Antioxidant activity of crude fucoidan extract from brown seaweeds (Phaeophyceae), *Sargassum* sp. and *Turbinaria* sp.

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Abstract. Seaweed is one of the highly commercial aquatic commodities, accounting for 51.3% of total marine and coastal aquaculture production. As much as 99.5% of seaweed production is concentrated in Asia, especially in East and Southeast Asian countries, one of which is Indonesia. The wide diversity of seaweeds has not been fully optimizing their potential to provide maximum economic benefits. This study aimed to determine the characteristics of crude extracts of fucoidan using two different solvents and to analyze the antioxidant activity of crude extracts of fucoidan using different solvents. The research method included the preparation of raw materials, extraction with distilled water and 2% $CaCl_2$ at 85 °C for 4 h, followed by quality analyses. Total sugar using the phenol-sulphate method obtained higher results in distilled water (67.88%). The total sulphate analysis showed higher results with 2% $CaCl_2$ (19.60%). The total phenol analysis yielded higher results with 2% $CaCl_2$ (978.20%). The antioxidant activity of the crude fucoidan were 312.01 ppm (distilled water), 193.52 ppm (distilled water), 54.653 µg/mL ascorbic acid/g extract ($CaCl_2$ 2%, 128.38 mol Fe^{2+}/g ($CaCl_2$ 2%).

1 Introduction

Seaweeds are one of the most popular aquatic commodities. Worldwide seaweed production is quite substantial with a total output of 32.4 million tons worth US$13.23 billion. Seaweeds currently represent 51.3% of the total marine and coastal aquaculture production. As much as 99.5% of seaweed cultivation is concentrated in Asia, particularly in East and Southeast Asian countries [1]. Indonesia is an Asian country that produces seaweeds. Indonesia is the second largest seaweed producer in the world, after China. Indonesia produces seaweed, with a total production of 9.6 million tons per year. In 2021, seaweed production will increase to 7.14%. Seaweeds have also contributed to the highest export growth during the fourth quarter of 2021, with a growth rate of 4.85% [2]. Seaweed production in Indonesia is spread across 15 provinces: Banten, Central Java, East Java, West Java, West Nusa Tenggara, East Nusa Tenggara, West Sulawesi, West Sulawesi, Central Sulawesi, South Sulawesi, Southeast Sulawesi, North Sulawesi, East Kalimantan,

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North Kalimantan, Gorontalo, and Maluku [3]. Indonesian seaweed production has great potential and plays an important role in national economic growth.

Seaweed diversity in Indonesia is a promising resource with great potential. Indonesia has an ocean area of 64.00.000 km^2 and a territory of 1.10.000 km, with a long coastline and tropical climate that makes it suitable for growing various types of seaweed [4]. There are a total of 782 species of seaweed, including 19 species of green algae (Chlorophyceae), 452 species of red algae (Rhodophyceae), and 134 species of brown algae (Phaeophyceae) [5]. The vast diversity of seaweed has not been fully realized when it comes to optimizing its potential for maximizing economic benefits for the seaweed development industry in Indonesia. Unfortunately, many seaweeds continue to be discarded as marine trash, either left to drift with currents or being stranded on beaches. Brown seaweed (Phaeophyta) is one of the various types of seaweed that is widely cultivated and traded in Indonesia. Diverse species of Phaeophyta are distributed worldwide, but only a few have been commercially exploited [6]. Coastal communities in Indonesia do not widely utilize brown seaweed (Phaeophyta) in the consumer market [7].

Several species of brown seaweed are abundant in the coastal waters of Indonesia, including *Padina* sp., *Sargassum* sp., *Laminarian* sp., and *Turbinaria* sp. Brown seaweeds are highly nutritious because they contain carbohydrates, proteins, vitamins, and minerals. Some components also possess bioactivity that can be beneficial to human health [8]. These components are polysaccharides of brown seaweeds, such as alginate, fucoidan, and laminarin. Fucoidan has recently attracted attention because of its various benefits and potential for industrial development in several fields. Several studies have proposed that fucoidan exhibits anticancer, antidiabetic, anti-inflammatory, and antioxidant activities [9–11]. Fucoidans are typically extracted using solvents such as acids, water, and calcium salts. Among these three methods, the extraction process using $CaCl_2$ produces the best results in terms of yield [12]. Although the antioxidant properties of fucoidan are known, there has been no analysis of the antioxidant activity of the crude extract of fucoidan extracted from distilled water and 2% $CaCl_2$. Therefore, this study aimed to determine the characteristics of the crude fucoidan extract from brown seaweed and analyze its antioxidant activity using distilled water and 2% $CaCl_2$ as solvents.

