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Characteristics and Cytotoxic Activity of Fucoidan from the Brown Seaweed *Sargassum hystrix* against MCF-7 Breast Cancer Cells

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ABSTRACT

Cancer is a disease that is considered a problem in the health sector. Cancer treatment methods still have side effects, so a safer alternative to anticancer treatment is needed. For example, fucoidan is a sulfated carbohydrate produced by brown seaweed and known to have a wide range of bioactivity, including anticancer activity. This study aimed to characterize fucoidan from the brown seaweed *Sargassum hystrix* and test its cytotoxic activity against the breast cancer cell line MCF-7. Fucoidan was extracted using distilled water at 85°C and characterized by determining the yield, total sugar, and total sulfate contents and analyzing its functional groups. Fucoidan was then subjected to a cytotoxic activity test on MCF-7 breast cancer cells based on an MTT method. Results demonstrated that *S. hystrix* fucoidan had a yield of 6.77% ± 1.47%, total sugar content of 69.35% ± 5.83%, and a sulfate content of 26.5% ± 2.73%. Functional group analysis revealed the existence of ester and sulfate in the extract, indicating the fucoidan content. Fucoidan elicited cytotoxic effects on MCF-7 breast cancer cells and had IC_{50} of 555.54 µg/mL. Fucoidan-exposed cancer cells shrank. They were round and had a limited spread pattern. Thus, *S. hystrix* fucoidan has an anticancer activity and should be further scrutinized as a potential alternative cancer therapy.

Keywords: Brown seaweed, Focoidan, Characterization, *Sargassum hystrix*, Cytotoxicity.

Introduction

Cancer is a disease that is considered a problem in the health sector in many countries and the medical field. Breast cancer is the most public cancer cases that affect women. In 2018, 2,088,849 new cases worldwide accounted for 11.60% of all newly diagnosed cancer cases.[1] In the same year, 626,679 patients died from breast cancer, and they were 6.60% of the total cancer deaths, making it the second-most frequent source of cancer mortality next to lung cancer. The occurrence of breast cancer in Western countries is higher than that in other countries, but the death rate is higher in developing countries than in other countries.[1] Breast cancer cases in Indonesia are the highest among women, i.e., 43 out of every 100,000 women.[2]
Several widely used cancer treatment methods include surgery, radiation, chemotherapy, and immunotherapy. However, these methods can cause side effects. As such, anticancer treatment methods with relatively fewer side effects should be developed. Anticancer agents obtained from natural ingredients can be used to treat the disease source by repairing damaged cells, tissues, and organs and by enhancing the immune system.[3] Various cancer treatment methods are continued to be developed; one of them is by exploring natural ingredients as anticancer agents. One of the natural ingredients that have the potential as an anticancer agent is fucoidan from brown marine algae,[4-7] such as *Sargassum hystrix*.[8] As an anti-breast cancer agent, fucoidan from *Eisenia bicyclis*, *Sargassum* sp., *Saccharina cichoriodes*,[9] *Sargassum cristaefolium*, *Turbinaria conoides*, *Padina fraseri*,[7] and *Sargassum polycystum*[10,11] has been investigated. The level of cytotoxic action of fucoidan extracted from brown marine algae is influenced by species, growth phase, geographic location, environmental conditions, and seasons.[12] However, the cytotoxic action of fucoidan from *S. hystrix* has yet to be fully explored. Therefore, this research aimed to establish the characteristics and cytotoxicity of fucoidan from *S. hystrix* against MCF-7 breast cancer cells

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Materials and Methods

Sample preparation
The sample used was the brown alga *S. hystrix*, which was obtained from Telukawur Beach, Jepara, Central Java, Indonesia, in May 2018, and identified at the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada. A voucher specimen (BS-S05) was deposited at the Department of Fisheries Faculty of Agriculture Universitas Gadjah Mada (Yogyakarta, Indonesia). It was rinsed, dried at room temperature without exposure to sunlight, stored in a jar, and wrapped in black plastic.

