fucoidan from brown algae offers several advantages in terms of separation and purification. The presence of CaCl$_2$ facilitates the removal of undesirable components, such as alginates, which are commonly co-extracted during fucoidan extraction from algae, thereby improving the purity of the extracted fucoidan. In a study examining *S. cristaefolium*, fucoidan was effectively extracted using an acidic extraction method. During this process, CaCl$_2$ was used in the precipitation step to facilitate the separation of alginates from fucoidan. The use of CaCl$_2$ allowed for cleaner extraction, enhancing the purity of the obtained fucoidan (Isnansetyo *et al.*, 2015). Similarly, in a study focused on *Cystoseira myrica*, CaCl$_2$ was used as part of a multi-step extraction process aimed at purifying fucoidan from other polysaccharides. Calcium chloride treatment was effective in avoiding the co-extraction of other polysaccharides, which is crucial for obtaining high-quality fucoidan extracts (Dhahri, 2023).

Consequently, incorporating CaCl$_2$ into the extraction process can significantly enhance the selectivity of fucoidan isolation and improve the overall quality of the extract by minimizing impurities. Such modifications to the extraction process are crucial for obtaining fucoidans that meet the purity standards required for potential therapeutic or industrial applications (Dhahri, 2023; Isnansetyo *et al.*, 2015). Previous research found that the crude fucoidan extract obtained using 2 M CaCl$_2$ had a higher yield (12%) than that obtained using 4 M CaCl$_2$ (5%). This is in contrast to other studies, where different extraction methods have also shown varying yields. For example, a study highlighted that enzyme-ultrasonic extraction from *Nizamuddinia zanardinii* provided a yield of 7.87%, whereas ultrasound-assisted extraction of fucoidan from *S. muticum* showed a yield ranging from 26% to 31% (Imbs *et al.*, 2014; Rodrigues *et al.*, 2015; Alboofetileh *et al.*, 2019; Flórez-Fernández *et al.*, 2017). Husni *et al.* (2022) posited that adjusting the solvent concentration constitutes an effective strategy for optimizing the extraction process in accordance with the desired characteristics of fucoidan. This study aimed to evaluate the physicochemical and structural characteristics of crude fucoidan extracted using different CaCl$_2$ concentrations, based on yield, physicochemical properties, and functional groups. This research is an important initial step in producing fucoidan extract by determining the concentration of CaCl$_2$, in addition to other technical steps such as precipitation time, extraction temperature, and the ratio of seaweed powder to water.

MATERIALS AND METHOD
Seaweed Flour Making

Seaweed flour was prepared according to the method described by Puspantari *et al.* (2020), with modifications. Briefly, dried *Sargassum* sp. seaweed from the waters of South Sulawesi, Makassar, Indonesia, was washed with distilled water to remove sand and dirt, cut into smaller pieces, and then drained and dried in a cabinet dryer at 50°C for 18 h. The moisture content of the dried seaweed was determined to be less than 4%. The seaweed was ground until smooth and filtered using a 100-mesh sieve to obtain the brown seaweed flour.

Crude Fucoidan Extraction

The crude fucoidan extraction process was based on the method described by Nguyen *et al.* (2020). Seaweed flour (150 g)

was added to distilled water (1:20) (b/v). The mixture was extracted for 4 h at 85°C while stirring. The mixture was filtered through a 500-mesh planktonic filter, and the filtrate was collected for further analysis. The filtrate was added to a $CaCl_2$ solution at three different concentrations, namely 2 M, 3 M, and 4 M, and stirred for 30 min at room temperature. After centrifugation at 8,000 rpm for 15 min at 4°C, the filtrate was collected, and the precipitate was discarded. Ethanol (Pro-Analysis, SMARTLAB) (ratio 1:2 for seaweed and ethanol) was then added to the filtrate, stirred, and allowed to stand overnight at room temperature. The solution was centrifuged at 6,000 rpm at room temperature for 15 min, the precipitate was collected, and the filtrate was discarded to obtain crude fucoidan.

