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Optimization of fucoidan by Microwave Assisted Extraction method using Box-Behnken Design and its potential bioactivity from Tropical Brown seaweed (Turbinaria decurrens) Indonesia

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Abstract

Fucoidan is a sulfated polysaccharide that occurs naturally in the cell wall of brown seaweeds and has substantial biological efficacy. The physical characterization of Turbinaria decurrens powder was carried out by determining the moisture content, ash content, and pH. The optimization of fucoidan extraction was applied on the brown macroalga *T. decurrens* using a Box-Behnken Design (BBD) to inspect the impacts of different power (200, 450, 700), extraction time (5, 17.5, 30 min), and solvent ratio (10, 30, 50 mL) on the yield, total sugar, and total sulfate content. The optimal conditions obtained from BBD were used for fucoidan extraction from T. decurrens. The chemical composition of extracted fucoidan was evaluated, and its bioactivity, including antioxidant potential, antidiabetic activity, and anti-*Helicobacter pylori* activity, was assessed. The RSM predicted the optimum method of fucoidan extraction was a solvent ratio of 44.5, during 24.5 minutes and 495.5-watt power. The predicted yield from the RSM was 14.00%, while the predicted total fucose and total sulfate content were 4.8 and 0.9%. The real fucoidan extracted with the optimum method has the highest yield 14.44% with fucose 5.2% and sulfate (2.0%). The extracted fucoidan has demonstrated inhibition activity of the ABTS assay (IC_{50} 50.7 ug ml^{-1}). For the inhibition of α-glucosidase enzyme activities, the IC_{50} obtained from fucoidan extract was >500 ug ml^{-1}, while the anti-Helicobacter pylori showed that fucoidan extract has inhibition activity against *Helicobacter pylori.* These findings could be used for various biomedical applications to improve the pharmaceutical industry.

Introduction

Seaweeds are a valuable natural resource with significant potential across various industries, including functional foods, nutraceuticals, pharmaceuticals, and cosmeceuticals, as well as offering environmental benefits (Gomez-Zavaglia et al. 2019). Seaweeds are categorized based on their pigment composition into three main groups, each with distinct characteristics. Red algae (Rhodophyta), such as *Glacilaria corticate*, contain sulfated polysaccharides; brown algae (Phaeophyceae), like *Sargassum ilicifolium*, are rich in omega-3 and omega-6 fatty acids like DHA and EPA; and green algae (Chlorophyta), such as *Ulva lactuca*, have high amounts of chlorophyll and ash (Premarathna et al. 2022).

Indonesia, an archipelago, is abundant in natural resources, including seaweeds. According to the Indonesian Nature Conservation Foundation, Indonesian waters host a variety of seaweed species, with 134 species of brown algae, 196 species of green algae, and 452 species of red algae found in locations such as the Seribu Islands (Jakarta), Spermode Archipelago (South Sulawesi), Maluku (Ambon), and Nusa Penida (Bali) (Basyuni et al. 2024; Nursafira et al. 2024). Among these, brown algae are the least

explored, despite its vast potential for various applications. In Indonesia, brown seaweed populations are distributed across several regions, including four genera found along the North and South coasts of Teluk Awur, with 17 species identified in Aceh and 8 species in Labuan Bajo (Jayawardena et al. 2019)

The chemical compounds found in brown tropical seaweed species such as *Padina* sp., *Sargassum* sp., and *Turbinaria* sp. vary, containing phenolic compounds, steroids, alkaloids, β-carotene, chlorophyll pigments, carotenoids, fucoxanthin, amino acids, polysaccharides like fucoidan and alginate, as well as vitamins and minerals. Brown seaweed cell walls contain polysaccharides, including laminarin, alginate, and fucoidan, with alginate being the most abundant at 49%. Among these, fucoidan exhibits the most bioactivity (Farid et al. 2013; Soamole et al. 2018; El-Beltagi et al. 2022). Fucoidan, a sulfated polysaccharide found in brown seaweed cell walls, is composed of L-fucose and sulfate groups, and qualitative analyses reveal functional groups such as carbonyl, hydroxyl, methylene, sulfoxide, and sulfate groups (Jayawardena et al. 2022). Specifically, Turbinaria species of macroalgae are known as potential biomass for health-protective compounds. In this study, the bioactive compounds studied were fucoidan compounds (Stranska-Zachariasova et al. 2017).

Fucoidan can be extracted through both conventional and non-conventional methods, with the latter, including Microwave Assisted Extraction (MAE), Ultrasound Assisted Extraction (UAE), Enzyme Assisted Extraction (EAE), and Hydrothermal Extraction (HE), yielding higher fucoidan quantities. Studies indicate that non-conventional methods, especially MAE, generate significantly higher yields compared to conventional methods. For instance, the MAE method produces 19.20% yield from *Sargassum plagiophyllum* compared to 19.10% using conventional extraction, and 56.06% from *Kappaphycus alvarezii* versus 44.53% with conventional extraction (Herawati and Pudjiastuti 2021). This method offers advantages such as reduced extraction time, enhanced yield, and an environmentally friendly process (Herawati and Pudjiastuti 2021; Kapahi et al. 2024). Fucoidan's bioactivity is influenced by its chemical structure, molecular weight, geographical origin, and extraction method. Its primary components L-fucose and sulfate groups play a critical role in its biological effects. Fucoidan derived from different brown seaweed species and origins exhibits varying levels of biological activity (Alboofetileh et al. 2019a; Tagliapietra et al. 2024). Fucoidan has shown promising bioactivities, including antibacterial effects against both gram-positive and gram-negative bacteria, inhibition of *Helicobacter pylori* colonization, antioxidant properties by scavenging reactive oxygen species (ROS), anti-inflammatory effects by reducing leukocyte infiltration, anticancer activities by inhibiting cell proliferation, and antidiabetic effects by inhibiting carbohydrate-degrading enzymes (Alboofetileh et al. 2019b; Apostolova et al. 2020; Sun et al. 2025)This study utilizes brown tropical seaweed *Turbinaria* sp. and explores the effects of varying extraction parameters, such as power, extraction time, and solvent ratio, using

the MAE method to evaluate its antioxidant, anti-*Helicobacter pylori*, and antidiabetic properties.

Materials and methods

Chemicals and reagents

The chemicals used were fucose (total sugar content standard), K_2SO_4 (total sulfate content standard), distilled water, HCl, $CaCl_2$, trifluoroacetic acid (TFA), NaOH, phenol, H_2SO_4, $BaCl_2$, gelatin, trichloroacetic acid (TCA), methanol, Folin-Ciocalteu reagent, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Kalium persulfate ($K_2S_2O_8$), Mueller Hinton agar, α-glucosidase enzyme (Megazyme, USA), PNP, Phospate buffer, Na_2CO_3 and Ultrapure water was purified using a Milli-Q system (Millipore, USA). Other analytical grade chemicals and solvents for extraction and analysis were of analytical grade and purchased from local chemical suppliers.

Samples collection and Preparation

Seaweed samples *Turbinaria decurrens*, were collected from from local fishermen at Binuangeun Beach, Banten Province, Indonesia (6∘50′ 40″ S, 105∘53′ 30″ E) in September 2024. Fresh brown seaweed (*T. decurrens*) was collected from the coastal waters of Binuangen and subsequently subjected to a cleaning process to eliminate contaminants such as other seaweed species, coral debris and sand particles. The cleaning process was performed using continuous running water until the seaweed was visibly clean and the rinse water became clear. The cleaned brown seaweed was the manually chopped and dried in a convention oven at 50°C for 24 hours. The dried material was milled and sieved to obtain brown seaweed powder with a similar particular size of 60 mesh, which was then used for further analysis.

