MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Prombanchong *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 21 (2025) 2857–2869

RESEARCH ARTICLE

Extraction and Characterization of β-glucan from *Ulva rigida* and *Gracilaria fisheri*: A Comparative Study of Two Extraction Methods

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Abstract *Ulva rigida* and *Gracilaria fisheri* are very common algae and are considered potential sources of β-glucans. In this study, acid hydrolysis with 0.1 M HCl (Method 1) and alcohol precipitation with $CaCl_2$-assisted extraction (Method 2) were used to isolate β-glucan from both algae and followed by characterization. The crude extract from *U. rigida* was whiter than the β-glucan crude from *G. fisheri*, which had a faint brown color. In comparison to Method 2, Method 1 produced a higher percentage of both algae and contained phenolic compounds (0.32 ± 0.00–1.35 ± 0.04 µg gallic acid/mg sample). It also demonstrating significant antioxidant activities properties via DPPH, ABTS, and reducing power assays. The existence of β-glucan functional groups was established by FT-IR analysis, and the extracts from *G. fisheri* (both methods) and *U. rigida* (method 1) have been determined to contain β-glucans by LC-MS. These findings highlight *U. rigida* and *G. fisheri* as promising alternative sources of β-glucan for potential applications in the food, pharmaceutical, and cosmetic industries. The results suggest that acid hydrolysis (Method 1) is a more effective method for extracting β-glucan, resulting in a higher yield while preserving its bioactive properties.

Keywords: β-glucan, extraction, seaweeds, *Ulva rigida*, *Gracilaria fisheri.*

Introduction

These are bioactive polysaccharides known as β-glucans which are composed of D-glucose monomers linked by β-(1→3), β-(1→4), or β-(1→6) glycosidic bonds [1]. These polysaccharides are widely distributed in nature, primarily in the cell walls of fungi, yeast, bacteria, cereals, and marine algae [2]. β-glucans exhibit various structural types, which contribute to their diverse biological activities, including immune system modulation, cholesterol reduction, and antioxidant properties. As a result, they are valuable for use in functional foods, pharmaceuticals, and cosmetic uses [3,4].

Marine algae are becoming increasingly popular as an alternative source of β-glucans because their different structural arrangements may offer greater biological activity compared to β-glucans derived from cereals and yeast [5]. Among various marine species, *Ulva rigida* and *Gracilaria fisheri* are promising candidates due to their high polysaccharide content and availability [6]. However, the method used to extract β-glucan significantly impacts its effectiveness, as different extraction methods can alter the yield, purity, and bioactivity of the resulting product [7].

Typical methods for extracting β-glucans from plants include aqueous extraction, enzymatic hydrolysis, and acid or alkali treatments [8]. Each of these methods alters the structure and function of polysaccharide [9]. Acid hydrolysis is commonly used to disrupt the cell wall and enhance β-glucan solubility, although it may lead to structural degradation [10]. Alternatively, alcohol precipitation combined

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Received: 7 July 2025
Accepted: 2 Sept. 2025

with calcium chloride-assisted extraction is considered a gentler method that improves the purity of β-glucan while reducing the likelihood of degradation [11]. However, there is limited comparative research on the efficiency of these methods for extracting β-glucans from *U. rigida* and *G. fisheri*.

In this study, the effects of two extraction techniques, acid hydrolysis (Method 1) and alcohol precipitation with $CaCl_2$-assisted extraction (Method 2), on the yield and content of β-glucan from *U. rigida* and *G. fisheri* were assessed and compared. The yield, sugar content, total glucan composition, antioxidant activity, and properties of the extracted β-glucans from both algae using the two methods were examined. Liquid chromatography-mass spectrometry (LC-MS) and Fourier-transform infrared spectroscopy (FT-IR) were used to confirm the presence of β-glucan and study its structure.

Materials and Methods

Sample Preparation

Ulva rigida and *Gracilaria fisheri* were collected from the southern part of Thailand, specifically in Nakhon Si Thammarat Province during March–April 2024. After being washed three times with distilled water, the two types of algae were completely dried at 60°C and ground into a fine powder, passed through a 1 mm sieve, and stored in a refrigerator at 4 ± 1 °C with silica gel desiccants to control humidity until further extraction.

β-glucan Extraction

Extraction of β-Glucan was performed using two methods, namely Method 1 and Method 2, with the operation of each method described as follows:

Method 1 (acid hydrolysis): β-glucan extraction was carried out by slightly modifying the method of Ramalingam *et al*. (2021) [10]. Ten grams of algae powder was weighed and mixed with 200 ml of 0.1 M HCl solution. The mixture was then incubated in a temperature-controlled water bath at 60°C for 2 h. Afterward, the solution was removed, and the pH was adjusted to neutral (pH 7.00) using 1 M NaOH. The resulting solution was then freeze-dried. The obtained substance was in a refrigerator at 4 ± 1 °C with silica gel desiccants to control humidity for further analysis.

Method 2 (based on alcohol precipitation and $CaCl_2$-assisted extraction): β-glucan extraction was performed with slight modifications to the method as described in Bobadilla *et al*. (2013) [11]. Ten grams of seaweed powder were weighed into a 500 ml glass bottle, and 200 ml of 85% ethyl alcohol was added. The mixture was incubated at room temperature (32±2°C) for 24 h then stirred with a hand blender (Clarte' model FHM106S) at an approximate speed of 15,000 rpm, based on typical speeds of similar devices, for 1 min. After stirring, the solution was discarded, and the extracted sludge was collected by adding 200 ml of 2% w/v $CaCl_2$, blending for 1 min, and then incubating in a temperature-controlled water bath at 70°C. The sludge was extracted two more times by adding 100 ml of 2% w/v $CaCl_2$ each time. The clear solution obtained from the three extractions was combined, and the solvent was evaporated using a vacuum evaporator at 40°C. Then, 10 ml of chloroform solution was added, the mixture was shaken well, and the solution was centrifuged at 3,000xg for 10 min. The resulting solution was subjected to dialysis using cellulose dialysis bags (12,000 – 14,000 MWCO, Spectrum Spectra/Pro® 4) in deionized water for 16 h at 4 ± 1 °C in a refrigerator. The extracted substance was stored in a refrigerator at 4 ± 1 °C with silica gel desiccants to control humidity for further analysis.