Yield of Crude Fucoidan

The yield was calculated as the ratio of the weight of the crude fucoidan extract to the weight of the seaweed. Yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{weight of fucoidan extracted (g)}}{\text{weight of raw seaweed (g)}} \times 100$$

Moisture

Moisture content testing was performed using the oven method described by the AOAC (2005). The process started by drying an empty cup in an oven for 15 min, cooling in a desiccator for 30 min, and weighing (A). Two gram sample (B) was placed in a dish and heated in an oven at 105-110°C for 4 h. After heating, the cup was cooled in a desiccator and weighed (C). The moisture content was calculated using the following equation:

$$\text{Moisture (\%)} = \frac{C-B}{C-A} \times 100$$

Ash

The determination of ash content was performed according to AOAC (2005). The porcelain cup was cleaned, dried, and heated at 550°C for one hour to ensure that there were no contaminants, cooled in a desiccator, and weighed to a constant weight (W1). The weighed samples were then placed in a cup

and dried at 105°C to remove moisture (W2). Subsequently, the samples were burned in a furnace at a temperature of approximately 550 °C for 4–6 h until all the organic matter was burned out and only grayish-white ash remained. The cup containing the ash was cooled in a desiccator before weighing (W3). The ash content was calculated using the following equation:

$$\text{Ash (\%)} = \frac{W3-W1}{W3-W2} \times 100$$

pH

A sample of powdered crude fucoidan was mixed with 10 mL of distilled water. The samples were then homogenized using a glass stirrer. pH measurements were performed using a pH meter. The calibration process was completed when the pH value shown on the screen was in accordance with the neutral and stable pH value, and the electrode was then dipped into the sample. The pH values displayed on the screen were noted. Measurements were performed at room temperature (Nguyen *et al.*, 2020).

Color Analysis

The color testing process in digital color processing encompasses several stages. Initially, the images were captured using a 50×50×50 cm³ box equipped with light-emitting diode (LED) lights. The box had a white background, and the samples were positioned within it. Photographs of crude fucoidan samples at varying concentrations were taken using an iPhone XR (Goñi & Salvadori, 2016). Before capturing the images, white balance calibration (using a white standard) and a standard color reference, such as a Color Checker, were performed. This step is crucial for normalizing the images and ensuring that the color measurements are consistent and independent of lighting variations, as previously conducted by Cugmas and Struc (2020).

The camera was positioned 3 cm from the samples. Subsequently, the images were cropped to remove extraneous parts to facilitate the analysis. Image conversion was performed using three components: red, green, and blue components. This image processing method

aims to achieve homogeneous datasets. The results were converted into L* (lightness), a* (redness), and b* (yellowness) color models.

Functional Group by Fourier Transform Infra-Red (FTIR)

Crude fucoidan (2 mg) was ground evenly with 100 mg KBr until the particle size was <2.5 μm. Transparent KBr pieces were prepared at a pressure of 500 kg/cm^2 for FTIR spectroscopy. Fourier-transform infrared (FTIR) spectra were obtained using an FTIR Cary 630 ATR Diamond instrument (Agilent Technologies). Signals were collected automatically using 60 scans over the 4,000-400 cm^{-1} range at a resolution of 16 cm^{-1} and compared with the background spectra collected from KBr at room temperature (Yu *et al.*, 2021).

Data Analysis

This study used a Completely Randomized Design, where all data from three replicates were analyzed using GraphPad Prism 10.1.0. Data pertaining to the yield, moisture content, ash content, color, and pH were assessed for normality and homogeneity of variance. If the data were normally distributed and homogeneous, they were subjected to analysis of variance (ANOVA), followed by the Honestly Significant Difference (HSD) test to ascertain the differences.