Fucoidan extraction
Fucoidan was extracted using the method described by Sinurat and Kusumawati.[13] Algal powder (100 g) was placed in a 2 L beaker and soaked in distilled water at 1:20 (w/v) covered with aluminum foil. The sample was stirred at 85°C for 4 h using a water bath. The mix was strained using a planktonet, and the filtrate was collected. $CaCl_2$ crystals were added to the filtrate to form a 2% concentration while stirring for 30 min at room condition. The blend was next centrifuged at 8,000 rpm and 5°C for 15 min. The filtrate was conjoined, and precipitation was removed. Then, the filtrate was joined with ethanol (1:2) and precipitated overnight. The mixture was separated between the pellet (sediment) and the supernatant (filtrate) through centrifugation at 8,000 rpm for 15 min. The collected pellets were

dissolved in aquabides until they were completely dissolved and dried with a freeze dryer.

Yield determination
The yield was gained from the ratio of the load of the fucoidan extracts to the load of the evaporated sample. The yield of fucoidan was calculated with the following formulation: Yield (%) = (Load of fucoidan/Load of dried sample) x 100%

Determination of total sugar
The total sugar contentment of the *S. hystrix* fucoidan was evaluated with a phenol–sulfate assay following the method of Dubois *et al*.[14] In our study, the examined sugars were fucose and xylose. After the standard solutions of fucose, xylose, and the sample were prepared, the phenol–sulfate solution was assayed with 2.5 mL of dense H_2SO_4 supplemented in each of the solutions, which were whipped until uniformly and subsequently cooled for 20 min. Furthermore, 0.5 mL of 5% phenol was joined to the sample and allowed to cool for 30 min. The absorbance of all the formed solutions was recorded with a UV-VIS spectrophotometer (Perkin-Elmer Lambda 25, USA) at wavelengths of 480 and 490 nm for the xylose and fucose standards, respectively.

Determination of the sulfate contentment
The sulfate level of fucoidan from *S. hystrix* was examined with a $BaCl_2$–gelatin system following the methods described by Dodgson and Price.[15] The solution of the $BaCl_2$–gelatin system was formed by dispersing 2 g of gelatin in 400 mL of hot purified water (60–70°C). Afterward, 2 g of $BaCl_2$ was dissolved in a semi-gelatin liquid, and the obtained solution was deposited at 4°C for 1 week before it was used. Sodium sulfate as a standard solution was made with a concentration of 10,000 ppm in 1 N HCl, and a series of concentrations of 200, 400, 600, 800, and 1000 ppm was made. Each concentration series was taken as much as 10 µL and inserted into a microplate well containing 190 µL of 4% TCA. The solution mixture was then added with 50 µL of the $BaCl_2$–gelatin solution and whipped for 20 min. Samples of 1 mg of fucoidan were reconstituted in 1 mL of 1 N HCl and toasted in an oven at 105°C for 4 h. The hydrolyzed fucoidan was then used as a sample to measure the sulfate residue as a standard solution. The absorbances of the standard solution and the sample were evaluated at a wavelength of 360 nm by using a UV-VIS spectrophotometer (Perkin-Elmer Lambda 25, USA). The mixture composed of Na_2SO_4 standard and commercially available fucoidan was prepared with the same method as the sample mixture.

Investigation of functional groups
The investigation of the functional groups of fucoidan was done using a Fourier Transform Infrared (FTIR) spectroscopy (Perkin-Elmer 57, USA). This method was used to know the categories of functional groups in the *S. hystrix* fucoidan. A total of 1 mg of sample was crushed by 200 mg of potassium bromide (KBr) up to homogeneous. Afterward, the powder blend was prepared into lean and transparent tablets at a pressure of 7,000 Pa. Then, the tablets were placed into a simple pan to record the infrared spectra at wavenumbers 4000 - 500 cm^{-1}.[13]

Cell cultures
MCF-7 cell line was purchased from the Central Laboratory of Pharmaceutical and Medical Technology Agency for the Assessment and Application of Technology (BPPT, Jakarta). The cells were maintained in RPMI (Sigma-Aldrich, Germany) enhanced with 10% fetal bovine serum, streptomycin, penicillin, and fugizone in a saturated incubator with 5% CO_2 at 37°C.

Cytotoxicity assay
MCF-7 breast cancer cells were subjected to a cytotoxicity assay following the process described by Somasundaram *et al*.[16] with some modifications. The assay was set on the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), a pale yellow substrate split with live mitochondria to generate a dark blue formazan product. The cells were placed in 96-well culture plates at 10^4 cells per well and then incubated in an incubator with CO_2 for 24 h. Then, several concentrations of fucoidan (0–500 µg/mL) were added to a suitable well. The plate was subsequently placed in a CO_2 incubator at 37°C and a flow of 5% CO_2 for 24 h. The medium was disposed, and 100 µL of medium-soluble MTT was joined. Afterward, the plate was re-incubated for 4 h, and purple formazan was dissolved with 100 µL of 10% SDS and allowed to stand for 12 h at room temperature. The plate was read with a microplate reader at a wavelength of 570 nm, and the proportion of cell growth inhibition was counted with the following formulation:

$$\text{Inhibition activity (\%)} = ((\text{Absorbance of cell with sample}) / (\text{Absorbance of cell without sample})) \times 100\%$$

The half-maximal inhibitory concentration (IC_{50}) was determined through probit analysis in Microsoft Excel.