Determination of Sugar Content

The determination of sugar content was performed using the phenol-sulfuric acid method according to Dubois *et al*. (1956) [12]. The reaction mixture consisted of 0.5 mL of sample at 100 µg/ml and 0.25 mL of 6% (w/v) phenol in 99% ethanol. Then, 1.25 ml of concentrated sulfuric acid was added, and the mixture was incubated at room temperature (32 ± 2°C) for 20 min. The absorbance of the solution was determined using a spectrophotometer at 490 nm. The sugar content was determined by comparing the sample absorbance to a standard glucose concentration range of 0–60 µg/ml.

Determination of Antioxidant Activity of 1, 1-Diphenyl-2-picrylhydrazyl Radical (DPPHº)

The analysis of antioxidant activity using DPPH assay was modified from the method of Blois (1958) [13]. A 0.20 mM DPPH solution in 99% ethanol (0.5 ml) was mixed with 1.0 ml of sample solution at a concentration of 10 mg extract/ml. The reaction mixture was incubated in the dark at room temperature (32 ± 2°C) for 30 min. After incubation, the absorbance was measured at 517 nm using a

spectrophotometer (Libra Biochrome, UK), distilled water was used as the reference solution (blank). The inhibition percentage was calculated with the following formula:

Inhibition Percentage=((Acontrol-Asample))/Acontrol x 100 where Acontrol and Asample are the absorbance values of DPPH solutions without and with extracts, respectively. The IC_{50} value of synthetic antioxidant Butylated Hydroxy Toluene (BHT) was used as the reference agent.

Determination of the Antioxidant Activity of 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS)

The antioxidant activity of the samples was evaluated using the ABTS assay, following a method modified from Re *et al*. (1999) [14]. The ABTS radical solution was prepared by mixing equal volumes of a 7 mM ABTS solution with a 2.45 mM potassium persulfate solution. The mixture was incubated at room temperature in the dark for 12 h. After incubation, the ABTS radical solution was mixed with a sample solution at a concentration of 10 mg/ml or distilled water (control) at a volume of 1 ml. The mixture was incubated in a dark place for 5 min, and the absorbance was measured at 734 nm, distilled water was used as the reference solution (blank). The inhibition percentage was calculated with the following formula:

Inhibition Percentage=((Acontrol-Asample))/Acontrol x 100 where Acontrol and Asample are the absorbance values of ABTS solution without and with the extract, respectively. The IC_{50} value of BHT was used as the reference agent.

Reducing Capacity Measurement (Reducing Power)

The reducing capacity of the β-glucan extract was measured using a method slightly modified from Yen and Chen (1995). [15]. The reaction consisted of a 200 mM sodium phosphate buffer solution at pH 6.6 (1 ml), a sample solution with a concentration of 10 mg/ml (1 ml), and a 1% w/v potassium ferric cyanide ($K_3Fe(CN)_6$ solution (1 ml). The mixture was incubated in a 50°C water bath for 20 min. Then, 1 ml of 10% w/v trichloroacetic acid (TCA) solution was added, followed by aspirating 1 ml of the resulting mixture and transferring it to a test tube. Next, 1 ml of distilled water and 0.2 ml of 0.3% w/v ferric chloride ($FeCl_3$) solution were added. The mixture was incubated at room temperature for 10 min, then the light absorption was measured at 700 nm. Distilled water in the same reaction was used as the reference solution (blank). The light absorption value obtained was compared to the standard graph of ascorbic acid, with a concentration range of 0-0.25 mg/ml.

Determination of Total Phenolic Compounds

The total phenolic content was determined using a modified Folin–Ciocalteu method based on Singleton and Rossi (1965) [16]. In brief, 0.5 ml of the sample solution was combined with 0.5 ml of Folin–Ciocalteu reagent diluted tenfold with distilled water and allowed to react at room temperature (32±2°C) for 7 minutes. Following this, 0.5 ml of 10% (w/v) Na_2CO_3 solution was added, and the mixture was incubated for an additional 30 minutes at room temperature. The resulting blue solution was measured at 725 nm using a UV–Vis spectrophotometer. Phenolic content was calculated using a gallic acid standard curve (0–100 µg/mL) and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

Protein Content Measurement

Protein content measurement was measured according to the Lowry method as described by Lowry *et al*. (1951) [17]. In this method, 1 ml of sample solution (10 mg/ml) was reacted with a mixed solution consisting of 2% solution w/v Na_2CO_3 in 0.1 M NaOH, 0.5% w/v $CuSO_4.5H_2O$, and 1% w/v sodium potassium tartrate ($KNaC_4H_4O_6$) in a 98:1:1 ratio. After adding 2 ml of the mixed solution to the sample, the mixture was shaken well and incubated for 10 min. Folin-Ciocalteu's reagent (diluted with distilled water in a 1:1 ratio) was then added, and the solution was shaken again and incubated for 30 min. Then the absorbance was measured at 750 nm. The protein concentration was determined by comparing the absorption to a BSA standard curve, with a concentration range of 0-100 µg/ml, and expressed as milligrams of protein per gram of extract (mg protein/g extract).

Total β-Gulcans and α-Glucan Analysis

Using an assay kit (Megazyme© Ltd., Bray, Wicklow County, Ireland), the 1,3-1,6-beta-glucans were measured in triplicate according to the manufacturer's instructions.

Fourier-Transform Infrared Spectroscopy (FT-IR analysis)

The samples were scanned over the spectral range of 4,000 – 500 cm^{-1}.

LC-MS Analysis

β-glucan was dissolved in distilled water to a concentration of 1 mg/ml to prepare samples for injection analysis. The sample solution was then filtered using a PTFE 0.2 µm syringe filter and placed in vial bottles. A gradient mixed solution of 0.1% formic acid in water and 0.1% formic acid in acetonitrile was used as the mobile phase. After the sample solution was injected into an Agilent Poroshell 120 EC-C18 (4.6 x 150 mm, 2.7 µm) column with a volume of 1.0 µl, the following gradient was applied: for 0-9 min, a mixture ratio of 95; for 10-19 min, 83:17; for 20-26 min, 0:100; and for 27-33 min, 95:5, with a flow rate of 200 µl/min at 35°C. Data analysis was performed using the Agilent MassHunter Workstation Software (Qualitative Analysis, version B.08.00, Agilent) and the Personal Compound Database and Library (PCDL).

Data Analysis

The results were obtained by repeating the experiments three times, and the data were reported as the mean ± standard deviation. Statistical differences between the means were analyzed using one-way ANOVA at a 95% confidence level ($p<0.05$).