2 Materials and methods

2.1 Materials

The research was carried out using the primary raw materials, namely *Sargassum* sp. and *Turbinaria* sp., in powder form with a particle size of 25 mesh of 50 g. Other materials used for the extraction process included distilled water, $CaCl_2$ 2% (Merck) as a solvent, and 96% ethanol and double-distilled water (IKA 500). Materials used for determination of total sulphate, namely $BaCl_2$ 0.25 M (Merck), distilled water, gelatine, Tricarboxylic Acid (TCA) 4%, and standard K_2SO_4 (Merck). Materials used for the analysis of total phenols were Folin 50%, distilled water, Na_2CO_3 5% (Merck), and standard acid error (Merck). Functional group analyses were performed using potassium bromide (Merck, Darmstadt, Germany). The materials used for antioxidant analysis were ammonium acetate buffer (Samarth), tri(2-pridil)-s-triazine (TPTZ) solution (Sigma), $FeCl_{3.6}H_2O$ (Merck), $CuCl_{2.2}H_2O$ (Merck), ethanolic neocoproin (Sigma), ABTS (2.2 –azinobis 3-ethylbenzathiazolin-6-sulfonic acid) (Merck), potassium persulfate, absolute methanol, DPPH (1,1-diphenyl-2-picryhydrazyl) (Sigma). The research was also conducted using various pieces of equipment, including analytical scales (Sartorius TE64, Germany), dry blender (Miyako), water bath (Memmert), refrigerator (LG MA53LHJG), thermometer,

magnetic stirrer (Basco), centrifuge (Hettich EBA), vortex (IKA Genius 3), 14 MWCO dialysis bag (Sigma), freeze dryer (Telstar), Perkin Elmer Lambda 25 UV-VIS spectrophotometer, and FTIR (Perkin Elmer 577).

2.2 Sample preparation

The raw materials for seaweed, specifically *Sargassum* sp. obtained from the waters of Klui Beach, Lombok, and *Turbinaria* sp. obtained from Pramuka Island, Seribu Islands, DKI Jakarta, were collected. The samples were thoroughly cleaned with coral, wood, sand, and salt, and then washed with clean seawater. Subsequently, the seaweed was sun-dried for 40 h until the moisture content was reduced to a minimum of 50%. The dried seaweed was ground using a blender and sieved through a 25-mesh sieve to obtain seaweed powder.

2.3 Extraction method

The seaweed extraction process uses the [13] method with modifications. Extraction using distilled water was initiated by soaking the seaweed in 1:20 (w/v) distilled water. The mixture was stirred for 4 h at 85 °C using a magnetic stirrer to accelerate the contact between the sample and the solvent. The mixture was filtered through a filter paper. The filtrate was collected, and the residue was discarded. Subsequently, a 2% $CaCl_2$ solution (1:20) was added to the filtrate to separate fucoidan from alginate, while stirring for 30 min at room temperature. The filtrate was then centrifuged for 15 min at 5 °C and 8000 rpm. The filtrate was mixed with 96% ethanol (1:2) to eliminate impurities. The obtained precipitate was dissolved in water until it was completely dissolved as a precipitate wash. The obtained samples were then centrifuged for 15 min at 8000 rpm. The residue was discarded, and the filtrate was obtained for dialysis. The dialysis process involves separating the colloids and impurity ions based on the differences in molecular weight. The dialysis stage began with preparation of the dialysis bag. A total of 5 g of Na_2CO_3 was added to distilled water, which was heated to a temperature of 100 °C, and then the bag was soaked for 10 min. The soaking process was repeated using distilled water as solvent. The bag was then stored at chilling temperature for an hour until it was ready to use. The dialysis process starts by placing the filtrate obtained from extraction into a dialysis bag and tying it with a wool thread. The bag was then placed into a glass beaker and filled with distilled water until it was submerged. Samples were soaked for 2×24 h. The dialysis filtrate was dried using a freeze dryer to obtain the crude fucoidan extract.

Extraction using $CaCl_2$ was initiated by soaking the seaweed in $CaCl_2$ 2% (1:20) (w/v). The mixture was then stirred for 4 h at 85 °C using a magnetic stirrer. The mixture was filtered through filter paper. The filtrate was then centrifuged for 15 min at 5 °C and 8000 rpm. The filtrate was mixed with 96% ethanol (1:2) to eliminate impurities. The obtained precipitate was dissolved in water until it was completely dissolved as a precipitate wash. The obtained samples were then centrifuged for 15 minutes at 8000 rpm. The residue was discarded, and the filtrate was obtained for dialysis. The dialysis stage began with preparation of the dialysis bag. A total of 5 g of Na_2CO_3 was added to distilled water, which was heated to a temperature of 100 °C, and then the bag was soaked for 10 min. The soaking process was repeated using distilled water as solvent. The bag was then stored at chilling temperature for an hour until it was ready to use. The dialysis process starts by placing the filtrate obtained from extraction into a dialysis bag and tying it with a wool thread. The bag was then placed in a beaker and filled with distilled water until it was submerged. The sample for 2x24 hours. The dialysis filtrate was dried using a freeze dryer to obtain the crude fucoidan extract.