Statistical analysis
Data were presented as means ± standard deviation (SD) of three repetitions and statistically analyzed through one-way analysis of variance. They were recognized significantly different when $p < 0.05$. Statistical analysis was done by SPSS.

Results and Discussion

Yield and composition of fucoidan
The yield of fucoidan was obtained from the percentage yield of seaweed extract in the form of fucoidan to seaweed powder before extraction. The yield of fucoidan obtained from *S. hystrix* was 6.77% ± 0.85% (Table 1). The yield of fucoidan from *S. hystrix* extracted using distilled water at 85°C was higher than that extracted by Suhaila *et al*.[8] with the same solvent (4.67% ± 0.23%). In another study with the same solvent, *Sargassum binderi* has a yield of 3.36%.[13] The yields of fucoidan from other *Sargassum* species are as follows: 3.75% ± 0.44% from *S. cristaefolium*,[17] 9.4% ± 1.90% from *S. cinereum*,[16] 3.68% from *S. tennerium*,[18] and 10.59% from *S. wightii*.[19] The yield obtained in this study was different from that determined by Suhaila *et al*.[8] although the same type of sample and extraction method was used because of differences in harvesting time/season. Each species of brown algae has unique physiological and biochemical mechanisms of metabolite production. The number of metabolites produced by brown algae is also directly influenced by the nutrients available in their growing environment.[20]
The total sugar was set on with a simple phenol–sulfuric acid assay. The principle of this method is that sugar is dehydrated with concentrated sulfuric acid and then hydrolyzed to produce furfural derivatives. The furfural derivative is then reacted with phenol to give a pale yellow solution. The total sugar of *S. hystrix* fucoidan and commercially available fucoidan was 69.35% ± 5.83% and 71.22% ± 5.20%, respectively (Table 1).
The total sugar level of fucoidan from *S. hystrix* and commercially available fucoidan were 69.35% ± 5.83% and 71.22 ± 5.2%, respectively. Based on the results of statistical analysis, the total sugar level of *S. hystrix* and commercially available fucoidan was not significantly different ($p > 0.05$). This result is higher than that reported by Suhaila *et al.*[8] which has a total sugar value of 58.57% ± 1.50%. This difference might be due to variations in sampling times/seasons. Other studies with similar methods but different seaweed species have obtained the following total sugar values: 76.25% for *S. binderi*,[13] 26.21% ± 0.95% for *S. wightii*,[19] 21.41% ± 0.60% for *S. cristaefolium*,[17] and 32.23% ± 1.68% for *S. cineureum*.[16] The total sugar content in each brown algal species varies because each seaweed has a heterogeneous content of fucoidan.[22]
The main framework of the fucoidan is composed of straight or occasionally branched fucose chains, where the sulfate groups are bound to the C-2, C-3, or C-4 atoms of the fucose. In the sulfate content test, K_2SO_4 was used as the standard curve. The calculation of the residual sulfate concentration was obtained from calculations using linear regression on the standard curve. The total sulfate contents of *S. hystrix* fucoidan and commercially available fucoidan were 26.5% ± 2.73% and 26.52% ± 1.35%, respectively (Table 1).

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The total sulfate contents of *S. hystrix* fucoidan and commercially available fucoidan were 26.5% ± 2.73% and 26.52% ± 1.35%, respectively. Statistical analysis revealed that *S. hystrix* fucoidan was not significantly different from commercially available fucoidan ($p > 0.05$). Suhaila *et al*.[8] used similar samples and methods and obtained a total sulfate content of 24.95% ± 0.68%. Other studies involving a similar method have obtained the following total sulfate contents: 8.10% from *S. binderi*,[13] 18.44% ± 0.44% from *Nizamuddinia zanardinii*,[23] 3.7% ± 1.54% from *S. cinereum*,[16] 11.58% ± 0.59% from *S. cristaefolium*,[17] and 16.96% ± 0.30% from *S. tenerrimum*.[18] The total sulfate contents of fucoidan from various algal species vary widely because of different biochemical and physiological mechanisms in the absorption of nutrients from their environment. The sulfate content of fucoidan is greatly affected by the differences in growth sites, sample ages, and extraction methods.[24] The sulfate content in fucoidan greatly influences its bioactivity.[25]