Results and Discussion

Characteristics and Percentage of Yield

The physical characteristics of crude β-glucan extracted from both algae using the two different methods showed slightly different. The crude β-glucan from *U. rigida* was whiter than the crude β-glucan from *G. fisheri*, which had a faint brown colour (Figure 1). This difference may be attributed to the variations in the chemical composition and metabolite content of the algae species, as well as potential degradation occurring during extraction.

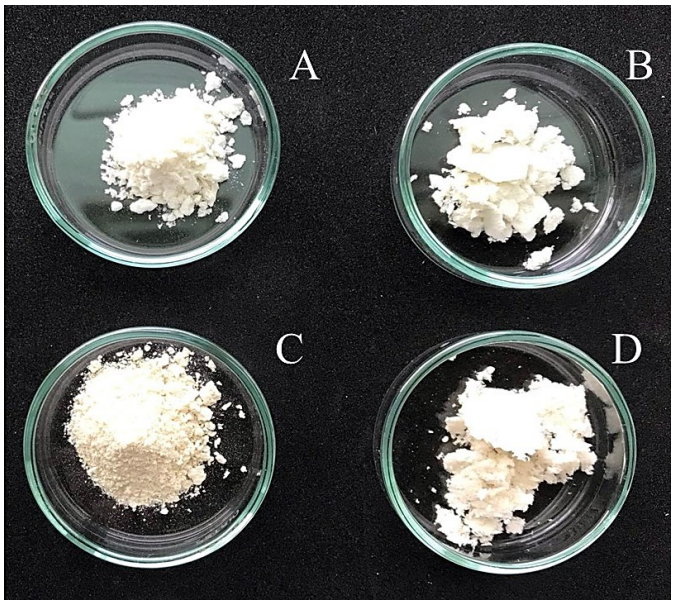

Figure 1. Crude β-glucan from *U. rigida* (A and B extracted using methods 1 and 2, respectively) and *G. fisheri* (C and D extracted using methods 1 and 2, respectively).

The extraction of β-glucan from two species of algae using two different methods revealed that Method 1 (acid hydrolysis) showed a significantly higher yields than Method 2 (alcohol precipitation and $CaCl_2$-assisted extraction) (Table 1). Crude β-glucan from *G. fisheri* extracted using Method 1 had the highest yield (53.43%), while Method 2 resulted in a much lower yield (14.27%). The yield of crude β-glucan from *U. rigida* also showed a similar trend, with the yields of Methods 1 and 2 being 44.27% and 10.13%, respectively. This difference is likely due to the fact that acid hydrolysis breaks down cell walls more efficiently, releasing more β –glucan [13]. The lower yield from Method 2 might be attributed to incomplete dissolution of the β-glucan, with some parts lost during the precipitation and dialysis steps [4].

Total Sugar, Total Glucan, β-Glucan, α-Glucan and Protein Content

Both types of algae extracted using Method 2 had more total sugar than those extracted with Method 1. The extract from *G. fisheri* obtained by Method 2 had the highest total sugar content (89.27%), while *U. rigida* extracted by Method 2 had a lower value (31.17%), and *U. rigida* extracted by Method 1 showed the lowest total sugar content (24.32) (Table 1). Since β-glucans are polysaccharides, the total sugar content gives an estimate of how much of the extract consists of carbohydrates. A high total sugar content indicates a higher proportion of polysaccharides, such as β-glucans, relative to impurities. A low total sugar content may suggest a higher level of impurities [18]. According to these results, Method 2 provides a purer β-glucan fraction than Method 1, which yields a mixture of glucans and other components. It has been found that β-glucans from various sources have different structures, which affect their biological activity and usefulness [3]. We found that the protein content in *G. fisheri* β-glucan was higher than *U. rigida* β-glucans. Both algae extracts showed higher protein content in Method 1 than Method 2. This could be due to differences in the structural composition of the algal cell walls, with *G. fisheri* potentially having more protein-associated polysaccharides or cell wall-bound proteins [7].

Table 1. % Yield and Chemical Composition of β-glucan Extracted Using Methods 1 and 2 of *G. fisheri* and *U. rigida*

Type of algae	*G. fisheri*		*U. rigida*	
Extraction method	1	2	1	2
% yield	53.43±0.12^{d}	14.27±0.06^{b}	44.27±0.06^{c}	10.13±0.15^{a}
% sugar	40.95±2.48^{c}	89.27±7.81^{d}	24.32±0.58^{a}	31.17±0.61^{a,b}
% DPPH scavenging at 10 mg extract/ml	21.76±0.50^{c}	10.46±0.50^{a}	13.46±1.09^{b}	13.46±0.63^{b}
% ABTS scavenging at 10 mg extract/ml	34.04±0.08^{a}	36.85±0.77^{b}	50.42±2.51^{c}	98.96±0.04^{d}
Reducing power (µg ascorbic acid)	36.08±2.73^{a}	48.38±5.13^{b}	48.44±5.00^{b}	37.67±1.78^{a,b}
Total phenolic compounds (µg gallic acid/ mg sample)	1.35±0.04^{d}	0.53±0.10^{c}	0.32±0.00^{a}	0.41±0.00^{b}
Protein contents (mg/g sample)	13.54±0.22^{b}	13.40±0.74^{b}	3.29±0.08^{a}	4.35±0.15^{a}
Total glucan (%w/w)	26.46±2.22^{b}	38.36±0.35^{c}	1.88±0.53^{a}	57.17 ±1.87^{d}
β-glucan (%w/w)	26.42±2.22^{b}	38.30±0.35^{c}	1.84±0.53^{a}	57.14±1.87^{d}
α-glucan (%w/w)	0.037±0.002^{c}	0.058±0.001^{d}	0.032±0.001^{b}	0.014±0.001^{a}

* IC_{50} DPPH of BHT = 0.05 mg/ml
IC_{50} ABTS of BHT = 8.95 µg/ml
Reducing Power: The standard curve for ascorbic acid was represented by y= 5.6364x, with an R^2 value of 0.9956.

Total glucan content from *U. rigida* (Method 2) had the highest percentage, at 57.17%. The total glucan content of *G. fisheri* (Method 2) was lower (38.36%). The highest β-glucan content (57.14%) was found in *U. rigida* (Method 2), while *G. fisheri* (Method 2) had a 38.30% content, suggesting that a significant portion of its recovered polysaccharides was β-glucan. All samples had very small levels of α-glucan content, with *G. fisheri* (Method 2) having the highest amount at 0.058% (Table 1). These results indicate that while Method 1 removes a mixture of glucans and other components, Method 2 yields a purer beta-glucan fraction.