RESULTS AND DISCUSSIONS
Physicochemical Characterization of Crude Fucoidan

Physicochemical characterization of crude fucoidan involves a series of analytical techniques aimed at elucidating its structural features, composition, and functional properties. Table 1 shows that the variation in CaCl$_2$ concentration during the extraction process had an impact on the yield of crude fucoidan ($p<0.05$). The crude fucoidan extract yields for each CaCl$_2$ concentration were 8, 4.7, and 3.4%, respectively. As the CaCl$_2$ concentration increased, the yield of crude fucoidan extract decreased proportionately. Similarly, the moisture contents of crude fucoidan extracts 2, 3, and 4 M were 13.3%, 11.6%, and 8.01%, respectively. The concentration of CaCl$_2$ did not alter the ash content in the crude fucoidan extracts ($p>0.05$). The pH levels of the extracts varied with different CaCl$_2$ concentrations, especially between results 2 and 3 M. However, there was no difference between 3 and 4 M.

The concentration of CaCl$_2$ significantly affects the yield of crude fucoidan extracted from brown seaweed. Elevated CaCl2 concentrations were correlated with a reduction in fucoidan yield. The yield of fucoidan in this study was high because the use of molar concentration determined the extraction conditions that could optimize the yield. As explained previously, in the context of fucoidan extraction, variations in the CaCl$_2$ concentration in molar units determine the number of Ca^{2+} ions available to interact with the sulfate and carboxylate groups on the fucoidan molecule (Hifney *et al.*, 2016; Brown *et al.*, 2018; Nguyen *et al.*, 2020). This interaction affects the precipitation efficiency and extract quality. In contrast, excessively high CaCl$_2$ concentrations can decrease the yield of crude fucoidan extract. This is due to the possibility of co-precipitation or coagulation of other unwanted compounds

Table 1 The physicochemical characterization of crude fucoidan

Sample treaments (M)	Yield (%)	Moisture (%)	Ash (%)	pH
2	8.00±0.08[c]	13.28±0.07[b]	0.54±0.02[a]	7.02±0.02[a]
3	4.70±0.28[b]	11.57±0.08[b]	0.57±0.02[a]	7.12±0.02[b]
4	3.35±0.18[a]	8.01±0.01[a]	0.50±0.01[a]	7.08±0.03[b]

The means of the three replicate groups are reported alongside their standard deviations; Statistically significant differences for each parameter were identified using the honestly significant difference test at a significance level of $p<0.05$, as denoted by distinct lowercase letters within the same column.

and the potential degradation of the fucoidan structure due to excessive calcium ion interactions.

The moisture content of the crude fucoidan extract is closely related to the strength of CaCl$_2$ ions, which affects the hydrophilic or hygroscopic nature of the polysaccharide extract. In the presence of CaCl$_2$, the water-binding capacity may be altered, influencing the final moisture content of the extracted crude fucoidan. In the present study, crude fucoidan 4M had the lowest moisture content. This indicates that CaCl$_2$ has hygroscopic properties. Therefore, the manipulation of ionic concentration and temperature during extraction is crucial for tailoring the extraction to obtain fucoidan with the desired properties and minimum moisture levels, facilitating its downstream processing and handling (Nguyen *et al.*, 2020).

In the present study, no differences were observed in the ash content of crude fucoidan ($p > 0.05$). The ash content of crude fucoidan was 0.54%, 0.57%, and 0.50% for 2, 3, and 4 M, respectively. This indicates that the concentration of CaCl$_2$ used in this study did not significantly affect the composition and purity of the final product. The use of CaCl$_2$ in the extraction process, particularly for separating alginate from fucoidan, has been extensively documented. For instance, in one study, CaCl$_2$ was used during the extraction procedure to precipitate alginate, allowing the production of a 2.75% fucoidan product (Isnansetyo *et al.*, 2015). Generally, the molarity of CaCl$_2$ used in such processes can affect the ash content of crude fucoidan. A higher concentration of CaCl$_2$ may lead to a more effective removal of impurities, such as alginate, which can contribute to the ash content. In studies where CaCl$_2$ is used to eliminate alginate, the resulting fucoidan generally exhibits a reduced ash content due to the minimal presence of co-extracted substances. This is crucial because the ash content serves as an indicator of inorganic materials that are not part of the desired fucoidan (Nguyen *et al.*, 2020). The lack of standardization in reporting extraction properties and composition, such as ash content, continues to pose challenges in comparing fucoidans across studies. Efforts are underway to standardize extraction techniques and reporting methods to enhance the reproducibility and comparability of fucoidan research (Chadwick *et al.*, 2025).