Investigation of functional groups

The patterns of the functional groups in *S. hystrix* fucoidan were determined through FTIR analysis. In this study, the infrared spectrum was obtained to identify the existence of sulfate ester groups as an attribute of fucoidan, which indicated sulfated polysaccharides. In addition to sulfate ester groups, the characteristic of fucoidan was amplified by a wave of 800 cm^{-1} (C–O–S) to indicate the equatorial or axial position of sulfate. Figure 1 shows the functional groups detected in *S. hystrix* fucoidan. The FTIR spectra present a strong absorption at several wavenumbers (Table 2).

The FTIR spectra of *S. hystrix* fucoidan show the absorption value following the general spectra of fucoidan.[26] The absorption at a wavelength of 3411.61 cm^{-1} denotes the presence of an O–H stretching band originating from the hydroxyl group.[27] The strong absorption at a wavelength of 2926.47 cm^{-1} corresponds to the presence of C–H bonds originating from the pyranose ring.[28] The absorption at the wavelength region of 1400–1600 cm^{-1} indicates the presence of a carbonyl group.[27] Pretsch *et al*.[26] stated that the absorption at a wavelength of about 1600 cm^{-1} refers to the possible presence of uronic acid. Fucoidan contains sulfate ester groups which are shown in the absorption area at a wavelength of 1255–1258 cm^{-1}. Fucoidan extracted from *S. hystrix* has a weak wavelength absorption because of the effects of extraction methods and unrefined fucoidan.[8] The absorption band at a wavelength of 1116.02 cm^{-1} is an indication of the presence of C–O also C–C bonds from the pyranose ring including C–O–C from the glycoside bonds.[28] The absorption at a wavelength of 820 cm^{-1} indicates a C–O–S bond.[29]

Fucoidan cytotoxicity

S. hystrix fucoidan was assayed for its cytotoxic activity against MCF-7 breast cancer cells. The inhibition of the growth of MCF-7 breast cancer cells by *S. hystrix* fucoidan, commercially available fucoidan, and doxorubicin is presented in Figure 2, and their IC_{50} is shown in Table 3. The morphological attributes of the cells were monitored under an inverted microscope after the cells were incubated. The morphological attributes of MCF-7 cells that were exposed and not exposed to fucoidan are illustrated in Figure 3.

The MTT test is a method that can be used to determine the toxic properties of a compound. The MTT test results of *S. hystrix* fucoidan, commercially available fucoidan, and doxorubicin on MCF-7 breast cancer cells (Figure 2) showed that the dose given to cancer cells was directly proportional to the inhibition of cancer cell growth. *S. hystrix* fucoidan and commercially available fucoidan with a dose of 500 µg/mL could hinder the growth of cancer cells by 47.80% ± 0.96% to 49.60% ± 1.54%, and doxorubicin (as a standard drug) at a dose of 4 µg/mL could hinder the growth of cancer cells by 94.13% ± 0.56%.

Probit analysis revealed that the crude extract of *S. hystrix* fucoidan, commercially available fucoidan, and doxorubicin had IC_{50} of 555.54, 564.13, and 1.11 µg/mL. Other studies regarding the cytotoxic activity against cancer cells have been carried out. For example, Palanisamy *et al*.[10] used a sample of *S. polycystum* fucoidan extract against MCF-7 cells and obtained IC_{50} of 50 µg/mL. Roshan *et al*.[30] utilized a crude fucoidan extract from *Fucus vesiculosus* against HepG2 hepatocyte cancer cells obtained IC_{50} of 4 mg/mL. Crude fucoidan extracted with hexane and ethyl acetate from *S. siliquosum* against MCF-7 cells has IC_{50} of 18.75 ± 5.77 and 7.16 ± 0.72 µg/mL, respectively.[31] The crude extract of fucoidan from *S. cinereum* yields IC_{50} of 75 ± 0.90 µg/mL.[16] Focoidan from *Cladosiphon okamuranus* has IC_{50} of 2 and 4 mg/mL against Huh7 and HepG2 cells, respectively.[32] Fucoidan from *F. vesiculosus* has IC_{50} of 34 µg/mL against HCT-15 cells.[33] Focoidan fraction 2 from *S. polycystum* has IC_{50} of 20 and 50 µg/mL toward MCF-7 and HCT-15 cells.[11] Focoidan from *S. cristaefolium* and *Turbinaria conoides* toward MCF-7 cells has IC_{50} of 461 and 547 µg/mL, respectively.[7]