Phenolic Content and Antioxidant Activity (DPPH, ABTS, and Reducing Power)

The crude β-glucan from *G. fisheri* (Method 1) had 1.35 µg gallic acid/mg sample, which was the highest amount of total phenolics. The crude β-glucan *U. rigida* (Method 1) had the least gallic acid per mg (0.32 µg gallic acid/mg sample). Phenolics are known antioxidants, which help the extracts perform their functions [4]. The higher phenolic content in *G. fisheri* suggests that it may have stronger antioxidant potential compared to *U. rigida*, which also correlates with higher DPPH scavenging activity. *U. rigida* (Method 2) exhibited the highest ABTS scavenging activity, indicating strong antioxidant potential. This aligns with previous studies showing that marine algae contain significant amounts of polyphenols, which contribute to their functional properties [2]. The crude β-glucan from *G. fisheri* (Method 1) exhibited the highest total phenolic content (1.35 µg gallic acid/mg sample), whereas *U. rigida* (Method 1) had the lowest (0.32 µg gallic acid/mg sample). Given that phenolic compounds are well-known antioxidants [4], the relatively higher phenolic content in *G. fisheri* (Method 1) likely contributed to its greater DPPH radical scavenging activity (21.76%) compared to *U. rigida* (Method 1) (13.46%). This observation supports the role of phenolics as major contributors to antioxidant potential. However, this trend was not consistent across all assays. In the ABTS assay, *U. rigida* (Method 2), despite its relatively low phenolic content (0.41 µg gallic acid/mg sample), demonstrated the highest radical scavenging activity (98.96%), far exceeding that of G. *fisheri* (Method 1) (34.04%). These results indicate that antioxidant activity cannot

be explained solely by phenolic content. Other bioactive constituents, particularly polysaccharides such as β-glucans, likely play significant roles. Indeed, β-glucans from *U. rigida* (Method 2) accounted for 57.14% (w/w), the highest among all samples, which likely contributed to its remarkable ABTS scavenging activity.

These findings highlight the complexity of antioxidant activity in marine algae. Multiple constituents—including phenolics, polysaccharides, and proteins—contribute to radical scavenging. While DPPH scavenging was strongly associated with total phenolic content, ABTS scavenging appeared to be more closely related to polysaccharide content, particularly β-glucans, as observed in *U. rigida* (Method 2). This dual behavior underscores the importance of considering both hydrophilic and lipophilic antioxidants when evaluating the antioxidant potential of algal extracts. Similar patterns have been reported in previous studies; for example, polysaccharides extracted from *Padina gymnospora*, *Kappaphycus alvarezii,* and *Kappaphycus striatus* exhibited strong ABTS-scavenging activity, with *P. gymnospora* showing the highest efficacy [19] Additionally, exopolysaccharides from microalgae such as *Chlorella sorokiniana* achieved >90% ABTS radical removal, further underscoring the antioxidant potency of polysaccharide-rich extracts [20]. The reducing power of *U. rigida* (Method 1) and *G. fisheri* (Method 2) was quantified as 48.44 µg and 48.38 µg ascorbic acid equivalents/mg sample, respectively. These values suggest a substantial electron-donating capacity, indicative of their potential to act as reducing agents in antioxidant reactions. Such activity is crucial for mitigating oxidative stress by neutralizing free radicals and reactive oxygen species. Overall, the results suggest that the antioxidant activities of *G. fisheri* and *U. rigida* are influenced by both species and extraction methods. Phenolic compounds predominantly contribute to DPPH radical scavenging, whereas polysaccharides, especially β-glucans, are more influential in ABTS radical scavenging. The reducing power of the extracts further indicates their electron-donating capacity, which can help neutralize reactive oxygen species. These findings, consistent with previous studies on marine algae polysaccharides [19]. Antioxidative study of polysaccharides extracted from red (*Kappaphycus alvarezii*), green (*Kappaphycus striatus*) and brown (*P. gymnospora*) marine macroalgae/seaweed [20], underscore the importance of using multiple assays for a comprehensive assessment of antioxidant potential.

Fourier-Transform Infrared Spectroscopy (FT-IR Analysis)

The FT-IR spectra of β-glucan obtained from the two different extraction methods were comparable with that of standard β-glucan, indicating that both methods successfully preserved the characteristic functional groups and structural features of β-glucan (Figures 2**)**. The spectra exhibit broad absorption bands in the range of 3600–3200 cm^{-1}, which can be attributed to the stretching vibrations of hydroxyl (-OH) groups, indicating the presence of polysaccharides. The characteristic peaks around 3000–2800 cm^{-1} correspond to C-H stretching vibrations. Additionally, the absorption bands observed in the range of 1200–1000 cm^{-1} are assigned to C-O and C-O-C stretching vibrations, which are typical of β-glucan structures [21, 22]. The fingerprint region below 900 cm^{-1} may indicate the presence of β-(1→3)- and β-(1→6)-glycosidic linkages, which are essential structural features of β-glucans. The observed differences in peak intensities and slight shifts in band positions between the different spectra suggest variations in the molecular structure of β-glucan depending on the extraction method and fungal source.

Minor variations in peak intensities and slight band shifts suggest subtle differences in molecular conformation, branching, or hydrogen-bonding interactions. These variations likely result from extraction conditions—such as temperature, solvent polarity, and ionic environment—that affect polymerization and solubility [23].