2.4 Fucoidan yield measurement

Fucoidan yield was determined by dividing the weight of the fucoidan extract by the weight of the dried seaweed material. The yield that can be obtained using the formula.

$$Yield\ (\%) = \frac{weight\ of\ fucoidan\ extract\ (g)}{weight\ of\ dry\ seaweed\ (g)} \times 100\% \quad (1)$$

2.5 Phytochemicals analysis

Phytochemical analysis was performed using the method in [14]. The analysis included Alkaloids, flavonoids, phenols, and tannins were analyzed. Alkaloid analysis was performed using the Meyer, Wagner, and Dragendroff methods. Total (0.05 g samples were added to 3 mL chloroform and 3 drops ammonia. The chloroform fraction was separated and acidified using 10 drops of 2 M H_2SO_4. The acid fraction was collected and subjected to the Meyer, Wagner, and Dragendroff's reagent. The alkaloids were identified by the formation of a white precipitate with Meyer's reagent, brown precipitate with Wagner's reagent, and red precipitate with Dragendroff's reagent. Flavonoid analysis began by added methanol 30% to a 0.05 g sample until submerged and heated for 5 min. The resulting mixture was then filtered to obtain the filtrate. The extract filtrate was added to 1 drop of 10% NaOH. The formation of a red color in the filtrate after the addition of 10% NaOH indicated the presence of flavonoid compounds. Phenol analysis began by dissolved 0.05 g sample in 2 mL of 70% ethanol. Then, 1 mL of the solution and 2 drops of 5% $FeCl_3$ solution were added. Green or blue green indicates a positive result. Tannin analysis was initiated by dissolved 0.05 g samples in 5 mL distilled water. The mixture was heated to 100°C for 5 min, filtered, and then five drops of 1% $FeCl_3$ were added to the filtrate. Positive results were indicated by the production of dark blue, green, and black colors.

2.6 Determination of total sugar

The total sugar content was determined using the phenol-sulphate test according to a previous study [15]. The test began by added 0.2 mg of crude fucoidan to the test tube, followed by the addition of 1 mL of distilled water. As much as 2.5 mL of concentrated H_2SO_4 was added to the mixture and allowed to stand for 20 min. Then, 5% phenol (0.5 mL) was added, and the mixture was cooled in ice water for 30 min. The samples were then stirred using a vortex, and their absorbance was measured using a PerkinElmer Lambda 25 UV-VIS spectrophotometer. For comparison, a standard glucose solution was used at concentrations of 20, 40, 60, 80, and 100 ppm and treated in the same way as the samples. Sample measurements were taken at a wavelength of 490 nm, and the absorbance values were converted into total sugar, expressed in %.

2.7 Determination of total sulphate

The protocol is reported in [16]. $BaCl_2$-gelatin was prepared by dissolving 2 g of gelatine in 400 mL of hot water (60-70°C) and allowing it to reach a temperature of 4°C for 6 h. $BaCl_2$ (2 g) was dissolved in gelatine solution. This solution was stored at 4°C for one week before use. Sample preparation was carried out by dissolving 2 mg of the sample in 2 mL of distilled water in a closed tube, after which 5 mL of 4% TCA was added and heated in a water bath at 100°C for 4 h. The solution mixture was cooled to room temperature for 5 min and homogenized before the tube was opened. One millilitre of the hydrolysis results was collected, and 1 mL of $BaCl_2$-gelatin and 1.4 mL of TCA were added. Subsequently,

the mixture was mechanically stirred, and the turbidity was measured using a turbidimeter. The standard uses K_2SO_4 to treat the same samples were treated with 5, 10, 15, 20, and 25 ppm. The turbidimeter data obtained can be interpreted by making a K_2SO_4 standard curve and then converted into total sulphate expressed in %.

2.8 Quantitative determination of total phenols

The protocol reported in [17] was performed with slight modifications. The protocol began by mixing 1 mL of the sample extract with 1 mL of 99.9% ethanol and 5 mL of distilled water. The mixture was then homogenized with 0.5 mL of 50% Folin-Ciocalteu reagent and allowed to stand for 5 min. Then, 1 mL of 5% Na_2CO_3 was added, homogenized, and allowed to stand in the dark for 60 min. Gallic acid was used as the standard. Sample measurements were recorded at a wavelength of 725 nm, with absorbance values converted into total phenol expressed in mg GAE/g sample weight.

2.9 Antioxidant analysis

There are three different methods for testing the antioxidant activity. The DPPH test uses the method of [18], with some modifications. The crude fucoidan extract was dissolved in ethanol p.a. to prepare solutions with concentrations of 200, 250, 300, 350, 400, and 450 ppm. Ascorbic acid was used as a comparator and dissolved in ethanol to prepare solutions with concentrations of 1, 2, 3, 4, 5, and 6 ppm. DPPH solution was prepared by dissolving DPPH crystals in ethanol at a concentration of 0.3 mM. The manufacturing process was performed at low temperatures and protected from sunlight. To test the antioxidant activity, 4.50 mL of the extract and ascorbic acid antioxidant solutions were each mixed with 0.5 mL of 0.3 mM DPPH solution in separate test tubes. The reaction took place at 37°C for 30 min, and the absorbance was measured at a wavelength of 517 nm using a UV-VIS spectrophotometer. The absorbance of the blank solution was measured to calculate the percentage of inhibition. The blank solution was prepared by mixing 4.50 mL of sample solvent (distilled water and 2% $CaCl_2$) with 0.50 mL of 0.3 mM DPPH solution in a test tube. Antioxidant activity is expressed as the inhibition percentage and can be obtained using the following formula:

$$Inhibition\ (\%) = \frac{Abs\ blank - Abs\ of\ sample}{Abs\ blank} \times 100\% \quad (2)$$