Analysis of brown seaweed *T. decurrens* characteristics

pH

The pH was determined using methodologies from García, Hern´andez, Palacios, & Rold ´an, 2001 in Tagliapietra et al, 2024 with modifications. Using benchtop pH meter (HI 2221-02, Hanna instruments, USA), previously calibrated using buffer solutions of pH 4 and 7. For the analysis, 10 mg seaweed was weighed in 50 mL Falcon™ tubes followed by the addition of 10 mL of distilled water. Subsequently, the tubes were subjected to shaking for a period of 30 min then they were centrifuge for 6 min at 3000 rpm, and then the pH was measured in the resulting supernatant (Tagliapietra et al. 2024)

Moisture content

The analysis of water content uses the thermogravimetry, refers to AOAC 925.09 (AOAC 2005). Weighing 1 gram each macroalgae sample that has been mashed using porcelain dish which weight has been identified. The next step is drying the sample in an oven at 100°C temperature for 3–5 hours, then chilled in a desiccator, and weighed afterwards the sample is re-heating the sample in the oven for 30 minutes, then chilled in a desiccator, and weighed again. The treatment is repeated until a constant dish and sample weight is obtained with less than 0.2 mg or 0.0002 g difference, then the percentage of the water content is calculated using the equation.

$$Watercontent\,(\%) = \frac{weightofthedishandinitialsample\,(g) - weightofthedishanddriedsample\,(g)}{weightofthedishandinitialsample\,(g) - weightoftheemptydish\,(g)} x100\% \ldots (1)$$

Total ash

The analysis of ash content uses the gravimetry method. This refers to AOAC 942.05 (AOAC 2005) Each cleaned macroalgae sample is mashed and put in an oven at 80°C temperatures until dry, thereafter 1 gram of each macroalgae sample is weighed, then the fresh sample placed into the porcelain crucible. The analysis is continued by annealing the sample to wrap at 500°C temperatures until the color changes into white. Afterwards the crucible is placed into the desiccator until the temperature is lowered, weighing the obtained ash, then comparing the ash content in fresh sample and processed sample using the following equation.

$$Ashcontent = \frac{weightofthedishandprocessedsample(g) - weightoftheemptydish(g)}{weightofthefreshsample} x100\% \ldots\ldots(2)$$

Extraction of fucoidan

Various weight of samples was dissolved in 100 mL of aquadest, then the filtrate obtained from the extraction was filtered through planktonet 200 mesh. $CaCl_2$ were added to the filtrat obtained from the filtration process to precipitate the alginate, then the filtrate was stirred for 30 minutes. The filtrate containing the alginate prepicatate was subjected to centrifugation to ensure complete phase separation. The resulting supernatant was subsequently collected and freeze-dry to obtained the crude extract of fucoidan (Sinurat and Kusumawati 2017).

$$Extractionyield\,(\%) = \frac{Weightofextractedpolysaccharide\,(g)}{Weightofseaweedpowder\,(10g)} x100\%$$

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Optimization of fucoidan extraction

The Box-Behnken Design (BBD) from the response surface method (RSM) was used to determine the optimum condition to extract fucoidan from brown seaweed *Turbinaria decurrens* for this study (Table 1). The optimal condition was use for fucoidan extraction from brown seaweed *Turbinaria* sp. The parameters and levels that were used to analyze the optimum conditions is energy (200,450, and 700 watt), extraction time (5, 17,5, and 30 min) and the solvent ratio (10, 30, 50 mL). The temperature used for this study is 80 ℃. Thirteen treatments were included in the total number of experiments. The introductory experiments were performed to detect that control the fucoidan yield.

Table 1
The Box-Bhenken Design from response surface methodology (RSM) using levels of independent central composite design (CCD) variables for optimization of fucoidan extraction with MAE method.

Run	Temperature (℃)	Power (watt)	Extraction time (minutes)	Solvent ratio (mL)
1	80	450	17.5	30
2	80	450	5.0	50
3	80	700	5.0	30
4	80	200	17.5	10
5	80	200	17.5	50
6	80	700	17.5	50
7	80	200	5.0	30
8	80	450	5.0	10
9	80	450	30.0.	50
10	80	700	30.0	30
11	80	700	17.5	10
12	80	450	30.0	10
13	80	200	30.0	30

Analysis of fucoidan characteristics

Total sugar analysis

Total polysaccharides were analyzed using phenol-sulfuric acid method (Sinurat and Kusumawati 2017). Fucoidan extract 10 mg dissolved in 10 mL purified water, subsequently add 2,5 mL H_2SO_4 and 0,5 mL of phenol 3%. The mixture was incubated for

30 minutes and subsequently measured using a UV spectrophotometer (UV-2550, Shimadzu Co., Tokyo, Japan) at a wavelength of 480–490 nm. L-fucose (Sigma Aldrich Co., St. Louis, MO, USA) were used as the standard.

Sulfate content analysis

Total sulfate content was determined using the barium chloride-gelatin method (Dodgson and Price 1962). The Barium chloride gelatin solution was prepared by weighing one gram of gelatin and dissolved into the 200 mL of hot water 60℃ and allowed to stand over night at 4 ℃ before use. One gram of barium chloride was added to gelatin solution and left to stand for two to three hours. The fucoidan extract were dissolved in 1 M HCl 0.1 N and hydrolyzed for 5 hours at 110℃. 200 ul of TCA 3% was added to microplate 96-well, subsequently add 10 ul of hydrolyzed fucoidan extract then add 50 ul of barium chloride-gelatin solution. Incubated for 30 minutes and measure using an ELISA Reader (Varioskan Flash, Thermo Scientific) at 360 nm. K_2SO_4 were used as a standard.

Fourier transform infrared spectroscopy (FTIR)

FT-IR spectra of the pellet samples were collected using NicoletTM iS5 (Thermo scientific, Madison, WI, USA). Fourier transform mid-infared spectrometer equipped with diamond crystal attenuated total reflectance (ATR) accessory (iD7 ATR, Thermo scientific Madison, WI, USA). Single beam reflectance spectra were recorded in the wavelength range 550–4000 cm-1 with a resolution of 4 cm-1. Air black reference used for background calibration before each measurement. During each measurement, 64 scans were performed and averaged. Spectral data were recorded using the supplied OMNIC software 9.2.98 (Thermo fisher scientific Inc., USA). Each sample was measured in triplicate and the mean value was acquired for chemometric analysis (Zhao et al. 2021).

High performance liquid chromatography (HPLC)

Fucoidan extract were hydrolyzed using trifluoroacetic acid (TFA). UV-VIS detector and two LC pumps on the HPLC-Agilent 1100, United States. 4.6 mm x 250 mm x 5 m C18 column manufactured by Agilent were used for the analysis. Agilent ChemStation was use to collect and analyze chromatograms. At 35℃, the eluent flow rate is 1mL/min. Using linear decreases at 0,4,5, and 20 min, gradient elution was performed at 94, 94, 88, and 88% B. First, mobile phase A was 100% acetonitrile. Second, mobile phase B was a 90:10 v/v mix of distilled water and acetonitrile with 0,0045% potassium dihydrogen phosphate – 0,05% triethylamine buffer (pH 7.5). Uv detection were conducted at a wavelength of 245 nm

^{1}H nuclear magnetic resonance (NMR) spectroscopy

NMR spectra were recorded at 70 solvents ∘ C on Avance NEO (700 MHz) NMR spectrometer using Topspin 4.1.1 software (Bruker ASCEND 700Mhz, Fallanden, Switzerland). The concentration of fucoidan was 3 mg/mL in a of $D_2O + 1\%$ TSP (sodium 3-(trimethylsilyl)propionate) was used as an internal standard. The water signal was suppressed by a presaturation sequence at the water signal frequency. All pulse sequences were supplied by Bruker (Sinurat et al. 2025).

Determination of bioactivities fucoidan

ABTS assay *(2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)*

The sample were examined for ABTS assay using Sinurat et al (2025) with slight modifications. The ABTS reagent was prepared by mixing ABTS (7.4 mM) with $K_2S_20_8$ (2.6 Mm) and allowed to react for 18 hours at room temperature. The reagent was then diluted with aquadest until the absorbance 0.80 at wavelength of 734 nm. For the assay, 1 mL extract sample was prepared at concentrations 250, 500, 750, 1000, 1250 ppm. Each samples received 1 mL of the ABTS reagent and incubated at room temperature for 10 minutes before measurement (Sinurat et al. 2025).

$$ABTSradicalscavengingactivity\,(\%) = 1 - \left(\frac{Acontrol - Asample}{Asample}\right) x100$$

A control = aquadest + ABTS reagent

A sample = sample solutions

$A_{control}$ indicates the absorbance of the aquadest solution of ABTS without sample, and A_{sample} is absorbance of the aquadest solution of ABTS with tested samples.

Anti-Helicobacter pylori activity of fucoidan (Agar diffusion method)

Preparation of *Helicobacter pylori*

The *Helicobacter pylori* strain CPY6081 was inoculated into Nutrient Agar (NA) medium using the streak plate method. The petri plate was incubated at 37°C for 24 hours, after incubation, a liquid bacterial stock culture was established. The rejuvenated bacterial culture from the NA plate was transferred using a single loop into 50 mL of sterile Nutrient Broth (NB) in a 250 mL Erlenmeyer, which was sealed with cotton to maintain sterility. This culture was incubated in an anaerobic jar for 24 hours at a temperature range of 27–30°C. The resulting culture was then preserved as a bacterial stock for further experimental use.