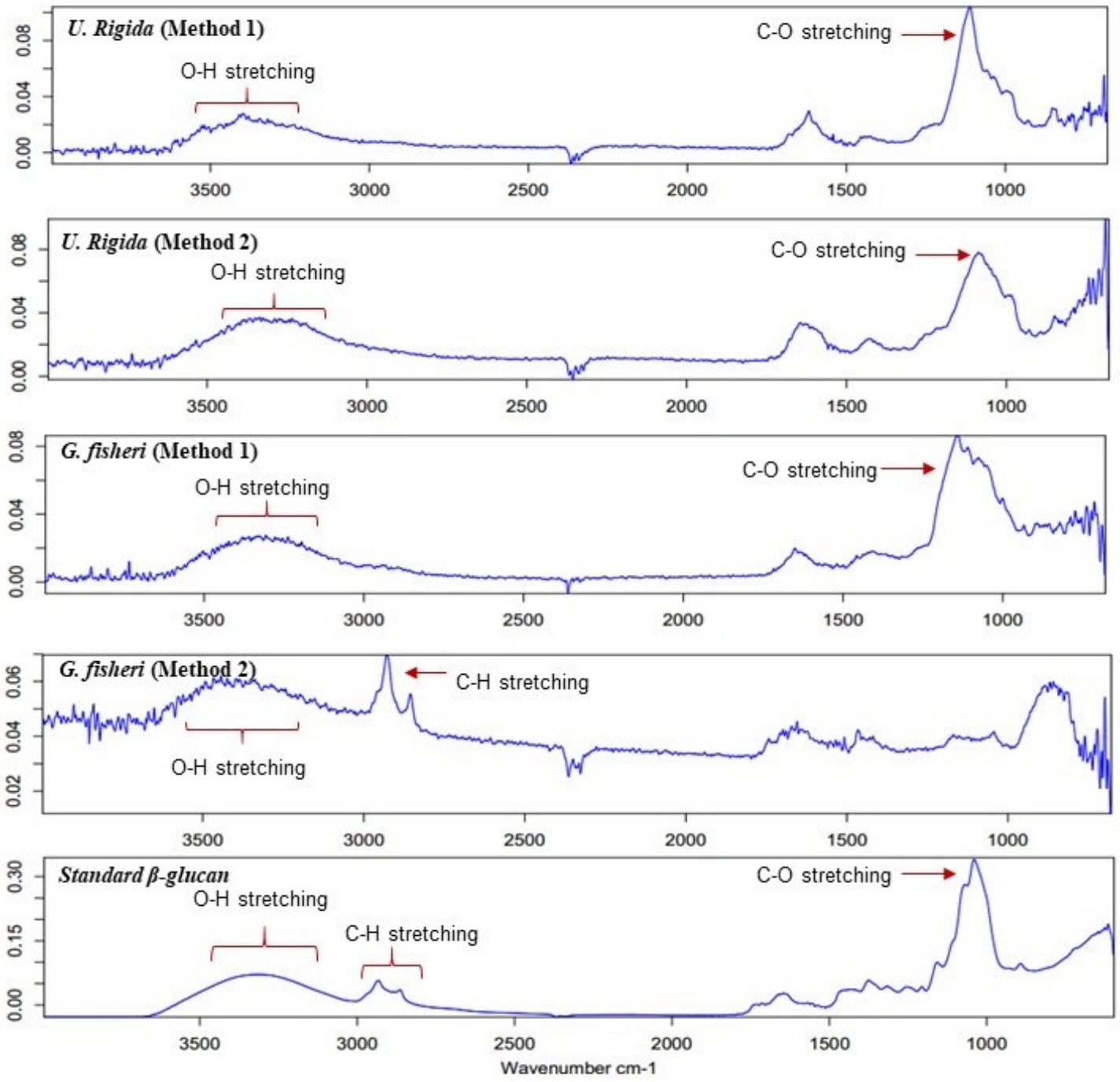

Figure 2. FT-IR spectra of crude β-glucan extracted from *G. fisheri* and *U. rigida* using Methods 1 and 2, compared with β-glucan standard

LC-MS Profiling of *G. fisheri* and *U. rigida*

The LC-MS analysis of *G. fisheri* and *U. rigida* using two different analytical methods (Method 1 and Method 2) (Tables 2 and 3 and Figure 3-6) provided comprehensive profiles of their bioactive compounds, including polysaccharides, fatty acids, amino acids, phenolics, and other secondary metabolites. The comparative analysis revealed both common and distinct bioactive components, highlighting the unique metabolic signatures of each species.

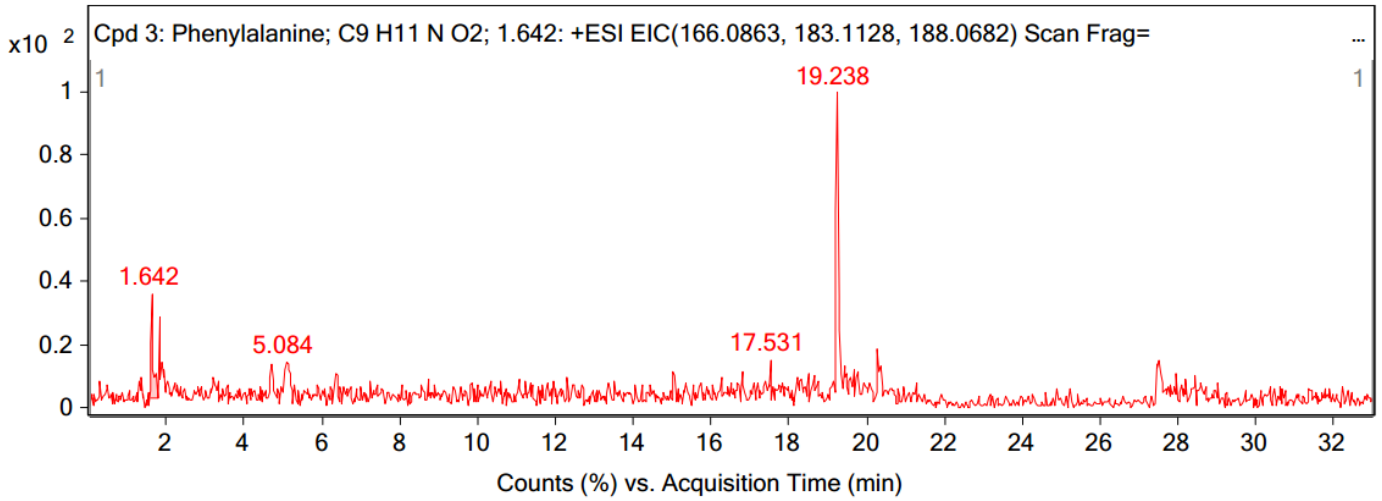

Figure 3. LC-MS chromatogram of crude β-glucan from *G. fisheri* extracted using Method 1

