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Characterisation of *Sargassum elegans* fucoidans extracted using different technologies: Linking their structure to α-glucosidase inhibition

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ABSTRACT

Fucoidan, a sulphated polysaccharide found in brown seaweeds, has gained attention for its potential as an α-glucosidase inhibitor (AGI), which is significant for managing type 2 diabetes mellitus (T2DM). Although the use of brown algae for fucoidan extraction is becoming more popular, a few challenges remain, such as low extraction yields and a lack of understanding of the structure-activity relationship of fucoidan as an AGI. This study compared fucoidan extraction yields from *S. elegans* using three novel extraction methods (citrate buffer, EDTA, and enzyme-assisted extraction) and two well-established extraction protocols (hot water and mineral acid extraction). The structure-activity relationships of the fucoidan extracts as potential AGIs were then investigated. The fucoidan extraction yields for enzyme-assisted, citrate buffer, EDTA, hot water extraction and mineral acid extraction were 28 %, 20 %, 15 %, and 2.5 % and 3 %, respectively. Distinct structural features were noted from the fucoidan extracted by different techniques, according to their chemical profiles confirmed by their carbohydrate content, FTIR and NMR, and physical characteristics. Each extract exhibited unique chemical and structural properties. With IC_{50} values of 25 and 88 μg/ml, respectively, the fucoidans extracted by water and citrate buffer demonstrated the highest potency as AGIs, whereas the fucoidans extracted with the assistance of enzymes and EDTA showed IC_{50} values of 186 and 273 μg/ml. In summary, the extraction technology affected the fucoidans' structural and chemical compositions, impacting their biological activity as AGIs. Fucoidan's potent activity as an AGI represents a promising strategy for mitigating the burden of diabetes by reducing or preventing hyperglycaemia. Leveraging fucoidan could contribute meaningfully to developing alternative, natural therapeutic interventions for managing T2DM.

1. Introduction

Fucoidan comprises several distinct structural units denoting a family of fucose-containing sulphated polysaccharides [1]. The polysaccharides have backbones which mainly consist of α-(1→3) or alternating α-(1→3) and α-(1→4)-linked *L*-fucopyranose residues. These fucoidan backbones may be substituted with sulphate and acetate or have side branches composed of fucopyranoses or other glycosyl moieties, such as glucuronic acid [1]. Several fucoidan structures reported in the literature contain small amounts of various other monosaccharides, such as glucose, galactose, xylose, and mannose [2,3]. When fucoidans are associated with various other monosaccharides they can be categorised under the broader term "heterofucans" [4].

Fucoidan polysaccharides are mainly found in brown seaweeds and are negatively charged, rendering them soluble in water and acid solutions [5], with a highly hygroscopic and mucilaginous nature. In addition, the structural properties of fucoidan vary within different brown seaweed species. Fucoidans extracted from brown algae may have different compositions under the same algal classification (i.e., order). Structural features, in turn, cannot be categorised or predicted by algal order [6]. Furthermore, geographic location, harvest time, and especially the extraction and purification methods used [6,7], influence fucoidan's structure and biological activity.

The biological activity of fucoidan has been linked to three main attributes: molecular size, sulphate content and monosaccharide composition [8]. More specifically, smaller fucoidans are more effective at inhibiting α glucosidase, which is explained by their ability to reach the enzyme's active site [9]. As fucoidan's negative charge strengthens its binding to enzymes through electrostatic forces, it has also been shown that a higher sulphate content in its structure improves its AGI

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[8]. Additionally, the distribution of monosaccharides in the polysaccharide has been considered significant in its capacity as an AGI, with higher fucose content being linked to substantial activity [8]. However, fucoidans with a low fucose content have demonstrated remarkable bioactivity in our earlier research [3,10].

Fucoidan's functional characteristics are fundamentally interconnected, underscoring the critical role these compounds play in developing targeted inhibitors for the effective treatment of conditions such as type 2 diabetes. A recent study showed that the structural integrity and chemical properties of fucoidan are affected by the extraction technology employed [11]. In recent decades, various extraction techniques have been used to extract fucoidan and other polysaccharides from brown algae. Some commonly used fucoidan extraction technologies are hot water, dilute acid, enzyme-assisted, alkaline, and microwave-assisted extraction [12].