Evaluation of anti-*Helicobacter pylori* activity (Agar diffusion method)

The anti-microbial efficacy of fucoidan against *Helicobacter pylori* (CPY6081) was assessed using the agar well-diffusion method on Mueller Hinton Agar (MHA) plates. The test organism was subcultured in Nutrient Broth and incubated at 37℃, the turbidity of the suspension was adjusted to 0,5 McFarland standard. The standardized inoculum was lawn cultured on MHA plates, after which wells of 6 mm diameter were aseptically prepared. Each well was filled with 50 ul of fucoidan extracts at concentrations 100, 75, 50, 25 mg/mL prepared in aquadest. Plates were allowed to stand at room temperature for 30 minutes to facilitate diffusion, then incubated at 37℃ for 18–24 hours. Following incubation, the antimicrobial activity was evaluated by measuring the diameter of inhibition zones (ZOI) in millimeters, with the presence of a clear zone around the wells indicating the inhibitory effect of fucoidan on *Helicobacter pylori*.

In vitro antidiabetic activity of fucoidan Inhibition of *α-glucosidase*

The inhibition activity of fucoidan against α-glucosidase was determined by measuring the amount of p-nitrophenol liberated from 4-nitrophenyl α-D-glucopyranoside according to the method Koh et al (2020) with modifications (Koh et al. 2020). A series test on various substrate (4-nitrophenyl α-D-glucopyranoside) and inhibitor (fucoidan) concentrations were conducted to determine the inhibition type. The working enzyme solution was prepared freshly prior to use by diluting the stock α-glucosidase solution (Mega zyme, Ireland) to 1 U ml^{-1} in sodium phosphate buffer (SPB, 0.1 M, pH 6.9). The concentrations of substrate solutions are 5 mM and prepared by solubilizing 4-nitrophenyl α-D-glucopyranoside into SPB. The fucoidan solutions various concentrations 31,5; 62,5; 125; 250; 500 ppm were prepared by solubilizing the fucoidan sample in SPB. Fucoidan solution of 25 ul, SPB 25 ul, and 25 ul substrate were added into the microplate and incubated for 5 minutes at 37℃, afterwards added α-glucosidase solution of 25 ul into the microplate then incubated for 15 minutes at 37℃, to allow the interactions between fucoidan and the enzyme. Subsequently, 100 ul of Na_2CO_3 0.1 M was added into the microplate to terminate the reaction, and the absorbance was read with ELISA reader (Thermo Scientific) at 400 nm. Positive control used is quercetin with various concentrations (1.25; 2.5; 5.0; 10.0 and 20.0 ppm). Negative control was prepared by replacing the α-glucosidase enzyme with SPB. A blank was prepared by replacing the sample with SPB This is to avoid any interference caused by the color of fucoidan. A standard curve of various concentrations of p-nitrophenol was constructed. The absorbance of sample reaction mixtures was fitted into the standard curve to determine the enzyme activity (Kumar and Sahoo 2017a).

Statistical analysis

The data were shown as a mean of three replicates ± SD (standard deviation). The coefficient regression for the linear and the quadratic variables as well as the interaction

between terms were determined using multiple linear regression. Analysis of variance (ANOVA) were used to validate the model. SPSS was used for the analysis. The ANOVA test was utilized to evaluate the quantitative variables, and Duncan's test ($p < 0.05$) was used to determine the differences between the variables.

Results and discussion

Properties of brown seaweed *T. decurrens* powder

Physical analysis of brown seaweed *T. decurrens* powder include moisture content, total ash and pH were evaluated. The moisture and total ash content of *Turbinaria* sp powder is analyze with gravimetric method. The result showed that the moisture content is 11.32% which is in line with the research of (Nagahawatta et al. 2021) the moisture content of *Padina arborescens*, *Sargassum autumnale*, *Sargassum thunbergii*, and *Ishige okamurae* is ranged from 8.15–11.95%. The moisture content was highly dependent on the drying temperature used. Higher temperatures accelerated water loss and reduced drying time (Gupta et al. 2011). Oven drying experiments have demonstrated that temperatures ranging from 25 to 60 ℃ are applicable, with the optimal choice depending on the seaweed species and the intenden processing objectives (Silva et al. 2019) Regulating the moisture content through appropriate drying or preservation strategies is essential for prolonging the shelf life of brown seaweed and safeguarding its freshness and quality (Subbiah et al. 2023). The total ash 9.68% which is lower than brown seaweed *Ascophyllus nodosum* 21.1% and *Sargassum latissum* 25.2% (Bojorges et al. 2025), which may be caused by the difference of environmental conditions. The ash content is widely regarded as an indicator of the total mineral composition and is strongly influenced by the environmental conditions in which the seaweed develops. The factor influence was geographical location, water temperature, nutrient availability, exposure contaminants and season and growth stage (Kumar and Sahoo 2017b; Ismail et al. 2023). The pH is 6,0, these results indicate that the resulting pH is within the neutral range, as reported to the previous studies, the intrinsic pH of brown seaweeds such as *Saccharina latissimi* and *Alaria esculenta* typically falls within a moderately neural range of 6.1 to 6.4 (Syahrir 2024). Gravimetric method involved subjecting the sample to oven drying under controlled temperature conditions, followed by quantification of moisture content based on the mass loss incurred during the drying process and pH was measured with pH meter (Martines-López et al. 2024).

Optimization of fucoidan extraction and validation of the optimum Conditions

The Box-Behnken Design (BBD) from the response surface method (RSM) was used to find the best conditions to extract fucoidan from *T. decurrens* for this study (Fig. 1).

The acquired optimized conditions were used for extraction of fucoidan from *Turbinaria* sp. The parameters and levels use for this study were solvent ratio (10, 30, and 50 mL), power (200, 450, and 700 watt) and extraction time (5, 17.5, and 30 minutes). Thirteen treatments were included in the total number of experiments. Significant differences in fucoidan content were observed among the different extraction conditions. The yield and biochemical composition of fucoidan extracted from *Turbinaria* sp is given in Table 3. Yield obtain from the fucoidan extraction process is the ratio of its extract to the weight of dry seaweed powder presented in percent (%). The fucoidan yields obtain from the optimization process with MAE method were above 7%, within the range of 4.40% and 14.74%. The highest fucoidan yield was obtained from the run 9 (14.74%) which was conducted using 450 watts of power for 30-minutes extraction, and a solvent ratio of 50 mL.

The result consistent with the result conducted by Rodriguez-Jasso (Yuan and Macquarrie 2015 and Dobrincic et al. 2021) where the fucoidan yield obtain from brown seaweeds *Ascophyllum nodosum*, *Fucus virsoides*, and *Cystoseira barbata* which was extracted with the same method, resulted in a yield of 16.08%, 13.19%, and 6.43%. Utilization of microwave power within the moderate to high range and up to 1000 W in certain applications substantially enhances extraction efficiency, thereby yielding higher concentrations of bioactive compounds (Mali and Kumar 2023), however excessive microwave power or prolonged irradiation can lead to polysaccharide degradation, manifested by the loss of sulfate groups or reduction in molecular weight, which in turn diminishes their biological activity and overall quality (Rodriguez-Jasso et al. 2011). The yield in this study was lower than that reported for the brown seaweed *Sargassum binderi Sonder* under the same solvent ratio but with a longer extraction time, which reached 58.44%. The characterization of the extracted fucoidan revealed that the total sugar content ranged between 4.88–6.85%, with the highest value was 6.85% obtained under extraction condition 450-watt power for 5 minutes and a 10 mL solvent ratio. Another research found that total sugar of fucoidan extracted from *Cystoseira barbata* using the same solvent and extraction method but with longer extraction time is 7.16%. Meanwhile, the sulfate content of extracted fucoidan range from 0.33% − 8.53%, with the highest sulfate concentration was 8.53% achieved after 700-watt power used for 17.5 minutes and 10 mL solvent ratio. This result was higher than the sulfate content obtained from fucoidan extracted from *Sargassum binderi Sonder* under UAE conditions with the same solvent but longer extraction period (Sinurat et al. 2025). Extending extraction time enhances solvent penetration and promotes cellular disruption, thereby facilitating polysaccharides release. Nevertheless, excessively prolonged exposure can induce thermal degradation and cleavage of glycosidic linkages, ultimately reducing both yield and biological activity (Tran et al. 2024), at the initial stages, extending extraction time tends to increase polysaccharide yield, as prolonged contact promotes deeper solvent

penetration into the biomass and facilitates a more effective release of polysaccharides from a raw material [40].An increased solvent-to-solid ratio typically enhances the recovery of bioactive compounds by facilitating more efficient mass transfer (Bhadange et al. 2024).