Prasetyaningrum *et al*.[34] indicated that the cytotoxicity of a substance based on its IC_{50} is divided into three levels: potential cytotoxic (IC_{50} <100 µg/mL), moderate cytotoxic (100 µg/mL < IC_{50} <1000 µg/mL), and low cytotoxic (IC_{50} >1000 µg/mL). Furthermore, according to the National Cancer Institute,[35] a compound can be classified as a strong anticancer agent if its IC_{50} is <20 µg/mL. The cytotoxicity test on the crude extract of *S. hystrix* fucoidan showed moderate cytotoxic values. A substance with moderate cytotoxicity can be used as a chemo-preventive agent. The chemo-preventive ability indicates that the crude extracts of fucoidan can be used to prevent and hinder the growth of cancer cells also trigger apoptosis.[34]

The cytotoxic ability of the fucoidan extract from *S. hystrix* can still be improved. Tantengo *et al*.[31] demonstrated that the cytotoxic activity of purified *S. siliquosum* fucoidan (IC_{50} = 7.16 µg/mL) is better than that of the crude extract of fucoidan (IC_{50} = 25.18 µg/mL). Palanisamy *et al*.[11] conducted a similar study and found that the cytotoxic activity of purified *S. polycystum* fucoidan (IC_{50} = 20 µg/mL) is better than that of the crude extract of fucoidan (IC_{50} = 50 µg/mL). Tantengo *et al*.[31] also Palanisamy *et al*.[11] indicated that the purification stage of fucoidan likely has a positive correlation with the cytotoxic activity of fucoidan. Fucoidan is purified to eliminate components such as alginates that are usually left in crude extracts during extraction. After the extract is refined, fucoidan with higher sulfate levels is produced, possibly increasing its bioactivity.

Doxorubicin (DOX) is an anti-cyclic anticancer medicine and an important agent for the therapy of malignant breast cancer.[36] The anticancer action of DOX has been described with various molecular pathways, covering the interaction mechanism of DOX with DNA, DNA-related enzymes, and cell membranes.[37] Another study has shown that *Cladosiphon okamuranus* fucoidan has strong antiproliferative and apoptotic reactions on MCF-7 cells in certain doses and does not affect normal cell proliferation in human mammalian epithelial cells.[6] The cell pattern is a process that requires high energy and involves four sequential stages that change from the stationary stage (G0 stage) to the proliferation stage (G1, S, G2, and M stage) and return to rest.[38] Fucoidan increases the population of hepatocarcinoma (Huh7) cells at the G0/G1 stage and decreases their population at the S stage; this result indicates that fucoidan can induce the cell pattern to persist at the G0/G1 stage.[32]

Banafa *et al*.[39] explained that fucoidan can inhibit the proliferation of some cancer cells and also activate their apoptosis. In the present study, the effect of fucoidan on cell proliferation was explored through flow cytometry, which showed that fucoidan induced a significant number of MCF-7 cells at the G1 phase.

Table 1: Yield and chemical composition of fucoidan from *Sargassum hystrix* and commercially available fucoidan

	***S. hystrix* fucoidan (%)**	**Commercially available fucoidan (%)**
Yield	6.77 ± 0.85	-
Total sugar	69.35 ± 5.83[a]	71.22 ± 5.20[a]
Fucose	63.12 ± 5.25[b]	64.98 ± 5.49[b]
Xylose	6.33 ± 0.58[c]	6.24 ± 0.29[c]
Sulfate	26.5 ± 2.73[d]	26.52 ± 1.35[d]

[a–d]The same symbol in the same row indicates no significant difference ($p > 0.05$).[21]

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The accumulation of the cells at the G1 phase can be caused by genes associated with the cyclin D1, G1 phase, and CDK-4. Fucoidan also causes cells to miss their phospholipid membrane asymmetry. Phosphatidylserine exposure outside the plasma membrane was determined via annexin V–FITC tinting in MCF-7 cells. Annexin V tinting showed that fucoidan induced a significant increase in the total count of apoptotic cells. The fucoidan-induced apoptosis of MCF-7 cells was validated with a fluorescence photomicrograph of the cells tinted with Hoechst 33258. The MCF-7 cells exposed to fucoidan produced stronger bright fluorescence than the negative controls. Thus, these results proved that fucoidan can cause the apoptosis of MCF-7 cells.