Therefore, adjusting the molarity of CaCl$_2$ is critical for optimizing the extraction process to enhance fucoidan purity while reducing the ash content. This optimization is necessary to ensure that the fucoidan extract is of high quality for its intended applications, such as in nutraceuticals and other health-related products, where efficacy and safety are paramount (Nguyen *et al.*, 2020).

The CaCl$_2$ concentration had a slight effect on the pH of the crude fucoidan extract in this study ($p < 0.05$), where 2 M was significantly different from 3 and 4 M, while there was no significant difference between 3 and 4 M. As shown in Figure 1, the pH of the crude fucoidan extract was almost the same at 7.01, 7.11, and 7.08 for samples 2, 3, and 4 M, respectively, indicating the stability of the pH of the extracted crude fucoidan. Fucoidan, a sulfated polysaccharide derived from brown algae, contains acidic sulfate ($-SO_3H$) and carboxylate($-COOH$) groups. When the concentration of CaCl$_2$ is increased, Ca^{2+} ions interact with the negative groups on fucoidan, especially sulfate and carboxylate groups, resulting in the neutralization of the negative charge. In the present study, the pH of the crude fucoidan extract was neutral. This supports the findings of Salehi *et al.* (2019), who reported that the interaction between Ca^{2+} ions and phosphate or carboxylate groups could reduce the ionization of acidic groups on fucoidan, causing the pH of the solution to increase due to the decrease in the number of free H$^+$ ions in the solution. Fucoidan, a sulfated polysaccharide with acidic groups, may interact with calcium ions when CaCl$_2$ is added to its solution, affecting the pH. Nevertheless, the pH of fucoidan solutions is generally stable under a wide range of conditions (Zayed *et al.*, 2016). Furthermore, the pH of the fucoidan extract in this study was higher than that of the crude fucoidan extract using a Box-Behnken Design (BBD) from El-Sheekh *et al.* (2024), which was 5.00 and 6.38.

Color of Crude Fucoidan

Different molar concentrations of $CaCl_2$ can lead to variations in the precipitation of the extracted fucoidan, potentially affecting its color. The visualization of the extracted crude fucoidan in a recent study is shown in Figure 1. The color quantification using MATLAB is presented in Table 2.

It is important to note that while fucoidan is extracted from pigmented brown seaweeds, the fucoidan compound itself does not possess a color pigment. The brown coloration in seaweeds is primarily due to other pigments, such as fucoxanthin, but these pigments are not part of the fucoidan molecule (Li *et al.*, 2008). Figure 1 shows the color of the fucoidan crude extract, which was dark to greenish-black and paste-shaped after precipitation with $CaCl_2$. As the $CaCl_2$ concentration increased, the color of the fucoidan extract changed from a solid greenish-black to a greenish-brown. Overall, both heating and phenolic content are crucial in influencing pigment degradation and the resultant color change during fucoidan extraction. Currently, there is no conclusive information regarding the color of crude fucoidan extract, considering various factors such as extraction techniques, seaweed origin, and additives used in the process. (Nguyen *et al.*, 2020). The color visualization in Figure 1 is supported by the color data obtained using the Lab system, as listed in Table 2.

Table 2 illustrates that the *a* (redness) value became increasingly positive with increasing 4 M CaCl₂ concentrations, indicating a shift in color towards green. The extraction of *Sargassum* sp. with 3 M CaCl₂ exhibited the most negative a* value (-0.56), followed by samples 2 M (-0.47) and C (-0.28). This variation can be attributed to the role of calcium ions (Ca²⁺) in fucoidan precipitation. At the optimal CaCl₂ concentration (3 M), Ca²⁺ ions facilitate the binding of the sulfate group, forming a more stable complex, thereby reducing pigment retention and resulting in a clearer or greenish hue. Conversely, in samples treated with 4 M CaCl₂, an excess of Ca²⁺ ions may lead to excessive coagulation or non-selective binding to other components, causing residual pigments to remain trapped in the matrix and produce a brownish or greenish-yellow color (Xing *et al.*, 2013).