Table 2
Model fit statistics of fucoidan yield, total fucose and total sulfate content

Parameters	Model of yield	Model of fucose	Model of sulfate	Interpretation
Std. Dev	1.00	0.11	0.49	-
Mean	14.0	5.37	2.01	-
C.V (%)	6.54	2.05	27.71	-
R^2	0.98	0.99	0.98	-
Adjusted R^2	0.95	0.98	0.96	-
Predicted R^2	0.56	0.88	0.90	Less than 0.2
Adeq Precision	23.11	31.02	19.80	> 4 strong signals to noise ratio

The optimal extraction conditions for fucoidan yield, total fucose and total sulfate content were generated when solvent ratio, power and extraction time were kept within ranges. Three variables (solvent ratio, power and extraction time) were tested for their effects on the yield, total fucose, and total sulfate content of fucoidan using BBD from the response surface methodology (Fig. 2). The result of fit statistics (Table 2) shows that the coefficient of determination (R^2) for model of yield, model of fucose and model of sulfate was 0.98, 0.99, and 0.98. The adjusted R^2 was 0.95, 0.98, and 0.96 was close to the R^2 value, indicating that the model is appropriate and does not exhibit overfitting, considering the number of variables involved. Furthermore, the predicted R^2 from the model of yield was 0.56 which means that the difference from the R^2 is more than 0. Suggesting that the model has limited predictive capability, the predicted R^2 from model of fucose and sulfate was 0.88, and 0.90 with a difference of less than 0.2. This indicates that the model possesses good predictive ability for new data points. The adequate precision value from each model was above the recommended threshold of 4, demonstrating that the signal-to-noise-ratio is sufficient and the model can be reliably used for design space navigation.

The predicted response for the extraction conditions based on the numerical graph from Response Surface Methodology shows at Fig. 3. The predicted optimal parameters for fucoidan extraction were 600-watt power and 50 mL solvent ratio that predicted obtain

fucoidan yield was 26.61% total fucose content was 5.4% and total sulfate content was 0.4%. The actual result of the highest fucoidan yield obtained were 27.74%, with 6.85% total fucose and 8.53% total sulfate content (Table 3).

Table 3

The yields, total fucose, and total sulfate (SO_3^-) response of Box Behnken Design from response surface methodology (RSM)

Run	Factor			Response		
	A.Solvent Ratio	B.Power	C.Time	Yields	Total fucose	Total sulfate
	mL	watt	Minutes	%	%	%
1	30.0	450.0	17.5	10.5	4.8	1.3
2	10.0	700.0	30.0	12.4	6.7	8.5
3	50.0	700.0	5.0	15.7	4.9	0.6
4	1.7	450.0	17.5	5.5	3.8	1.3
5	30.0	450.0	35.2	11.3	5.1	5.9
6	50.0	200.0	30.0	7.8	6.4	0.9
7	30.0	803.6	17.5	10.7	6.8	0.5
8	30.0	450.0	17.5	12.3	4.8	0.3
9	10.0	200.0	5.0	7.7	4.9	0.4
10	30.0	450.0	0.2	4.4	5.1	3.8
11	58.3	450.0	17.5	14.7	5.1	1.2
12	30.0	96.4	17.5	7.8	6.4	0.9
13	30.0	450.0	17.5	12.1	5.0	0.5

Based on the yields and total fucose of the extracted fucoidan, there was no noteworthy difference between the predicted and actual, but the result of total sulfate significantly different from the software prediction. Consequently, BBD might be used to successfully optimize the fucoidan yield, and its total fucose and sulfate content from *Turbinaria* sp.

A practical and affordable process for extracting polysaccharides that produce high yields and maintain the original fucoidan structure is preferable. The fucoidan yields reach the optimum yield at 450-watt power and decreases at the higher power. The yields of fucoidan effected by the solvent ratio and extraction time, where the p-value for solvent ratio, power and extraction time were < 0.0073, 0.2621 and 0.0161, respectively, on the

other hand, the fucose content of the extracted fucoidan was significantly effect by the solvent ratio, where the p-value for solvent ratio, power used and extraction time was < 0.0036, 0.0828, and 1.0000, subsequently the total sulfate content of the extracted fucoidan effected by the time extraction, where the p-value were < 0.8935, 0.5990, and 0.0402 (Table 4).

Table 4
Analysis of variance for the effects of solvent ratio, power and time extraction on the yield, total fucose, and sulfate content of extracted fucoidan using the quadratic response surface model

Source	Sum of squares	df	Mean square	F-value	p-value	Significance
Model of yield	9.81	8.00	17.83	7.21	0.0368	*
A-Solvent ratio	0.84	1.00	62.72	25.37	0.0073	**
B-Power	0.08	1.00	4.20	1.70	0.2621	
C-Time	0.00	1.00	39.61	16.20	0.0161	*
AB	1.36	1.00	14.54	5.88	0.0723	
AC	0.00	1.00	1.53	0.62	0.4754	
BC	0.51	1.00	38.02	15.38	0.0172	*
B^2	5.40	1.00	1.64	0.66	0.4615	
C^2	0.13	1.00	19.75	7.99	0.0575	*
Residual	0.03	4.00	2.47			
Lack of fit	0.00	2.00	3.97	4.08	0.1969	Not significant
Pure error	0.02	2.00	0.97			
Model of fucose	9.81	9.00	1.09	89.67	0.0017	**
A-Solvent ratio	0.84	1.00	0.85	69.51	0.0036	**
B-Power	0.08	1.00	0.08	6.58	0.0828	
C-Time	0.00	1.00	0.00	0.00	1.0000	
AB	1.36	1.00	1.36	111.97	0.0018	**
AC	0.00	1.00	0.01	0.73	0.4569	
BC	0.57	1.00	0.57	47.02	0.0063	**
A^2	0.22	1.00	0.23	18.90	0.0225	*
B^2	5.40	1.00	5.40	444.24	0.0002	**
C^2	0.13	1.00	0.13	11.15	0.0444	*

Source	Sum of squares	df	Mean square	F-value	p-value	Significance
Residual	0.04	3.00	0.01			
Lack of fit	0.01	1.00	0.01	0.74	0.4815	Not significant
Pure error	0.03	2.00	0.01			
Model of sulfate	75.66	8.00	9.46	38.44	0.0016	**
A-Solvent ratio	0.01	1.00	0.0	0.02	0.8935	
B-Power	0.08	1.00	0.08	0.33	0.5990	
C-Time	2.20	1.00	2.20	8.96	0.0402	*
AB	3.69	1.00	3.69	14.98	0.0180	*
AC	8.75	1.00	8.75	35.55	0.0040	**
BC	6.59	1.00	6.59	26.77	0.0066	**
B^2	0.16	1.00	0.16	0.67	0.4595	*
C^2	25.68	1	25.68	104.36	0.0005	**
Residual	0.9843	4	0.2461			
Lack of fit	0.4243	2	0.2121	0.7576	0.5690	Not significant
Pure error	0.5600	2	0.2800			

*$P < 0.05$

**$P < 0.01$

The derived equation in terms of coded factors is provided below:

Fucoidan Yield $= 10.22 + 0.4596A + 0.1414\ B + 0.00C$ -0.8250 $AB + 0.0664\ AC + 0.5346\ BC$

Fucose content $= 4.85 + 0.\ 120155\ A - 0.010311\ B - 0.116579\ C - 0.000165\ AB + 0.000266\ AC - 0.1838\ A^2 + 0.8912\ B^2 + 0.1412\ C^2$

Sulfate content $= 0.9200 - 0.0354\ A - 0.1414\ B + 0.7425\ C - 1.36\ AB - 2.09\ AC + 1.81\ BC - 0.1538\ B^2 + 1.92\ C^2$

Where A is referred to Solvent ratio, B is referred to Power used for extraction, and C is referred to extraction time.

Based on the findings, the fucoidan yield obtained through the MAE method was significantly higher than that achieved with the UAE method, indicating the superior extraction efficiency of MAE. The fucoidan yield obtained through the MAE method has been widely reported in seaweed studies, as this technique is efficient, environmentally friendly, and capable of preserving the structure of the extracted compound (Gonzaga et al. 2025). MAE works by creating an interaction between microwave radiation and the sample matrix, which accelerates the extraction process and makes it more effective to those who sensitive to heat (Gonzaga et al. 2025). The crude extract of fucoidan shown a variation in color intensity, primarily influenced by extraction parameters such as the the time extraction and the solvent ratio. Prolonged extraction time has been shown to increase color intensity, likely due to the extended contact between the solvent and the sample matrix, which enhances the diffusion of pigment compound until the solvent approaches its saturation point (Husni et al. 2022).