To explain the fucoidan mechanism in MCF-7 cells, Banafa *et al*.[39] used Western blot and RT-PCR to check the levels of certain proapoptotic and antiapoptotic molecules. They found that their expression levels correspond to the trend of fucoidan-induced apoptosis of MCF-7 cells. The indication rate of antiapoptotic molecules, such as Bcl-2, slowly decreases, whereas the indication rate of the proapoptotic molecule Bax increases. Furthermore, Banafa *et al.*[39] explored the potency action of caspases in the cell mortality response triggered by fucoidan. Fucoidan-constrained apoptosis is associated with the gradually increasing activation of caspase-8 and cytochrome c. The activation of caspase-8 can cause the cleavage of several Bid proteins and lead to fucoidan-activated apoptosis of MCF-7 cells. Hereinafter, cell cycle-associated genes were observed through RT-PCR. The expression of cyclin D1 also CDK-4 was suppressed by dose-dependent fucoidan. These findings suggested that fucoidan-induced cell accumulation at the G1 phase is related with the decreased gene expression of cyclin D1 also CDK-4.

The differences in the morphological attributes of MCF-7 cells exposed to fucoidan and not exposed to fucoidan are illustrated in Figure 3. The morphological characteristics of MCF-7 cells exposed to fucoidan and the control cells not exposed to fucoidan differed. The morphological attributes of MCF-7 cells in the control cells not exposed to fucoidan were observed as an irregular polygonal and attached to the substrate. The morphological characteristics of the cells that were exposed to fucoidan varied, that is, the cells shrank, were round, and had limited distribution patterns compared with those of the control cells. This change in shape was consistent with that observed by Kim *et al*.[40] who stated that MC3T3 osteoblast cells exposed to fucoidan for 4 h have altered morphological characteristics, i.e., from an irregular shape to a round form with smaller sizes.

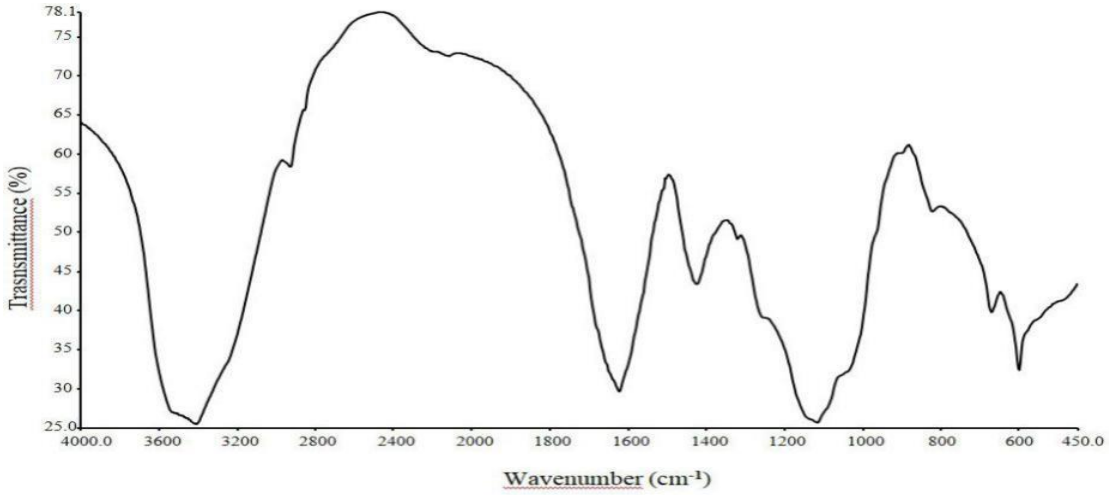

Figure 1: Infrared spectrum of *Sargassum hy*

Table 2: Elucidation of the functional group of fucoidan from *Sargassum hystrix*

Wavelength (cm^{-1})		Interpretation of functional groups[26]
***S. hystrix*[21]**	**Reference**	
3411,61	3200-3500	O-H (alcohol)
2926,47	2700-3000	C-H (alkanes)
1424,10	1350-1800	C=O (aldehyde, ketones, carboxylic acid, ester)
1116,02	1000-1300	S=O (sulfonate, sulfate)
820,93	700-850	S-O (sulfate)

Table 3: Inhibitory activity (IC_{50}) of fucoidan from *Sargassum hystrix*, commercially available fucoidan, and doxorubicin against MCF-7 breast cancer cells.