The Ferric Reduction Antioxidant Power (FRAP) method was carried out following [19] with several modifications. The FRAP method uses $FeSO_4.7H_2O$ as a standard. FRAP reagent was prepared in the form of 300 mM acetate buffer (3 mL CH_3COONa and 40 mL CH_3COOH) at pH 3.6, 10 mM TPTZ (2, 4, 6-tripiridyl-striazine) solution in 40 mM HCl, and $FeCl_3.6H_2O$ 20 mM. The solution was freshly prepared by mixing 40 mL of buffer acetate, 4 mL of TPTZ, and 4 mL of $FeCl_3.6H_2O$ solution. For absorbance measurements, 100 µL of the sample, 600 µL of distilled water, and 3000 µL of FRAP reagent were added. The sample and FRAP reagent were mixed using a vortex mixer and incubated in a water bath for 30 min at 37°C. The absorption was measured at a wavelength of 593 nm. To create a standard curve, $FeSO_4.7H_2O$ solutions with concentrations of 5, 10, 20, and 40 ppm were used.

The Cupric Reducing Antioxidant Capacity (CUPRAC) method was carried out following [20] study with several adjustments. This method begins by dissolving 0.3 mL of the sample in 99.9% ethanol and adding 1 mL of 0.01 M $CuCl_2.2H_2O$; 1 mL of neucoproin ethanol 0.0075 M, 1 mL ammonium acetate buffer pH 7 1 M and 0.8 mL distilled water.

The mixture and reagents were homogenized using a vortex mixer and incubated at room temperature in the dark for 30 min. The absorbance was measured at a wavelength of 450 nm. Calibration curves were generated using ascorbic acid solutions at concentrations of 8, 12, 14, 16, 18, and 20.

The 2,2 azinobis (3-ethylbenzothiazoline)-6- sulfonic acid (ABTS) method uses the [21] method with some modifications. To prepare ABTS reagent, ABTS (7.4 mM) was mixed with $K_2S_2O_8$ (2.6 mM) and incubated for 18 h at room temperature. The ABTS reagent was diluted with methanol to obtain a reagent absorbance value of 0.80 at 750 nm wavelength. To test a sample, 1 mL of sample extract was prepared at concentrations of 200, 250, 300, 350, and 400 ppm, and standard concentrations of 1, 2, 3, and 4 ppm. Add 1 mL) was added to each sample and the mixture was allowed to sit for 10 min at room temperature. The absorbance of the samples was measured at 750 nm. Percentage inhibition was calculated using the following formula:

$$Inhibition\ (\%) = \frac{Abs\ blank - Abs\ of\ sample}{Abs\ blank} \times 100\% \quad (3)$$

2.10 Functional group analysis

The functional group analysis method uses [22] with some modifications. A sample of fucoidan (1 mg) was crushed with potassium bromide (200 mg). The resulting powder was compressed into thin and transparent tablets at 7000 Pa. These tablets were then placed in a simple pan to record the infrared spectrum at wavenumbers ranging from 500 to 4000 cm^{-1}. The analysis was conducted using the FT-IR (Perkin-Elmer 577) KBr-pellet method and Specord M-80.

2.11 Statistical analysis

All data are summarized as mean ± standard deviation. All response data of fucoidan extracts from *Sargassum* sp. and *Turbinaria* sp. were tested using one-way ANOVA Completely Randomized Design (CRD) with the solvent used (distilled water and $CaCl_2$) as fixed factors. Statistical significance was set at $p \leq 0.05$. When significant effects of the factors occurred, ANOVAs were followed by Tukey's multiple comparisons test to identify differences between different solvent treatments.

3 Results and discussion

3.1 Yield of crude fucoidan extract

Extraction is the process of extracting active compounds from plants using specific solvents [14]. The solvent penetrates the cell wall and enters the cell cavity, which contains the active substance, leading to extraction [23]. To obtain better results, the extraction method should be based on the characteristics of the target organism. Analysis of the fucoidan crude extract treated with two different solvents is presented in **Table 1**.

The results obtained in this study showed that the yield of fucoidan in distilled water was higher than that in 2% $CaCl_2$ solvent. The highest yield of crude fucoidan extract was obtained from Sargassum sp., which was extracted with distilled water (4.79). The fucoidan extract is suspected to contain alginate and laminae. Alginate, fucoidan, and laminarin are cell wall constituents [25]. The alginate content in brown seaweed is also dominant (30-40%) [26]. The distilled water solvent that is varied at high temperatures only plays a role

as a breaker of interactions between fucoidan and cell walls; thus, alginate and other polysaccharides found in cell walls are also potentially extracted. Meanwhile, the 2% $CaCl_2$ solvent plays a role in separating fucoidan from alginate so that the resulting fucoidan content is higher [24].

Table 1. Characteristic of crude fucoidan extract.