Fucoidan FT-IR spectroscopy analysis

The result of FT-IR spectroscopy analysis presented (Fig. 4) that the fucoidan obtained from the MAE extraction method showed the same peak as the commercial fucoidan (*Fucus Vesiculosus*). The peak at 3729–3732 cm^{-1} showed the stretching O-H free, 3442–3443 cm^{-1} showed weak O-H. 1616, 1621, and 1626 cm^{-1} showed the carboxyl group (O-C-O), 1216 and 1237 cm^{-1} showed stretching sulphate groups. 1011, 1054 and 1056 cm^{-1} showed asymmetric ester sulfate. 527, 533, and 550 cm^{-1} showed asymmetric deformation of sulfate. The sulfate groups show the main functional groups of fucoidan 827 cm^{-1} from *fucus vesiculosus* shows the axial of C4 positions of fucose (Mohd Fauziee et al. 2021) FT-IR measurements determined which functional groups were present in the fucoidan generate from brown seaweed *T. decurrens*. The peak showed that the extracted fucoidan obtained from the MAE method was not pure. It is hypothesized that the observed peak is attributable to the carboxylate group of uronic acids present within the structure of fucoidan. The FTIR spectral region between 1850 and 1500 cm^{-1} is typically associated with carbonyl stretching vibrations, including C = O and conjugated C = C functional groups (Sinurat et al. 2025).

HPLC analysis

The monosaccharide compositions of brown seaweed *T. decurrens* were analyze with High-Performance Liquid Chromatography (HPLC). High-performance liquid chromatography (HPLC) was employed to characterize and determine the concentrations

of bioactive constituents in brown seaweed extracts, with emphasis on polyphenolic compounds and carbohydrate-derived metabolites

The crude fucoidan were hydrolyzed with TFA (trifluoroacetic acid) in order to liberate the monosaccharides units embedded within the fucoidan structure, a defined hydrolysis mechanism is to protonates the glycosidic oxygen, which increase the bonds reactivity and promotes its cleavage via nucleophilic attack by water, allowing the effective release of individual monosaccharides from the fucoidan backbone. The HPLC result of fucoidan extracted from *Turbinaria* sp (Fig. 5) was only shows a single sharp peak at 5 minutes retention time, indicating that the analyzed fucoidan was relatively homogenous and predominantly composed of one major monosaccharide, most likely fucose, which is the characteristic of fucoidan. *T.conoides* and *P. tetrastromatica* contain monosaccharides such as fucose, galactose, arabinose, glucose, glucuronic acid, mannitol, mannose, rhamnose and xylose (Hans et al. 2023).

^{1}H nuclear magnetic resonance (NMR) spectroscopy

In the ^{1}H NMR spectrum (Fig. 6), the signals corresponding to the anomeric protons (H-1) were observed between 5.50 and 5.00 ppm, originating from both α-L-fucose and β-D-sugar residues.

The spectrum also contained resonance characteristics of L fucopyranose ring protons (H-2 to H-5) ranging between 3.5 and 4.6 ppm. The sulfated ring protons could be localized between 4.60 and 4.10 ppm whereas from 4.20 to 3.50 ppm were of unsulfated pyranose ring protons. The presence of intense peaks of the methyl groups were observed between 0.8 and 1.4 ppm. The presence of intensive signal at around 4.19 ppm was indicated to the presence of *α–(*1 → 3)-linked-*L*-fucose residues, whereas the signal at around 3.94 ppm were assigned to (1 → 4)-linked-fucose residues. Signals observed between 2.20 and 2.40 ppm indicate the presence of O-acetyl functional groups. The acetyl groups can be found at various positions depending on the specific fucoidan. Acetyl groups attached to other fucose units, such as at C-4 of 3-linked fucose or C-2 of 1,2,3-linked fucose.

Determination of bioactivities fucoidan

ABTS assay *(2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)*

Table 5 presented the ABTS assay result for fucoidan extracted from *T. decurrens*, indicating variations in % for %inhibition and ug/ml for IC_{50} values under power and extraction times variations. The reaction mechanism of ABTS (2,2’-azino-bis (3-ethylbenzothiazoline-6-sulforic acid) reagent is converted into a stable radical cation through oxidation by potassium persulfate ($K_2S_2O_8$) (Cano et al. 2023). The result was indicated that the percentage of inhibition increased proportionally with the concentration

used of fucoidan extract (250–1250 ppm). The extract shows a strong radical scavenging activity, as the result of the IC_{50} obtained is 50.7 ug/ml for fucoidan obtained at 495.5 W for 24.5 minutes. Although fucoidan obtained from 495.5 W for 24.5 minutes have a higher yield (14.7%), but the fucoidan obtained have a higher sulfate content (8.53%) and have a slightly lower IC_{50} is in line with the study of (Husni et al. 2022) whereas *Sargassum hystrix* extracted with 85% ethanol have a lower yield but higher antioxidant activity than the *Sargassum hystrix* extracted with 0.1 N HCl who obtained the higher yield, this enhanced activity may be attributed to the co-extraction of secondary antioxidant compounds. Fucoidan enriched in sulfate groups demonstrates superior radical scavenging capacity, a property fundamental to their antioxidant activity. These findings are in agreement with previous report (El Rashed et al. 2021) that fucoidan extracted from *Ferula harmonis* have a strong antioxidant activity evaluated with ABTS assay, the IC_{50} obtained is 44.26 ug ml^{-1}.

Table 5. Antioxidant activity of fucoidan extracted from *T. decurrens*

Sample	Variable		Concentrations (ppm)	ABTS Assay	
	Power (watt)	Extraction time (minutes)		% Inhibition	IC_{50} (ug ml^{-1})
Extract Fucoidan from *T.decurrens*	495.5	24.5	250	3.7 ± 0.0	50.7
			500	10.9 ± 0.0	
			750	24.0 ± 0.1	
			1000	36.0 ± 0.0	
			1250	50.7 ± 0.0	
			500	18.4 ± 0.0	
			750	31.3 ± 0.0	
			1000	$42.8 \pm 0,0$	
			1250	$57.4 \pm 0,0$	

Anti-*Helicobacter pylori* activity of fucoidan

Fucoidan exhibits antimicrobial activity, as evidenced by the formation of inhibition zones (mm) on petri dishes against both gram positive and gram-negative bacteria. El-Sheekh et al (2024) reported that fucoidan derived from several species including, *Turbinaria turbinata, Sargassum cinerum, Padina pavonica* and *Dictyota dicotoma* produce strong

inhibition zone exceeding 20 mm against gram negative such as *Klebsiella pneumoniae* and *Escherichia coli* (El-Sheekh et al. 2024). Furthermore, (Table 6) summarizes the anti-*Helicobacter pylori* properties of fucoidan with various extraction condition. The results are expressed as inhibition zone diameters (mm) against *Helicobacter pylori*.

Table 6
Anti-gastric ulcer activity of fucoidan extracted from Brown Seaweed *T. decurrens*

Sample	Variable		Concentrations (mg mL^{-1})	Inhibition zone (mm)
	Power (watt)	Extraction time (minutes)		
Extract fucoidan from *T.decurrens*	495.5	24.5	25	-
			50	-
			75	3.6
			100	4.0

The anti-*Helicobacter pylori* activity from fucoidan is associated with its structural similarity to lewis b antigen, which normally serve as the binding site for *Helicobacte*r pylori. By mimicking this structure fucoidan have a same structure with lewis b antigent where the *Helicobacter pylori* usually get attach (Magalhes et al. 2010; Chua et al. 2015). Experimental results demonstrated that fucoidan extracted under 450-watt power for 24.5 minutes extraction exhibited inhibitory activity at concentrations starting from 75 mg mL^{-1}, producing a 3.6 mm inhibition zone, and showed slightly stronger activity at 100 mg $mL^{-1}$with a 4.0 mm zone. Since inhibition zones were < 5 mm, the findings indicate that fucoidan extracts from both treatments possessed relatively weak anti-*Helicobacter. pylori* activity. The weak inhibitory may be attributed to the relatively low sulfate content in both extracts. The extent sulfation represents a critical structural parameter that significantly modulates the antimicrobial efficacy of polysaccharides. Sulfate group give polysaccharides a negative charge, which allows them to attach to bacterial surfaces or capture nutrients (Ayrapetyan et al. 2021).

Antidiabetic activity of fucoidan

The result of antidiabetic activity of fucoidan against α-glucosidase enzyme shows in (Table 7). The Fucoidan extract obtained after 450-watt power for 24.5 minutes extraction have an IC_{50} value > 500 ug mL^{-1} due to its low sulfate content fucoidan from *Padina pavonica*, as reported by (Akl et al. 2025) exhibit 75% inhibition at a concentration of 10000 ug/mL and contained a sulfate content of 9.52%. This finding demonstrates that the

total sulfate content influences the inhibitory activity against α-glucosidase and α-amylase.