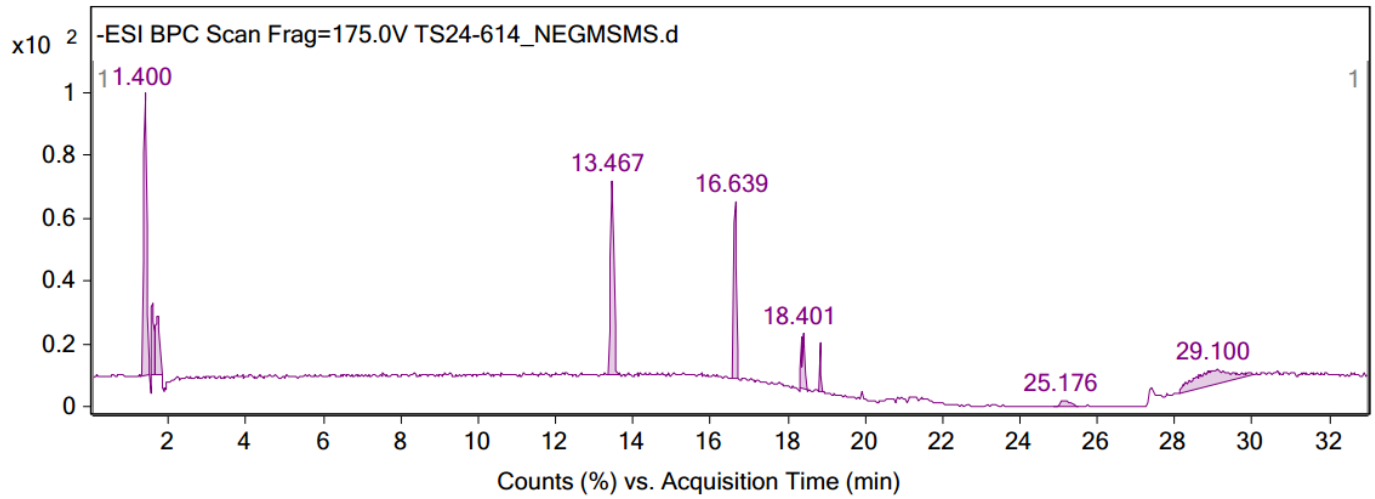

Figure 4. LC-MS chromatogram of crude β-glucan from *G. fisheri* extracted using Method 2

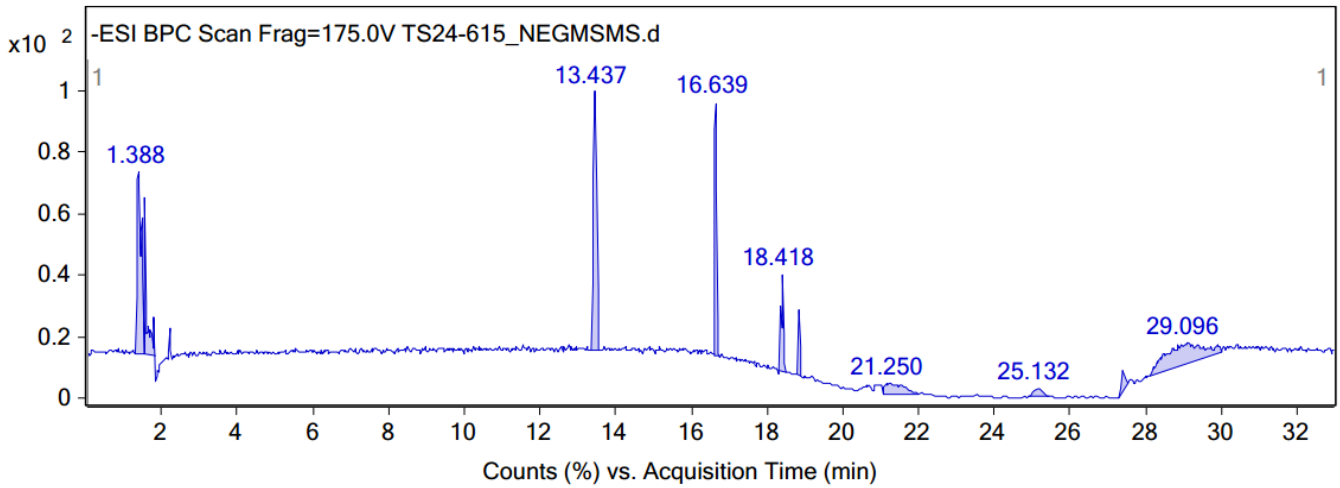

Figure 5. LC-MS chromatogram of crude β-glucan from *U. rigida* extracted using Method 1

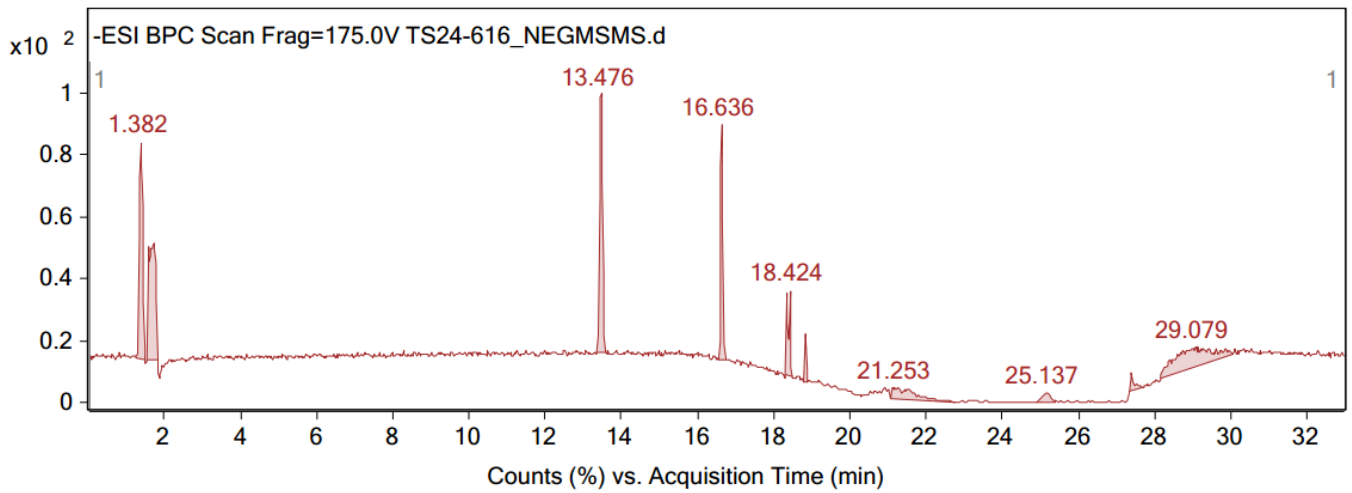

Figure 6. LC-MS chromatogram of crude β-glucan from *U. rigida* extracted using Method 2

Table 2. LC-MS analysis of bioactive compounds from G. fisheri using two extraction methods