Sample	Yield (%)	Color	Color[24]	Form	Form[24]
Sargassum sp./Distilled water	4.79	Brownish white	Brown	Powder	Powder
Turbinaria sp./Distilled water	3.66	Brownish white	Brown	Powder	Powder
Sargassum sp./$CaCl_2$	3.30	Brownish yellow	Brown	Powder	Powder
Turbinaria sp./ $CaCl_2$	2.90	Brown	Brown	Powder	Powder

A study by [27] found that the optimal temperature for fucoidan extraction was 85°C. The yield of fucoidan increases between 60-85°C, but decreased at 90°C. The yield of fucoidan increased with an extraction time of 3-4 hours but decreased at 5 h. This is because the cell walls of brown algae become more porous as the extraction temperature (70-85°C) and time (3-4 hours) increases, leading to more fucoidan being released from the intercellular tissue and dissolving in the solvent. However, when the extraction temperature was increased to 90°C and the extraction time was extended to 5 h, fucoidan began to degrade, resulting in a lower yield. It is important to avoid using temperatures that reach the boiling point (100°C) during the extraction process, as this can cause degradation of thermolabile compounds. To increase the contact between the solvent and solute within the cell structure, the sample used in the study was in powder form [28].

The research conducted has found that the color of the extracted fucoidan differed depending on the extraction solvent used. Fucoidan extracted using distilled water appeared whiter, whereas fucoidan extracted using 2% $CaCl_2$ solvent appeared brownish. The appearance of the crude extract of fucoidan was consistent with the fucoidan standard for food additives, which requires it to be in powder form and has a pale white to brownish color [29]. Previous research also showed that the color yield of fucoidan extract tended to be browner in 2% $CaCl_2$ solvent compared to fucoidan in distilled water [24]. It is believed that fucoidan extracted using a 2% $CaCl_2$ solvent exhibited higher fucoidan levels despite having a lower yield compared to the distilled water solvent. The higher the fucoidan content, the browner the fucoidan, which may be due to caramelization. When a solution contains saccharides, the concentration increases with the boiling point until all air is dissolved. If the heating continues, the liquid ceases to be air or solvent, but melts the monosaccharide. If the melted saccharide is consistently heated beyond its melting point, caramelization may occur [30].

3.2 Bioactive compounds of crude fucoidan extract

Phytochemical analysis refers to compounds that are not necessary for normal body function but have beneficial effects on health or play an active role in preventing diseases. In this study, the bioactive compounds in the crude fucoidan extract were qualitatively tested using color changes or precipitates formed in response to the administered reagents. The phytochemical tests performed in this study included alkaloids, flavonoids, phenols, and tannins. The results of phytochemical testing of the crude fucoidan extract are shown in **Table 2**.

The extract was subjected to solvent treatment with distilled water and 2% $CaCl_2$, which revealed the presence of active compounds, namely alkaloids and flavonoids. In a study conducted by [31], *Sargassum* sp. extract, when subjected to distilled water solvent,

contained active flavonoid compounds, but did not contain tannin. Another study conducted on the crude extract of *Turbinaria* sp. revealed that when subjected to distilled water solvent treatment, there were no active compounds, such as tannins, phenols, and flavonoids [32]. Distilled water and $CaCl_2$ 2% were not able to extract tannin and phenol phytochemical compounds. This is because tannin and phenol are semi-polar, and hence less extracted by polar (distilled water) and non-polar solvents [33]. Phenolic compounds are generally easier to extract using semi-polar organic solvents. Fucoidan is a carbohydrate polysaccharide that can trigger low levels of active phenolic compounds in fucoidan [34].

Table 2. Bioactive compound of crude fucoidan extract.

Phytochemical test	Distilled water		$CaCl_2$		Color standard
	Sargassum sp.	*Turbinaria* sp.	*Sargassum* sp.	*Turbinaria* sp.	
Alkaloid					
Meyer	+	+	+	+	white precipitate
Wagner	+	+	+	+	brown precipitate
Dragendoff	+	+	+	+	red precipitate
Flavonoid	+	+	+	+	The top has a darker color than the bottom
Phenol hydroquinone	-	-	-	-	Green/Blue
Tannin	-	-	-	-	Dark blue/Green/Black

Note : + : contain active compound
- Does not contain active compounds.

Alkaloids are a large group of secondary substances that are present in plants. They contain nitrogen atoms and are alkaline in nature; therefore, their extraction requires sulfuric acid. Generally, alkaloids exist in the form of crystals called alkaloid salts [35]. This compound has antihypertensive, antiarrhythmic, malarial, and anticancer activities. Flavonoids dissolve in polar solvents, such as methanol, ethanol, and distilled water. Flavonoids have antimicrobial, antihelminthic, antidiuretic, and antioxidant properties [26, 27]. Flavonoids act as antioxidants by donating hydrogen atoms [38]. Phenol is an acidic compound that does not easily dissolve at room temperature. The acidity of phenol is lower than that of carbonic acid; therefore, phenol can react with bases to form phenoxide. Phenol has antioxidant, anti-inflammatory, anticancer, and antimicrobial activities [36]. Tannin has been used for a long time as a quick treatment for diarrhoea, dysentery, bleeding, and tumour size [39]. Tannin can also be an indicator of antioxidant activity. The higher the tannin content, the greater the antioxidant activity, because tannin is composed of polyphenolic compounds that have free radical scavenging activity [40].