Table 7
The inhibition of α-glucosidase enzyme activity of fucoidan extracted from *T.decurrens*

Sample	Variable		Concentrations (ppm)	α – glucosidase inhibitor	
	Power (watt)	Extraction time (minutes)		% Inhibition	IC_{50} (ug ml^{-1})
Extract Fucoidan from *T.decurrens*	495.5	24.5	31.5	6.5 ± 6.3	> 500
			62.5	8.6 ± 13.6	
			125.0	10.7 ± 29.5	
			250.0	11.7 ± 6.0	
			500.0	12.3 ± 2.3	

Both crude and purified fucoidan extracts have been reported to exhibit strong inhibitory activity against the starch-hydrolyzing enzymes α-amylase and α-glucosidase. Recent investigations have demonstrated that fucoidan enhances insulin sensitivity and attenuates postprandial elevations in blood glucose, thereby mitigating hyperglycemia *in vivo* (Daub et al. 2020) (Mabate et al. 2021) The presence of sulfate groups enhances the inhibitory potential of fucoidan against carbohydrate-hydrolyzing enzymes, thereby attenuating postprandial elevations in blood glucose levels (Xing et al. 2015). Fucoidan is a competitive inhibitor of α-glucosidase enzyme, as previously reported by (Daub et al. 2020; Fajriah et al. 2021) (Koh et al. 2019) (Siratantri et al. 2014) that fucoidan extracted from *Ecklonia maxima*, *Caleurpa lentillifera*, *Undaria pinnatifida* shows an inhibition by showing the IC_{50} value 0.29 g L^{-1}; 0.253 g L^{-1} dan 0.137 g L^{-1}.

Conclusion

The study aimed to investigate the optimal variation conditions for fucoidan extraction with MAE as characterizes by its total sulfate and total sugar content and evaluate the potential bioactivity of fucoidan including antioxidant activity, anti-*Helicobacter pylori* activity, and anti-diabetic activity. The predicted yield from the RSM was 14.00%, and the predicted total fucose and total sulfate content was 5.4% and 0.4%. The actual data from this study was similar result, where the yield was 14.4%, the highest fucose and sulfate content were 6.66% and 8.53%. Based on the numerical graph from the RSM the optimal extraction condition was with 495.5-watt power and 24.5 mL solvent ratio. Fucoidan

exhibits strong antioxidant activity as demonstrated by its radical scavenging effect against the ABTS reagent, the IC_{50} obtained from fucoidan extracted with 450- watt power, extraction time 24.5 minutes was 50.7 ug mL^{-1}. The inhibition to α-glucosidase enzyme activities, the IC_{50} obtained from fucoidan extract was >500 ug ml^{-1} due to its low sulfate content ($<9\%$), the fucoidan extract displaying only limited antibacterial efficacy against *Helicobacter pylori*.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

ES , HEI, and SI Funding acquisition, project administration; ES, SY conceived the research idea. ES, HEI. EWS, SY, YA , and SI methodology, data curation, formal analysis, analyzed the data, investigation, and drafted the manuscript. Provided valuable insights during the manuscript writing process. ES, SY, EWS, YA and SI resources, software, supervision, validation, visualization, revised the manuscript. All authors read and approved of the final manuscript.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

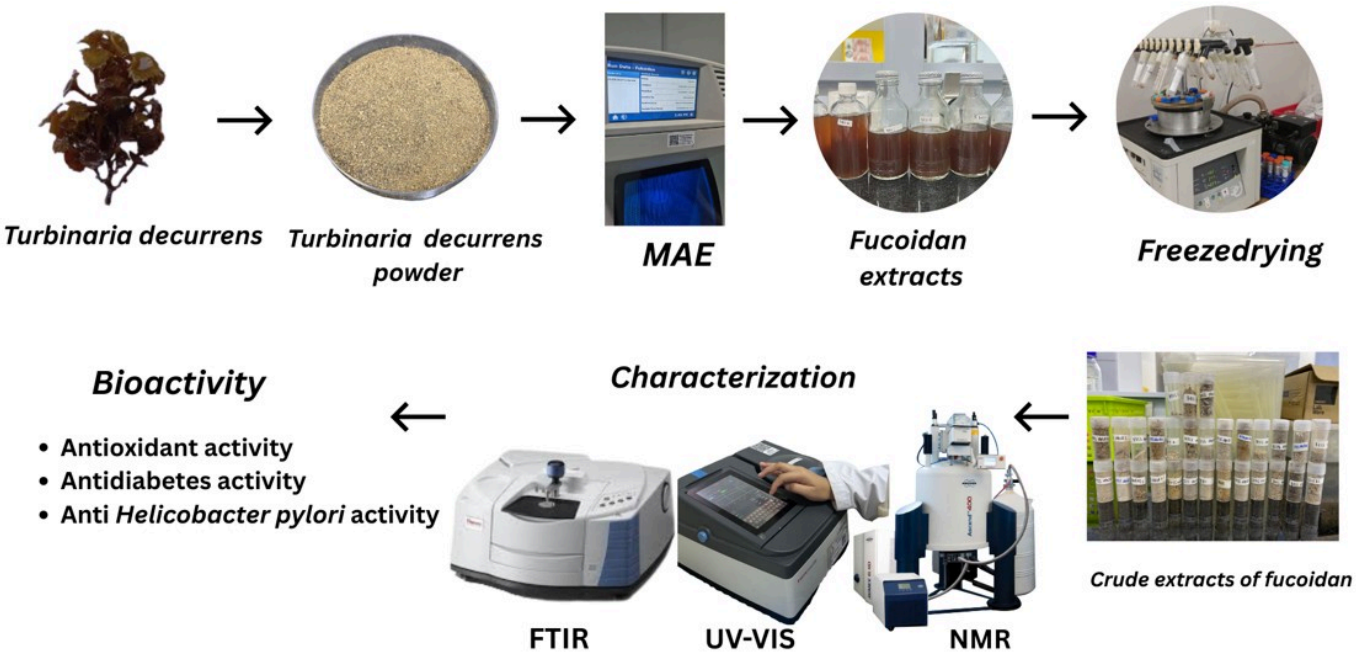


Figure 1

Graphical abstract of optimization fucoidan extraction using Microwave Assisted Extraction and its bioactivity