Method	Component Name	Analysis	Formula	Diff (ppm)
Method 1 (Negative mode)	β-glucan	mass: 162.0525, RT: 1.905, Abundance: 7.75%	$C_6H_{10}O_5$	-1.86
	Glycine	mass: 75.0324, RT: 7.306, Abundance: 1.90%	$C_2H_5NO_2$	4.8
	2-Hydroxyethyl benzoate	mass: 166.0632, RT: 10.626, Abundance: 2.01%	$C_9H_{10}O_3$	1.45
	(5RS,6RS)-5-Hydroxy-6-phenylmethyl-2-piperidinone	mass: 191.1071, RT: 18.850, Abundance: 66.08%	$C_{12}H_{15}O_2$	-0.76
	E-Dodec-5-en-4-olide	mass: 196.1463, RT: 19.140, Abundance: 13.49%	$C_{12}H_{20}O_2$	-0.27
	Palatinol IC [1,2-benzene dicarboxylic acid, di-(2-methylpropyl) ester]	mass: 278.1519, RT: 21.187, Abundance: 6.06%	$C_{16}H_{22}O_4$	0.43
Method 1 (Positive mode)	Syringic acid	mass: 198.0524, RT: 1.944, Abundance: 2.22%	$C_9H_{10}O_5$	-2.35
	(Z,Z,Z)-6,9,12-Octadecatrienoic acid	mass: 278.223, RT: 18.092, Abundance: 30.85%	$C_{18}H_{30}O_2$	-2.95
	Palatinol IC [1,2-benzene dicarboxylic acid, di-(2-methylpropyl) ester]	mass: 278.1532, RT: 20.799, Abundance: 63.82%	$C_{16}H_{22}O_4$	4.9
Method 2 (Negative mode)	Quercetin	mass: 302.0433, RT: 1.605, Abundance: 0.91%	$C_{15}H_{10}O_7$	2.16
	β-glucan	mass: 162.0531, RT: 1.921, Abundance: 1.36%	$C_6H_{10}O_5$	1.67
	Glycine	mass: 75.0322, RT: 4.672, Abundance: 1.48%	$C_2H_5NO_2$	1.79
	o-Xylene aromatic hydrocarbon	mass: 106.078, RT: 17.616, Abundance: 1.35%	C_8H_{10}	-2.24
	Benzaldehyde dimethyl acetate	mass: 152.0832, RT: 17.616, Abundance: 1.60%	$C_9H_{12}O_2$	-3.73
	(5RS,6RS)-5-Hydroxy-6-phenylmethyl-2-piperidinone	mass: 191.1071, RT: 18.846, Abundance: 74.92%	$C_{12}H_{15}O_2$	-0.5
	(E)-Dodec-5-en-4-olide	mass: 196.1462, RT: 19.131, Abundance: 3.14%	$C_{12}H_{20}O_2$	-0.85

Method	Component Name	Analysis	Formula	Diff (ppm)
Method 2 (Positive mode)	(Z, Z ,Z)–6,9,12- Octadecatrienoic acid	mass: 278.2234, RT: 20.424, Abundance: 1.27%	$C_{18}H_{30}O_2$	-4.22
	Tridecanoic acid	mass: 214.1925, RT: 20.813, Abundance: 1.53%	$C_{13}H_{26}O_2$	-3.74
	Linoleic acid	mass: 280.2392, RT: 20.813, Abundance: 1.83%	$C_{18}H_{32}O_2$	-3.8
	Palatinol IC [1,2-benzene dicarboxylic acid, di-(2-methylpropyl) ester]	mass: 278.1517, RT: 21.189, Abundance: 5.82%	$C_{16}H_{22}O_4$	-0.41
	Alanine	mass: 89.0479, RT: 31.040, Abundance: 1.48%	$C_3H_7NO_2$	2.87
	(Z,Z,Z)-6,9,12-Octadecatrienoic acid	mass: 278.2237, RT: 18.08, Abundance: 95.77%	$C_{18}H_{30}O_2$	-3.01

Table 3. LC-MS analysis of bioactive compounds from *U. rigida* using two extraction methods

Method	Component Name	Analysis	Formula	Diff (ppm)
Method 1 (Negative mode)	β-glucan	mass: 162.0531, RT: 11.432, Abundance: 0.30%	$C_6H_{10}O_5$	1.56
	Hexadeca-4,7,10,13-tetraenoic acid	mass: 248.1778, RT: 18.845, Abundance: 86.04%	$C_{16}H_{24}O_2$	0.51
	Phenol, 3,5-bis(1,1-dimethylethyl)-	mass: 206.1670, RT: 20.813, Abundance: 7.92%	$C_{14}H_{22}O$	-0.28
	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	mass: 278.1519, RT: 21.217, Abundance: 1.52%	$C_{16}H_{22}O_4$	0.33
Method 1 (Positive mode)	Phenol, 3,5-bis(1,1-dimethylethyl)-	mass: 206.1670, RT: 1.643, Abundance: 1.20%	$C_{14}H_{22}O$	-0.35
	Phloroglucinol	mass: 126.0320, RT: 2.834, Abundance: 1.91%	$C_6H_6O_3$	2.28
	Linolenic acid	mass: 278.2239, RT: 18.085, Abundance: 67.38%	$C_{18}H_{30}O_2$	-2.53
	Hexadeca-4,7,10,13-tetraenoic acid	mass: 248.1771, RT: 19.493, Abundance: 8.73%	$C_{16}H_{24}O_2$	-2.04
	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	mass: 292.2030, RT: 19.656, Abundance: 9.56%	$C_{18}H_{28}O_3$	-2.86
Method 2 (Negative mode)	Mannopyranose	mass: 180.0626, RT: 1.518, Abundance: 2.77%	$C_6H_{12}O_6$	-4.6
	Hexadeca-4,7,10,13-tetraenoic acid	mass: 248.1779, RT: 18.849, Abundance: 82.64%	$C_{16}H_{24}O_2$	0.91
	E-14-Hexadecenal	mass: 238.2296, RT: 20.788, Abundance: 3.00%	$C_{16}H_{30}O$	-0.26
	Phenol, 3,5-bis(1,1-dimethylethyl)-	mass: 206.1674, RT: 20.788, Abundance: 7.42%	$C_{14}H_{22}O$	1.83
	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	mass: 278.1519, RT: 21.184, Abundance: 1.60%	$C_{16}H_{22}O_4$	0.36
Method 2 (Positive mode)	Phloroglucinol	mass: 126.0320, RT: 1.888, Abundance: 1.18%	$C_6H_6O_3$	2.64
	1-Octadecene	mass: 252.2824, RT: 18.077, Abundance: 68.33%	$C_{18}H_{36}$	2.78
	Isophitol	mass: 296.3077, RT: 18.144, Abundance: 22.71%	$C_{20}H_{40}O$	-0.57
	2-Pentadecanone, 6,10,14-trimethyl-	mass: 268.2769, RT: 18.160, Abundance: 5.88%	$C_{18}H_{36}O$	1.06