3.3 Total sugar of crude fucoidan extract

The phenol-sulphate total sugar test is highly sensitive and reliable. It involves the use of concentrated sulfuric acid to dehydrate carbohydrates, which are then hydrolyzed to produce furfural derivatives. The resulting derivative was mixed with phenol, resulting in a dark-orange solution. The test results for total mad content of the crude fucoidan extract is shown in **Table 3.**

It is essential to determine the total sugar in a substance to process it appropriately for food or pharmaceutical purposes, predict its shelf life, and handle it correctly for further processing of fucoidan [30]. In this study, glucose was used as the standard because it is a monomeric compound comprising fucoidan [41]. According to previous research, the

fucoidan extract from *Turbinaria* sp. that was given a distilled water solvent had the highest total sugar value of 67.288 ± 0.02%. In contrast, the fucoidan extract from *Sargassum* sp. with 2% $CaCl_2$ had the lowest total sugar value of 44.424 ± 0.86%. The test results also showed that the total sugar content in the crude fucoidan extract prepared using distilled water as a solvent was higher than that in brown seaweed fucoidan prepared using 2% $CaCl_2$ solvent. The difference in the total fucoidan sugar content between distilled water and 2% $CaCl_2$ indicated that the total polysaccharide content was higher in the distilled water extractor. This is consistent with the statement that polysaccharides are more soluble in distilled water than in other solvents [42].

Table 3. Total sugar, total sulphate, and total phenol of crude fucoidan extract.

Sample	Solvent	Total sugar (%)	Total sulphate (%)	Total phenol (mg GAE/g)
Sargassum sp.	Distilled water	53.987 ± 0.06^{a}	14.671 ± 0.47^{b}	978.17 ± 0.64^{a}
	2% $CaCl_2$	44.424 ± 0.86^{b}	19.605 ± 0.93^{a}	503.73 ± 0.55^{b}
Turbinaria sp.	Distilled water	67.288 ± 0.02^{a}	14.012 ± 0.47^{b}	782.72 ± 0.32^{a}
	2% $CaCl_2$	60.377 ± 0.09^{b}	17.96 ± 0.47^{a}	272.79 ± 0.81^{b}

Analysis of variance indicated that the type of solvent used had a significant effect ($p < 0.05$) on the total sugar content in the crude extract of fucoidan samples from *Sargassum* sp. and *Turbinaria* sp. This means that the use of distilled water and 2% $CaCl_2$ solvents during the extraction process can affect the total sugar content in the crude fucoidan extracts of *Sargassum* sp. and *Turbinaria* sp. Similar research conducted by [24] also found that the total sugar content in the crude fucoidan extract of *Sargassum* sp. with distilled water and 2% $CaCl_2$ was 76.25% and 64.19%, respectively. The differences in the results were due to the variations in distilled water with different ethanol concentrations used in the extraction process. In this study, 96% ethanol was used, whereas absolute ethanol was used in the literature. The higher the ethanol concentration, the higher the sugar content. The total sugar content of fucoidan is influenced by several factors, including extraction methods, species differences, seasonal variations, growing/harvesting period, growing environment, and dissolution [43].

3.4 Total sulphate of crude fucoidan extract

Fucoidan is a sulphated polysaccharide containing L-fucose and sulphate [41]. One of the determinants of fucoidan activity is sulphate content. The greater the sulphate bound to fucose, the more active is the fucoidan. A high sulphate content results in higher bioactivity. The results for the total sulphate from the crude fucoidan extract are shown in **Table 3**. The results showed that the total sulphate content of the crude fucoidan extract with $CaCl_2$ was 2% greater than that of brown seaweed fucoidan with distilled water. This shows that the distilled water solvent can extract sulphate from the crude fucoidan extract better than the 2% $CaCl_2$ solvent. The fucoidan extracted from *Sargassum* sp. using 2% $CaCl_2$ as a solvent had the highest total sulphate value of 19.605 ± 0.93%. This is based on a statement by [44], which states that calcium salt solutions can precipitate alginate to increase the purity of fucoidan. Various analysis results showed that the different solvents provided had an effect ($p < 0.05$) on the total sulphate in the crude fucoidan extract samples from *Sargassum* sp. and *Turbinaria* sp. This shows that the difference in distilled water and 2% $CaCl_2$ affects the total sulphate content in the crude fucoidan extract of Sargassum sp. and *Turbinaria* sp.

According to some studies, the sulphate content in the crude extract of fucoidan was determined using two types of solvents: 2% $CaCl_2$ and distilled water. The measurements were taken using a spectrophotometer, and the results showed that the sulphate content in the crude extract using 2% $CaCl_2$ solvent was 8.69%, while the sulphate content in the crude extract using distilled water was 8.1% [24]. This finding supports the idea that the 2% $CaCl_2$ solvent has a higher sulphate content than distilled water. However, the differences in the results could be attributed to the different measurement methods used. The $BaCl_2$ method, which forms colloids, is easier to measure using a turbidimeter than a spectrophotometer. Solutions containing suspensions or colloids are more suitable for turbidimeters.

3.5 Total phenol of crude fucoidan extract

Phenolic compounds are rich sources of antioxidants that prevent free radicals and chelate metal ions. They inhibit lipid oxidation by donating hydrogen atoms to the free radicals. The total phenol content of the crude fucoidan extract is shown in **Table 3.** The total phenol content of the brown seaweed fucoidan crude extract with $CaCl_2$ was 2% higher than that with distilled water. Fucoidan extracted from *Sargassum* sp. with distilled water had the highest total phenol value of 978.17 ± 0.64. The results of the analysis of variance showed that the different solvents had an effect ($p < 0.05$) on the total phenols in the crude extract of fucoidan samples from *Sargassum* sp. and crude fucoidan extract of *Turbinaria* sp. This shows that the difference in distilled water and 2% $CaCl_2$ solvents used during extraction affects the total phenol content in the crude fucoidan extracts of *Sargassum* sp. and *Turbinaria* sp.