The highest L* value was observed in sample 3 M (25.31), indicating that the crude extraction results were brighter than those of the treatment samples 2 M (23.05) and 4 M
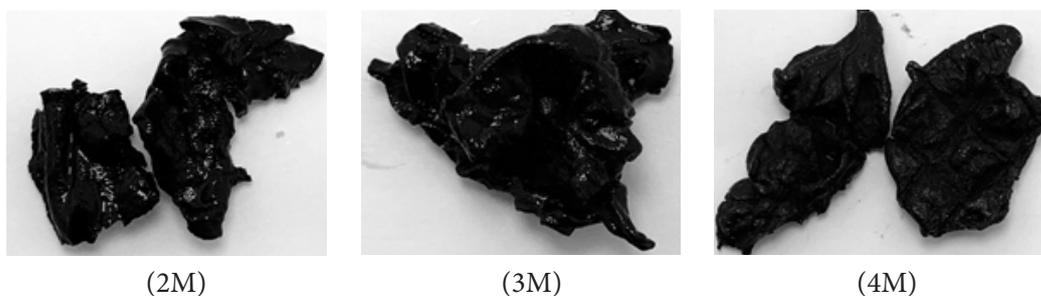

(2M)　　　　　　(3M)　　　　　　(4M)

Figure 1 The visualization of extracted crude fucoidan in the study

Table 2 The color of crude fucoidan

Sample treaments (M)	L*	a*	b*
2	23.05±0.07[a]	-0.47±0.01[a]	-4.20±0.02[a]
3	25.31±0.04[b]	-0.56±0.01[b]	-4.44±0.01[b]
4	24.31±0.03[c]	-0.28±0.03[c]	-5.14±0.02[c]

The means of the three replicate groups are reported alongside their standard deviations; Statistically significant differences for each parameter were identified using the honestly significant difference test at a significance level of $p<0.05$, as denoted by distinct lowercase letters within the same column.

(24.31). The increase in L* in sample 3 M is likely due to the optimal precipitation of fucoidan, which allows for more efficient removal of contaminants, such as melanin pigments, polyphenols, and proteins. According to Rioux *et al.* (2007), higher fucoidan purity correlates with a brighter color, approaching grayish white. The decrease in brightness in sample 2 M suggests suboptimal precipitation, whereas in sample 4 M, the brightness further decreased, possibly due to the coagulation precipitation of a mixture of fucoidan and contaminants caused by the excess calcium ions (Figure 2).

The yellowness parameter (b*) also exhibited significant changes. The fucoidan 4 M crude extract demonstrated the lowest b* value (-5.14), indicating a stronger yellow-green color tendency. This value was lower than the b* values of samples 3 M (-4.44) and 2 M (-4.20). The more pronounced yellow-greenish color in the sample with 4 M concentration may suggest that the precipitation process precipitates not only fucoidan but also non-polar pigments such as fucoxanthin that remain trapped in the final product. This implies that excess CaCl₂ may reduce the precipitation selectivity, as compounds other than fucoidan are also precipitated. In this context, 3 M proved to be more effective in minimizing residual pigments without altering the natural color of the fucoidan (Ale *et al.*, 2011).

Spectroscopy of Functional Groups through Fourier Transfrom -Infra Red (FTIR)