Sample	IC_{50} (µg/mL) against MCF-7
S. hystrix fucoidan	555.54[a]
Commercially available fucoidan	564.13[a]
Doxorubicin	1.11[b]

[a]The same symbol in the same row indicates no significant difference ($p > 0.05$).[21]

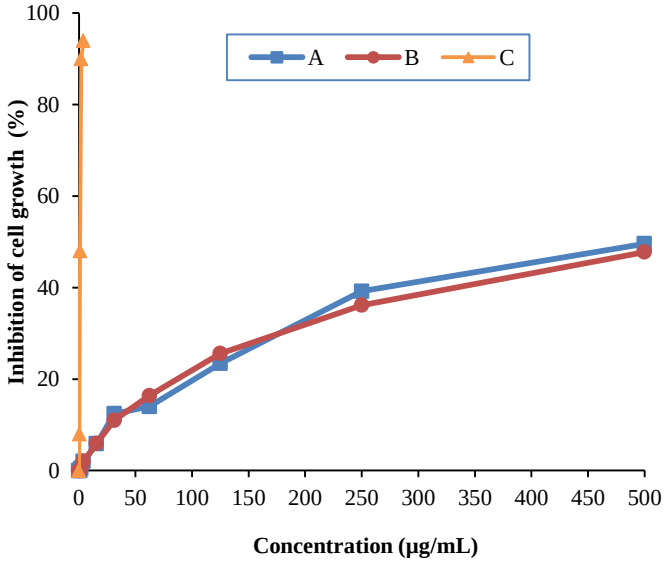

Figure 2: Cytotoxicity of *Sargassum hystrix* fucoidan (A), commercially available fucoidan (B) and doxorubicin (C) in MCF-7 breast cancer cells.[21]

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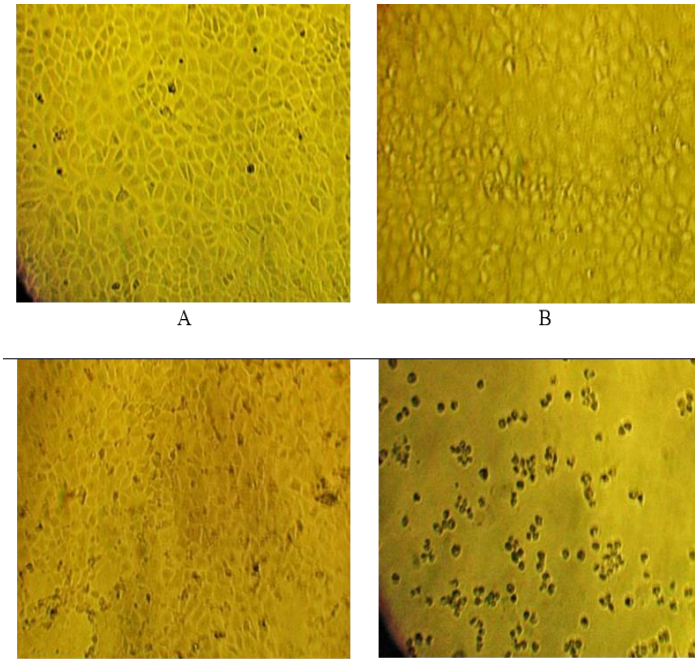

Figure 3: The morphology of breast cancer cell line MCF-7 in cells without exposure fucoidan (A), exposure of fucoidan of Sargassum hystrix 500 µg/mL (B), exposure of fucoidan commercial 500 µg/mL (C), and exposure to doxorubicin 4 µg/mL (D).[21]

Conclusion

S. hystrix fucoidan extracted with distilled water had a yield of 6.77% ± 1.47%, a total sugar of 69.35% ± 5.83%, and a sulfate content of 26.5% ± 2.73%. Fucoidan from *S. hystrix* had a growth-inhibiting activity against MCF-7 breast cancer cells at IC_{50} of 555.54 µg/mL equivalent to commercially available fucoidan (IC_{50} of 564.13 µg/ml).

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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