Crude fucoidan extracts are believed to require a semi-polar solvent to extract phenolic compounds. Phenolic compounds found in brown algae are naturally semi-polar, which means that they are not optimally extracted using polar distilled water solvents. In some research, the total phenolic value of brown seaweed (*Sargassum* sp.) distilled water extract was found to be 0.95 mg GAE/g, while other studies showed total phenols of 19.28 mg GAE/g [22, 36]. This difference is thought to be caused by the fact that the research samples were tested in the extract form, which means that protein, pigment, or fat could be an obstacle in the test, resulting in relatively lower phenol levels. The total phenol content did not consistently correlate with antioxidant activity in several tests in this study, indicating that other compounds in the extract, such as flavonoids, alkaloids, and tannins, played a role in influencing antioxidant activity. Various factors can affect total phenol testing, including genetic factors, pH of the surrounding environment, solubility, availability of phenolic compounds in the material, harvesting techniques, and the stability of phenolic compounds [46].

3.6 Antioxidant activity of crude fucoidan extract

Antioxidant tests on fourth crude extract samples from *Sargassum* sp. and *Turbinaria* sp. with different solvents (distilled water and $CaCl_2$ 2%) using four different test methods, namely DPPH, ABTS, CUPRAC, and FRAP methods. Relative radical scavenging activity was measured as the percentage of inhibition in the DPPH and ABTS assays. The IC_{50} value indicates the concentration of the sample required to cause a 50% loss in free radical activity [47]. A smaller IC_{50} value indicates a higher antioxidant activity. The crude fucoid extract was treated with distilled water and 2% $CaCl_2$, and ascorbic acid was used as the standard for the test. The research evaluated the antioxidant activity of fucoidan crude extract from *Sargassum* sp. and *Turbinaria* sp. samples using different solvents is summarized in **Table 4** and **Table 5**.

Each fucoidan sample had different antioxidant activities depending on the solvent used. The crude fucoidan extract of *Turbinaria* sp. had higher antioxidant activity when dissolved in distilled water, based on the DPPH test. On the other hand, *Sargassum* sp.*'s* crude fucoidan extract of Sargassum sp. has higher antioxidant activity when dissolved in distilled water based on the ABTS test. Various analyses showed that different solvents, namely distilled water and 2% $CaCl_2$, had a significant effect ($p < 0.05$) on the antioxidant activity of fucoidan crude extract of *Sargassum* sp. samples. and *Turbinaria* sp., using both the DPPH and ABTS methods. This shows that the difference in distilled water and 2% $CaCl_2$ solvents used during extraction affects the antioxidant activity using the DPPH and ABTS methods in the crude fucoidan extract of *Sargassum* sp. and *Turbinaria* sp.

Table 4. Antioxidant activity of crude fucoidan extract with DPPH and ABTS method.

Sample	Solvent	IC_{50} (ppm) DPPH method	IC_{50} (ppm) ABTS method
Sargassum sp.	Distilled water	341.44 ± 0.68^b	193.52 ± 3.6^b
	2% $CaCl_2$	424.90 ± 0.98^a	276.82 ± 2.7^a
Turbinaria sp.	Distilled water	312.01 ± 4.23^b	210.26 ± 4.12^b
	2% $CaCl_2$	423.12 ± 1.78^a	377.06 ± 4.23^a

Table 5. Antioxidant activity of crude fucoidan extract with CUPRAC and FRAP method.

Sample	Solvent	Antioxidant capacity (µg/mL ascorbic acid/g extract)	Antioxidant capacity (µmol Fe^{2+}/g extract)
Sargassum sp.	Distilled water	23.602 ± 1.20^b	83.830 ± 0.39^b
	2% $CaCl_2$	49.448 ± 0.14^a	128.389 ± 0.23^a
Turbinaria sp.	Distilled water	23.070 ± 1.73^b	73.275 ± 0.38^b
	2% $CaCl_2$	54.653 ± 0.57^a	92.440 ± 0.00^a

The results of testing antioxidant activity of the four crude extracts of fucoidan samples from *Sargassum* sp. and *Turbinaria* sp., each of which used distilled water and 2% $CaCl_2$ solvents, was higher in the crude extract of fucoidan *Turbinaria* sp. with distilled water solvent using the DPPH method. However, higher results were obtained for the crude fucoidan extract of *Sargassum* sp. using the ABTS method with distilled water as a solvent to test antioxidant activity. The results of other research using the DPPH method on fucoidan extract of *Sargassum* sp. with heated distilled water as a solvent showed that the IC_{50} value was 2.41 mg/mL [48]. Other studies have also shown that samples extracted with a 2% $CaCl_2$ solvent had an antioxidant activity of 10 mg/mL. Research has shown that fucoidan extract produced an antioxidant activity (IC_{50}) of 49.1 ± 1.1 ppm when mixed with distilled water. It also produced an antioxidant activity (IC_{50}) of 20 mg/mL when mixed with 2% $CaCl_2$ [49]. The different antioxidant activity results are thought to be due to the different sample habitats and extraction methods. The number of hydroxyl groups and conjugated double bonds in the test compound can influence the antioxidant activity. Apart from the hydroxyl group, the sulphate ester group is also a specific group of sulphated polysaccharides that can inhibit the cell damage caused by free radicals [50].