Infrared spectral data are instrumental in identifying the functional groups present within the molecular structure of a compound. The infrared spectrum of crude fucoidan extracted from the brown seaweed *Sargassum* sp. subjected to three different concentrations of CaCl₂ is depicted in Figure 2. This figure illustrates the presence of primary functional groups' characteristic of fucoidan compounds, as determined by FTIR analysis. The absorption peak observed at approximately 3,400 cm^{-1} signifies the presence of hydroxyl groups (O-H), indicating that the crude fucoidan extract has a polysaccharide structure. Additionally, carbonyl groups (C=O) were detected at
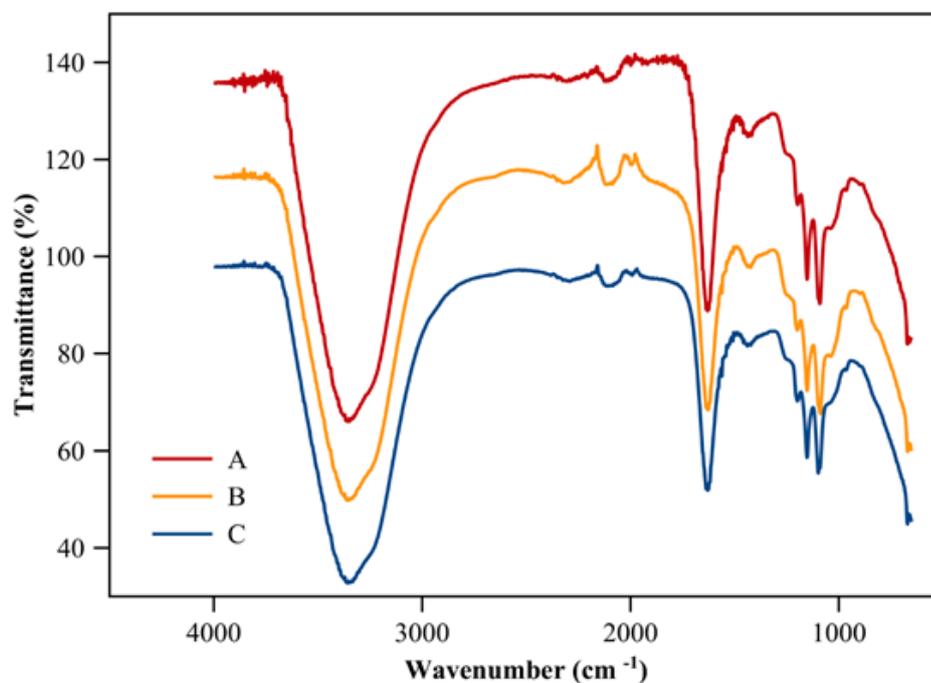

Figure 2 The FTIR spectrum of crude fucoidan: A (red line) represents crude fucoidan extracted with 2 M CaCl₂, B (yellow line) with 3 M CaCl₂, and C (blue line) with 4 M CaCl₂. The green dashed line indicates the fingerprint of the crude fucoidan extract.

approximately 1,640 cm^{-1}, which may originate from uronic acid or bound water within the structure of the crude fucoidan extract.

Figure 2 FTIR spectrum of crude fucoidan, A (red line): crude fucoidan extracted by 2 M of CaCl$_2$, B (yellow line): crude fucoidan extracted by 3 M of CaCl$_2$, and C (blue line): crude fucoidan extracted by 4 M of CaCl$_2$. The green dashed line indicates the fingerprint of the crude fucoidan extract.

Meanwhile, the sulfate ester group (S=O), a characteristic marker of sulfated fucoidan, was distinctly observable within the range of 1,220-1,250 cm^{-1}, suggesting the presence of significant biological activity. Additionally, the presence of glycosidic bonds (C-O-C) was identified at approximately 1,030 cm^{-1}, supporting the presence of polysaccharide structures in the three samples. The sample with a 4 M CaCl$_2$ concentration exhibited the lowest transmittance at the sulfate and hydroxyl groups, indicating a higher bioactivity potential. This observation aligns with previous studies demonstrating that fucoidans extracted from brown algae exhibit FTIR spectra with characteristic peaks in this range (Ale *et al.*, 2012; Nurhidayati *et al.*, 2020; Ramu *et al.*, 2020).