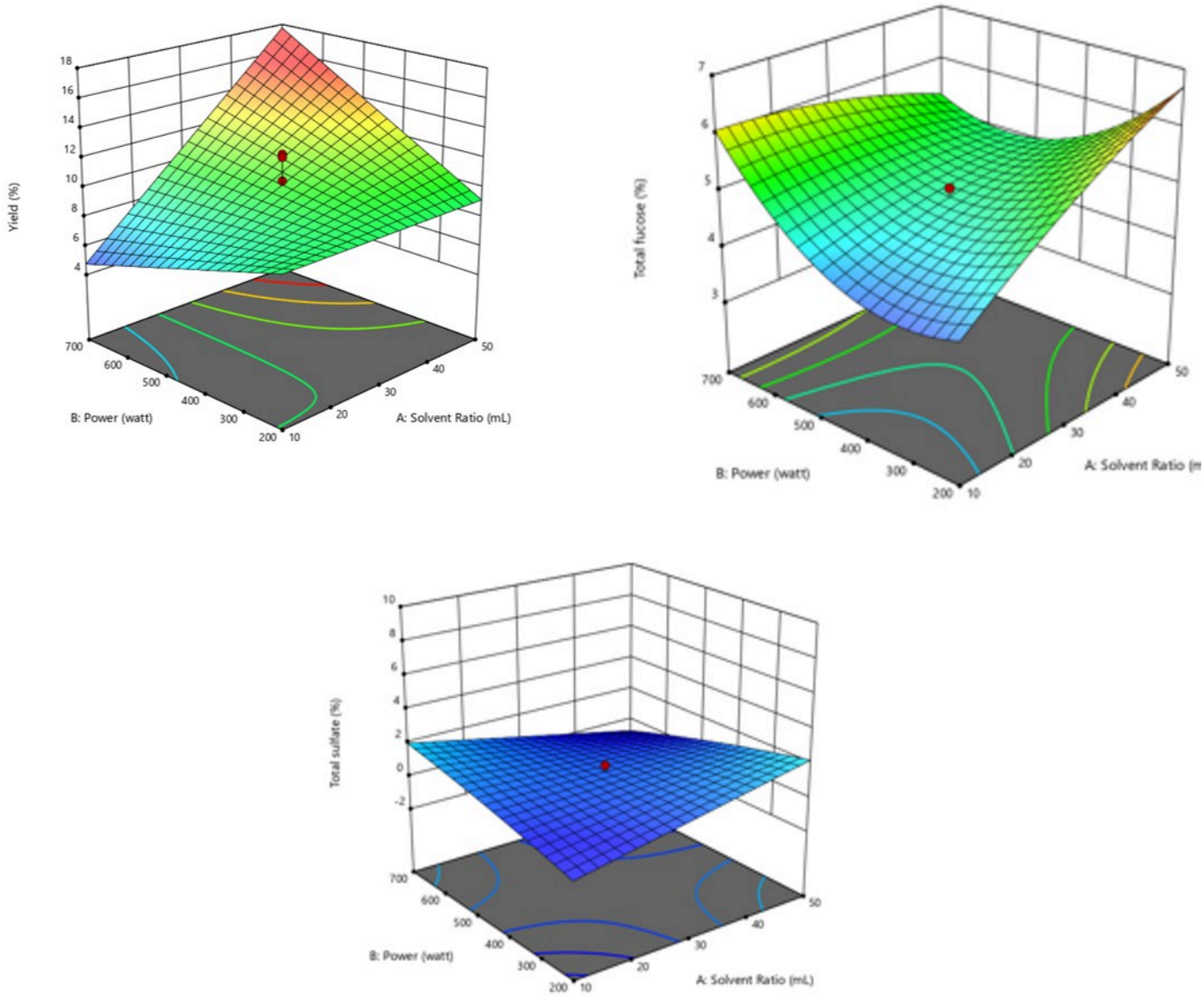


Figure 2

Response Surface Methodology graph showing the mutual effects of extraction conditions A: Solvent ratio, B: Power, and C: Extraction time on fucoidan yield, fucose content and sulfate content.

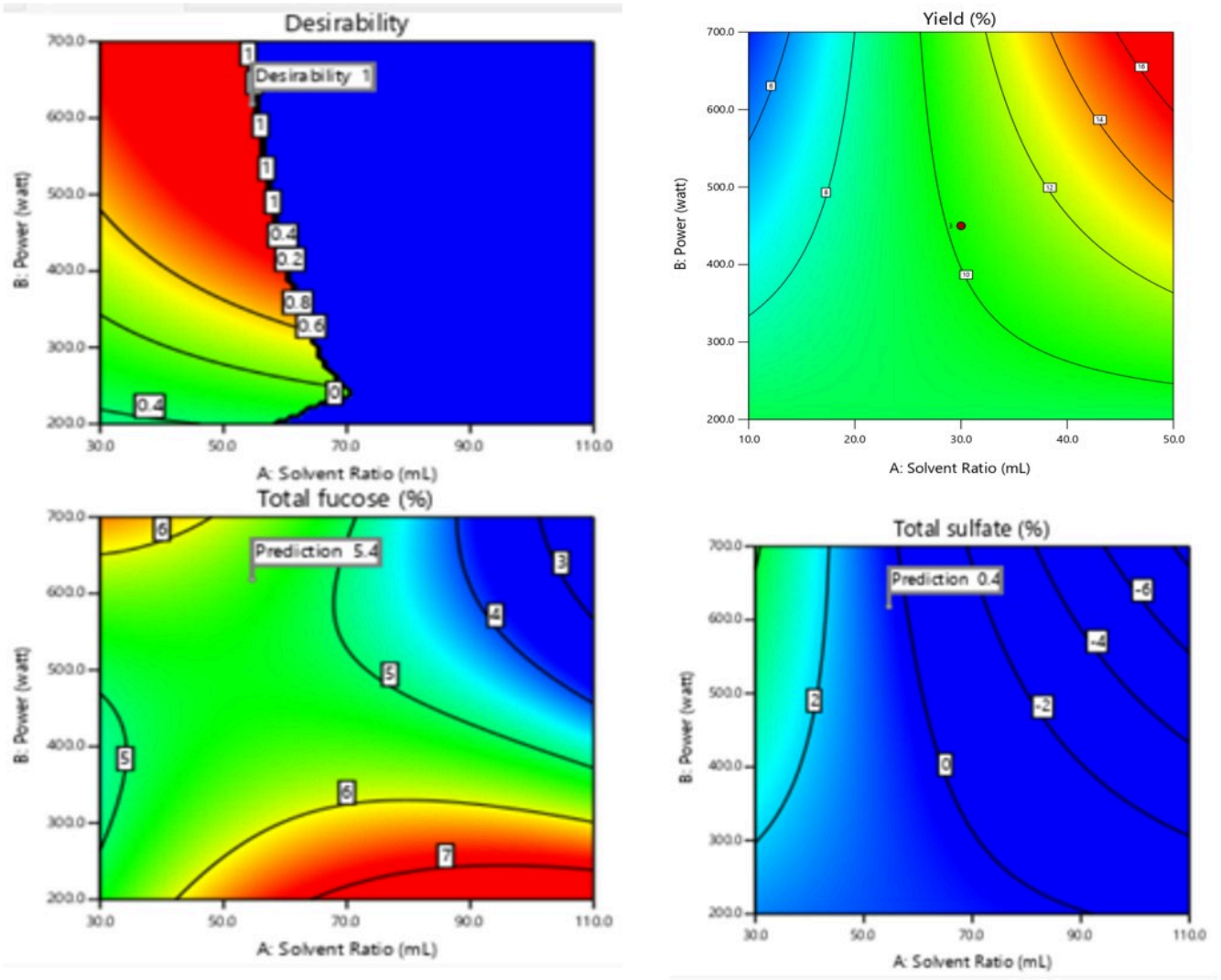


Figure 3

Response Surface Methodology prediction numerical graph of extraction conditions A. Solvent ratio, B: Power, C: Extraction time on fucoidan yield, fucose content, and sulfate content

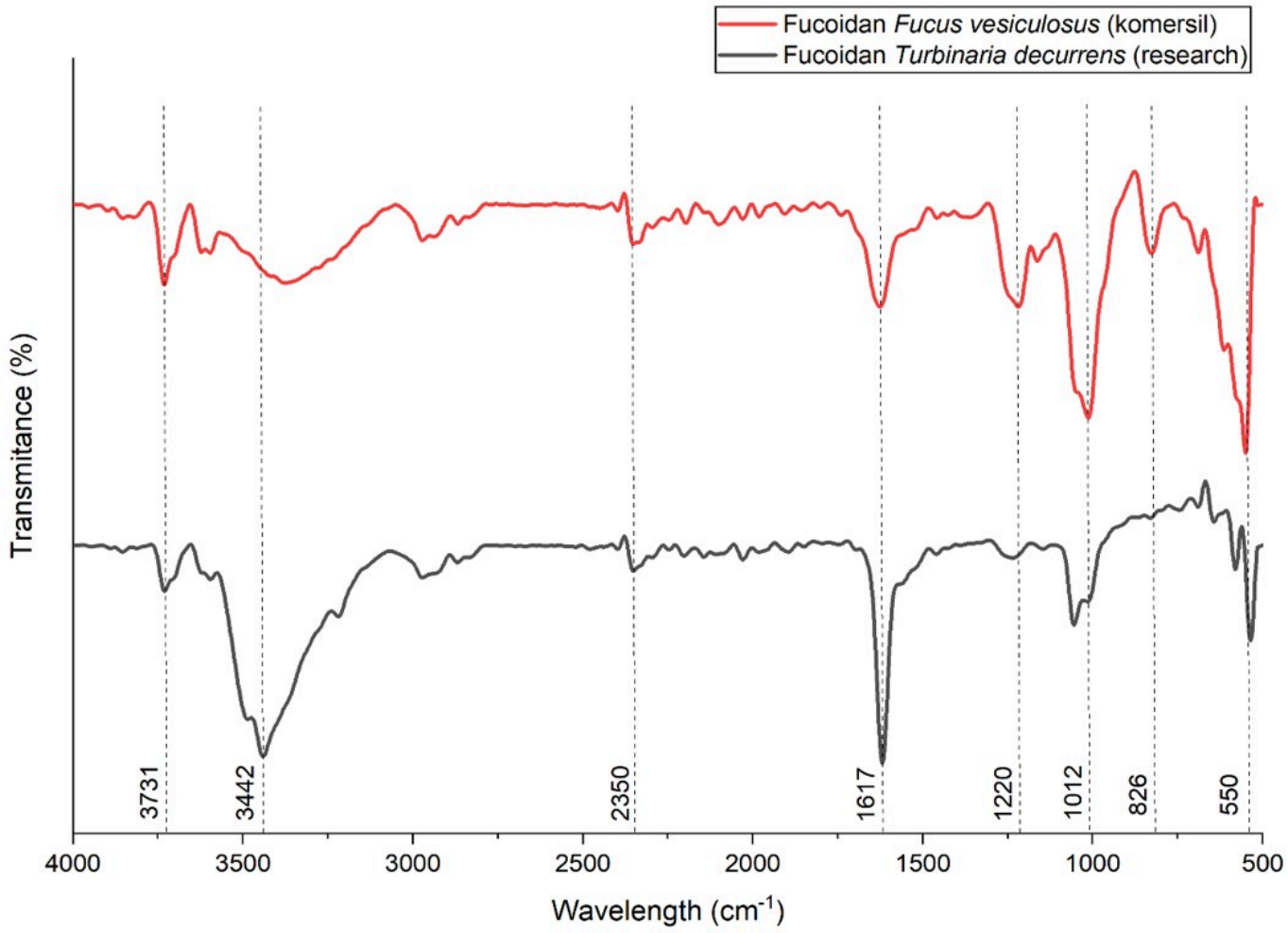


Figure 4

Fourier Transform Infra-Red spectra of the extracted fucoidan from *Turbinaria decurrens*