Antioxidant testing using the CUPRAC method showed higher antioxidant activity, namely crude extract from *Turbinaria* sp. with 2% $CaCl_2$ solvent. Antioxidant activity was tested using the FRAP method on the four crude extracts of fucoidan samples from *Sargassum* sp. and *Turbinaria* sp. using a higher solvent of distilled water and $CaCl_2$ 2%, namely crude extract from *Sargassum* sp. with 2% $CaCl_2$ solvent. Analysis of variance showed that the difference in distilled water and 2% $CaCl_2$ solvents had an effect ($p < 0.05$)

on the CUPRAC and FRAP test methods for *Turbinaria* sp. and *Sargassum* sp. This shows that the difference in distilled water and 2% $CaCl_2$ solvents used during extraction affects the antioxidant results obtained using the CUPRAC and FRAP methods on *Sargassum* sp. and *Turbinaria* sp.

Another study [51] used the CUPRAC method with samples of *Padina* sp. using a $CaCl_2$ solvent with an antioxidant activity of 0.395 ± 0.005 µg/mL ascorbic acid/g extract. These two results differ from research in that several factors can influence, for example, the type of solvent used, sample age, and sample habitat [52]. The results of other studies were also shown by [49], who carried out the FRAP test of fucoidan extract from *Padina* sp. using $CaCl_2$ solvent with lower antioxidant activity, namely 28 µmol Fe^{2+}/g. The sample habitat and extraction method influenced the antioxidant activity of the fucoidan extract. The FRAP test's antioxidant capacity (reducing power) is also influenced by the presence of flavonoid compounds in the sample, which can help reduce metal ions, such as iron [53].

The results of research on antioxidant activity using the four different methods were found to vary. The highest antioxidant activity was observed in the distilled water solvent during the DPPH and ABTS tests, whereas the 2% $CaCl_2$ solvent produced higher results during the CUPRAC and FRAP tests. These differences in results can be attributed to several factors, including variations in the mechanism of each test, variations in the antioxidant concentration of each fucoidan, and variations in temperature, oxygen pressure, air, and the structure of the fucoidan. These differences in antioxidant capacity lead to different mechanisms for counteracting free radicals and metals. The electron or hydrogen transfer mechanism affects the sensitivity of the four methods. The active compound components used as targets and the matrix components in the fucoidan extract are crucial factors for measuring the antioxidant capacity [54].

3.7 Functional group

The Fourier Transform Infrared (FTIR) method was used to detect the molecular structure of the compounds by identifying the functional groups that make up the compound. The results of FTIR spectrum measurements carried out at wave numbers 4000-450 cm^{-1} of crude fucoidan extracts of *Sargassum* sp. and *Turbinaria* sp. with different solvents (distilled water and $CaCl_2$ 2%) are shown in **Table 6**.

Table 6. Results of FT-IR analysis of fucoidan crude extract of *Sargassum* sp. and *Turbinaria* sp.

Vibration (cm^{-1})	Stretch	Functional group	Distilled water solvent		$CaCl_2$ Solvent	
			Sargassum sp.	*Turbinaria* sp.	*Sargassum* sp.	*Turbinaria* sp.
3200-3600	O–H	Alcohol	3421.37	3435.75	3435.73	3417.68
1622-1645	C=C	Uronic acid	1638.27	1635.39	1636.15	1638.26
910.34-1126.35/1200-1050	C–H	Fucose	1058.46	1058.71	1042.66	1061.76
599-660	O=S=O	Sulphate (significant amount)	606.00	603.25	603.25	611.55

According to a previous study [27], the absorption bands between 1622 – 1645 cm-1 indicate the presence of uronic acid. Analysis of the four fucoidan extracts revealed that they all had bands that fell into this category. Additionally, absorption bands at wavenumbers ranging from 1058.46 cm^{-1} to 1061.76 cm^{-1} were obtained, which further confirmed the presence of sulphate and CH vibrations of fucose. Fucose is known to have a

strong absorption peak in the range of 1200 – 1050 cm^{-1}, and the strong peak between 910.34 – 1126.35 cm^{-1} is indicative of fucose. The absorption band between 599 and 660 cm^{-1} indicates the presence of the O=S=O group, which was confirmed to contain significant amounts of sulphate [55].

4 Conclusions

Sargassum sp. and *Turbinaria* sp. produced a crude fucoidan extract with a higher yield using distilled water as a solvent compared to 2% $CaCl_2$ solvent. The crude extract prepared using distilled water as a solvent had a higher total sugar content and exhibited higher antioxidant activity. On the other hand, the crude extract of fucoidan with 2% $CaCl_2$ had a higher total sulphate and total phenol content. Additionally, fucoidan crude extract with 2% $CaCl_2$ demonstrated higher antioxidant activity.

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