Analysis of the transmittance spectra revealed that all samples exhibited spectral characteristics consistent with the fundamental structure of the crude fucoidan extract, a sulfated polysaccharide composed of L-fucose, sulfate groups, and other carbohydrate moieties (including the stretching vibration of S=O, which appears around 1,250 cm^{-1}, and the bending vibration of C-O-S, observed at approximately 818 cm^{-1}) (Alboofetileh *et al.*, 2018). The primary absorption band, observed within the wavenumber range of 3,200-3,400 cm^{-1}, was broad and intense, indicating the presence of hydroxyl (-OH) groups from both fucose and bound water. This feature is a principal characteristic of hydrophobic polysaccharides, such as fucoidan (Ale *et al.*, 2011). Notably, sample 4 M demonstrated the highest intensity for this band, suggesting a greater content of hydroxyl groups in the crude extract of fucoidan 4 M than in 2 M and 3 M.

Additionally, the absorption band observed at 1,600-1,650 cm^{-1} signifies the presence of a C=O group of carbonyls, likely originating from the uronate compounds. This group is commonly identified in fucoidan structures and contributes to the enhanced polarity and water solubility of fucoidan (Li *et al.*, 2008). The presence of bands within this range was pronounced and consistent across all samples, indicating a significant contribution of uronic acid to the structure of crude fucoidan. In the fingerprint region (indicated by the green dashed line in Figure 2), particularly between 1,200-1,000 cm^{-1}, complex absorption bands were detected, indicating C-O-C vibrations of the glycosidic bond as well as sulfate ester (S=O) groups. The absorption bands around 1,220-1,250 cm^{-1} are typical indicators of sulfate ester groups on fucoidan, which contribute to its bioactive properties, including its antioxidant and immunomodulatory activities (Wang *et al.*, 2010). The intensity of the sulfate band in the absorbance spectrum was the highest in treatment sample 4 M, followed by 3 M and 2 M, reflecting the variation in sulfate content among the samples and indicating that treatment sample 4 M possessed the highest degree of sulfation. This is corroborated by the transmittance spectrum, where sample 4 M exhibits the lowest transmittance in this region, signifying greater absorption of IR light by the S=O group. The absorption band at approximately 1,020-1,030 cm^{-1}, consistently appearing throughout the spectrum, indicates vibrations of the C-O group and can be attributed to the fucose structure in the main chain of fucoidan. Another characteristic that corroborates the presence of the fucoidan structure is the narrow band appearing at 820-840 cm^{-1}, which can be attributed to the vibrations of C-O-S sulfate groups at the axial or equatorial positions of the fucose ring (Choi *et al.*, 2014). The presence of this absorption in all samples, with the highest intensity in the treatment sample (4 M), indicates a direct relationship between the sulfate content and the strength of the FTIR signal in that range. The differences in absorption intensity between the samples can be attributed to

several factors, such as the effectiveness of the extraction process, the composition of the raw materials, and the environmental conditions under which the seaweed was collected. The FTIR analysis revealed that all three samples contained the main component of fucoidan; however, there were significant variations in the quality and functional group content. The FTIR spectrum of the treated sample 4 M consistently exhibited higher absorption intensity in the main bands, especially for the -OH, C=O, and S=O groups, indicating that fucoidan has the highest potential biological activity. These results align with the theory that high sulfate content is directly proportional to the bioactivity of fucoidan, including its antioxidant and anticoagulant properties (Choi *et al.*, 2014). These FTIR data not only serve as an identification tool but can also provide an initial indication of the quality and potential bioactivity of each sample, as well as support decisions in the selection of ingredients for the development of fucoidan-based pharmaceutical products or functional foods.

Analysis of crude fucoidan extracted with 4 M of concentration CaCl₂ revealed elevated peak intensities for sulfate and hydroxyl groups compared to those of the treatment samples (2 M) and (3 M). This observation suggests that sample 4 M possesses higher concentrations of sulfate and hydroxyl groups, which are typically linked to enhanced bioactivity, including antioxidant, anticancer, and immunomodulatory activities.

CONCLUSIONS

The 2 M concentration yielded the highest moisture content, while 4M showed the highest FTIR absorption of sulfate and hydroxyl groups, indicating higher bioactivity. The ash content remained stable across all treatments. The optimal CaCl₂ concentration should be aligned with the intended use, prioritizing either the extraction quantity or the quality of the bioactive compound. The peak intensity differences highlight the need to standardize extraction for consistent crude fucoidan quality.

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