G. fisheri LC-MS Analysis

The crude extracted from both extraction methods could identified various bioactive compounds with variations in their relative abundances suggested differences in sensitivity and selectivity. Polysaccharides, including β-glucan, and phenolic acids, such as syringic acid, were detected in the crude extract of *G. fisheri* obtained using Method 1. In contrast, Method 2 enabled the identification of flavonoids, including quercetin, along with aromatic compounds such as o-xylene and benzaldehyde dimethyl acetate. β-glucan, a key immunomodulatory and antioxidant polysaccharide, was detected in both methods [24]. Method 1 reported a significantly higher abundance of β-Glucan (7.75%) compared to Method 2 (1.36%), indicating better sensitivity for detecting structural polysaccharides. This suggests that Method 1 is more sensitive for detecting polysaccharides and phenolic acids, likely due to more efficient extraction and/or better ionization under LC-MS conditions. In contrast, Method 2 favored detection of flavonoids and aromatic compounds, indicating differences in selectivity between the methods. Notably, Method 1 also identified syringic acid (mass 198.0524, 2.22%), a compound with antioxidant, antimicrobial, anti-inflammation, anticancer, anti-diabetic properties [25], while Method 2 detected quercetin (mass 302.0433, 0.91%), a flavonoid with potent free radical scavenging activity [26]. The most abundant fatty acid detected in both methods was (Z,Z,Z)-6,9,12-octadecatrienoic acid (mass 278.2234), which reached 95.77% in the positive mode. Other notable fatty acids included linoleic acid (mass 280.2392, 1.83%), (Z)-9-octadecenoic acid (mass 282.2552, 0.95%), and eicosanoic acid (mass 312.3034, 0.54%). These fatty acids contribute to anti-inflammatory, antioxidant and cardioprotective effects [27, 28, 29], enhancing the nutritional value of *G. fisheri*. Amino acids such as glycine, phenylalanine, alanine, and arginine were identified in both methods, indicating the potential of *G. fisheri* to contribute to dietary protein intake. Glycine was consistently detected in both methods with slightly different retention times, with Method 1 showing a higher abundance (1.90%) compared to Method 2 (1.48%). Cholesterol (mass 386.3563, 0.05%) was detected in Method 1, suggesting the presence of sterols that may influence the membrane composition of *G. fisheri*. Palatinol IC (1,2-benzene dicarboxylic acid, di-(2-methylpropyl) ester, mass 278.1519) was one of the most abundant compounds detected, reaching 63.82% in the positive mode.

U. rigida LC-MS Analysis

The LC-MS analysis of *U. rigida* also revealed a diverse range of bioactive compounds, with variations between the two methods in their identification and relative abundances. In the negative mode, Method 1 detected hexadeca-4,7,10,13-tetraenoic acid ($C_{16}H_{24}O_2$) as the most abundant compound (86.04%), while Method 2 reported a slightly lower abundance (82.64%). In the positive mode, Method 1 identified linolenic acid ($C_{18}H_{30}O_2$) at 67.38%, while Method 2 detected 1-octadecene ($C_{18}H_{36}$) at a slightly higher abundance (68.33%). Phenol, 3,5-bis(1,1-dimethylethyl)- ($C_{14}H_{22}O$) was detected in both methods, with Method 1 reporting an abundance of 7.92% and Method 2 detecting 7.42%. Both methods also identified phloroglucinol ($C_6H_6O_3$), a key phlorotannin, albeit at different abundances—1.91% in Method 1 and 1.18% in Method 2. Phloroglucinol is known for its antioxidant and antibacterial properties [30]. Both methods identified β-glucan ($C_6H_{10}O_5$) and mannopyranose ($C_6H_{12}O_6$), polysaccharides with immunomodulatory properties. Method 1 detected β-glucan at mass 162.0531 (RT: 11.432 min), while Method 2 identified mannopyranose at mass 180.0626 (RT: 1.518 min). Other notable compounds detected included isophytol ($C_{20}H_{40}O$), identified at 22.71% in Method 2 (RT: 18.144 min), and benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester ($C_{18}H_{28}O_3$), detected in Method 1 at 9.56% (RT: 19.656 min).

The comparative LC-MS analysis of *G. fisheri* and *U. rigida* using two different methods provided complementary insights into their phytochemical composition. Method 1 demonstrated higher sensitivity for detecting polysaccharides, phenolic acids, and fatty acids, while Method 2 was more effective in identifying flavonoids and other secondary metabolites. Both methods identified essential fatty acids, phenolic compounds, and polysaccharides, reinforcing the potential therapeutic applications of both species.

Conclusions

The β-glucan extract from the two algae showed a slight difference in color, with the β-glucan from *U. rigida* algae being white, while the beta-glucan extract from *G. fisheri* algae had a slight light brown color. The percentage yields from the two types of algae extracted using Method 1 was higher than those from Method 2, and the yield from *G. fisheri* was higher than that from *U. rigida*. The β-glucan extracts had phenolic compound content ranging from 0.32±0.00 to 1.35±0.04 µg gallic acid/mg sample. Both Method 1 and Method 2 beta-glucan extracts exhibited antioxidant activity, including DPPH, ABTS, and reducing power. The FT-IR analysis confirmed that the extracts were β-glucan, and LC-MS analysis further confirmed that the extract from *G. fisheri* (using both Method 1 and Method 2) and the extract from *U.*

rigida (using method 1) were indeed β-glucan. Both methods are effective for β-glucan extraction, but they differ in complexity, purity, and potential for degradation. Method 1 is faster and simpler but may result in partial degradation due to acid hydrolysis. Method 2, while more intricate, ensures a higher purity product by employing sequential purification techniques. The choice of method depends on the intended application, whether focusing on high-yield extraction (Method 1) or high-purity extraction (Method 2).

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

This research is a part of a project funded by Rajamangala University of Technology Srivijaya through the Fundamental Fund, fiscal year 2024, under the National Science, Research and Innovation Fund (NSRF) (project code 192772).

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