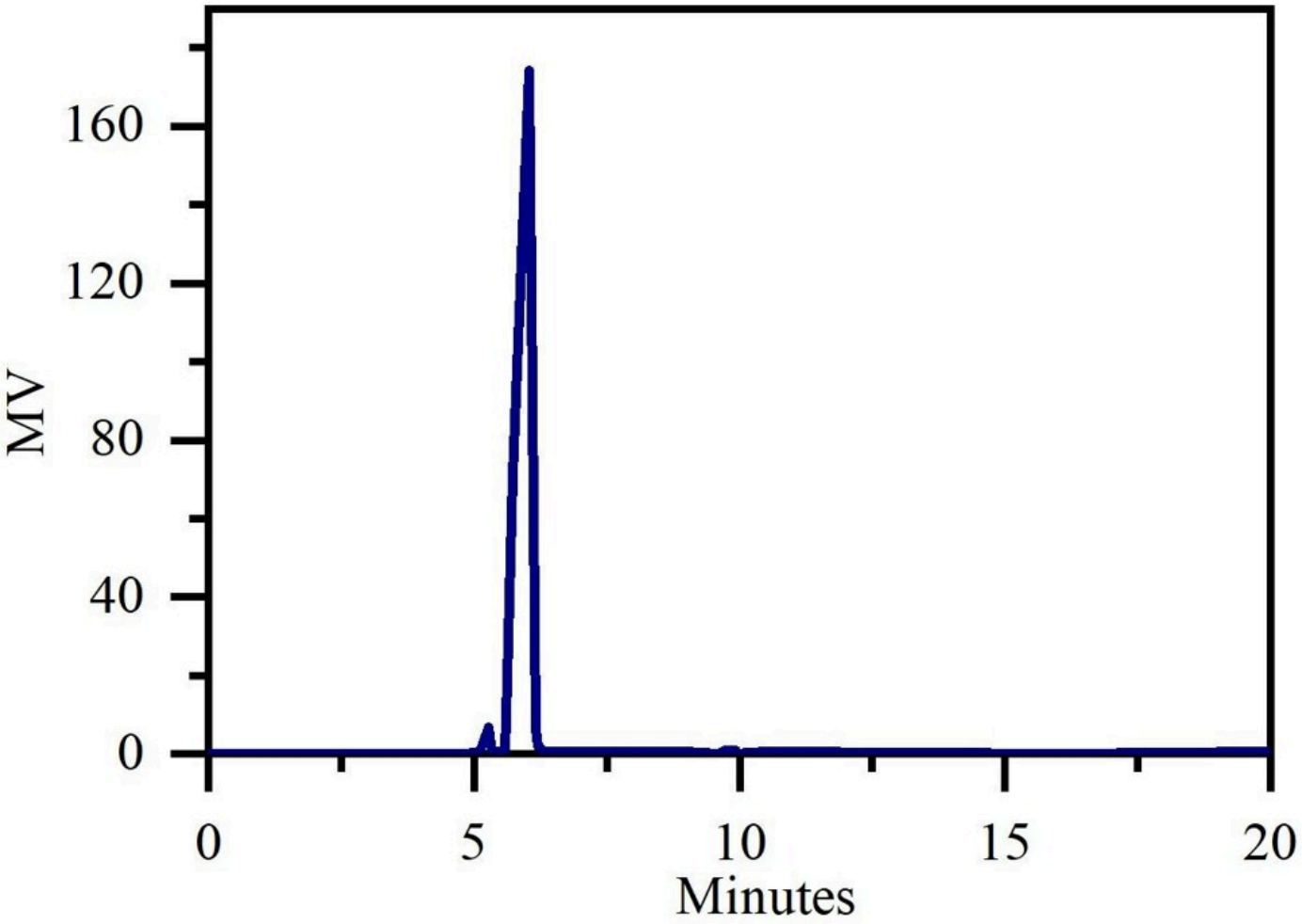


Figure 5

Monosaccharide chromatogram of fucoidan extracted from *Turbinaria decurrens*

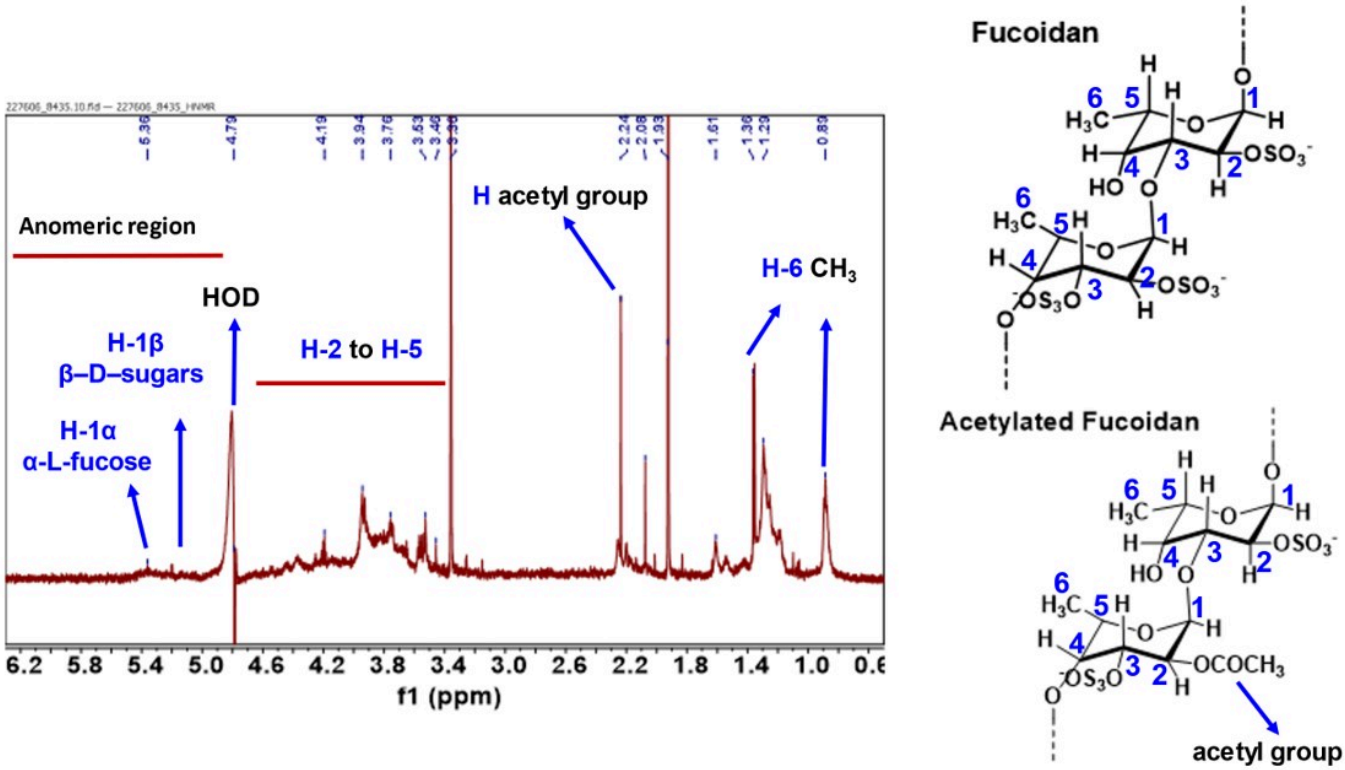


Figure 6

^{1H} NMR spectra of fucoidan *Turbinaria decurrens*