Challenges associated with producing fucoidan are its yield, biological activity, and environmental impact, which are associated with some extraction methods [13]. Thus, there is a need for novel extraction procedures and the improvement of existing conventional procedures to achieve better yields and maintain the biological activities of the extracted fucoidans. Although the literature has extensively characterised fucoidan from brown seaweeds, including *Fucus vesiculosus* and *Undaria pinnatifida*, *Sargassum elegans* fucoidan extraction is underexplored. Thus, this study highlights its unique biochemical composition.

Moreover, one of the prospective pharmacological applications of fucoidans is the alleviation of Type 2 diabetes mellitus (T2DM) [3]. T2DM is a chronic, emerging human disease projected to be one of the most burdensome in terms of mortality, morbidity, disability-adjusted life years and negative impact on the global economy [14,15]. T2DM is characterised by elevated glucose blood levels, leading to many complications, including organ damage and cardiovascular and nervous system complications [16]. Although several T2DM treatment approaches exist, including insulin secretagogues, insulin sensitisers, and inhibitors of amylolytic enzymes such as acarbose, and are clinically in use, there is a need for better therapeutics [17,18]. For example, acarbose use is associated with side effects, including bloating, abdominal discomfort, and diarrhoea [19].

New techniques like enzyme-assisted extraction (EAE) and EDTA-assisted extraction provide significant benefits by increasing the yield, especially considering the difficulties of low fucoidan recovery from seaweeds using conventional extraction methods (hot water extraction). Cellulases are used in enzyme-assisted extraction to release fucoidan from seaweed cell walls [20]. On the other hand, EDTA chelates the cell wall structure [21]. Both methods align with green chemistry principles as they do not use harmful solvents. Furthermore, considering the increasing incidences of T2DM and the associated side effects of some remedies like acarbose, better-tolerated treatments with improved therapeutic properties for T2DM are necessary. Thus, this study compared several fucoidan extraction technologies and evaluated the potential of the extracted fucoidans as inhibitors of α-glucosidase activity, an enzyme essential in the surge of post-prandial hyperglycaemia.

2. Materials and methods

2.1. Materials

The fucoidan used in the study was extracted from harvested *S. elegans* brown algae. Celluclast® 1.5 L (batch # CCN03208) was obtained from Novozymes. Acarbose (cat # LRAC2934) and *Saccharomyces cerevisiae* α-glucosidase (cat # G5003) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used in this study were obtained from Sigma-Aldrich, MERCK, Flucka Saarchem (Darmstadt, HE, Germany) and Celtic Diagnostic and Life Technologies (Cape Town, South Africa), and were reagent grade.

2.2. Harvesting and seaweed preparation

The *Sargassum elegans* brown algae used in this study were collected in March 2019 from Kelly's Beach in Port Alfred at coordinates (33 36′ 36.8424" S; 26 53′ 23.4996″ E) in the Eastern Cape, South Africa. The identification of the macroalgae was based on morphology and was confirmed by a macroalgal taxonomy specialist, Prof. John Bolton (University of Cape Town, South Africa). The harvested seaweed was stored on ice during transportation to the laboratory. Upon arrival at the laboratory, the seaweed was thoroughly washed with distilled water, cut into small pieces, and dried in an oven at 40 °C for 72 h. The dried algae samples were then pulverised using a coffee bean grinder and stored at room temperature until needed.

2.3. Fucoidan extraction

Five experimental protocols were used to extract fucoidans: hot-water extraction, EDTA-assisted extraction, dilute acid (HCl), citrate buffer, and enzyme-assisted extraction using the commercial cellulase preparation, Celluclast® 1.5 L. Hot water extraction, well known to extract bioactive fucoidan, was used as a proven standard. The dilute acid method is also a standard approach known to cleave important functional groups on the fucoidan extracts. The citrate buffer was used as a control since it was the buffer used in the experimental enzyme-assisted extraction. EDTA-assisted extraction was chosen to validate the novel method with our unique seaweed. Before fucoidan extraction, the dried algae were defatted using methanol (MeOH), chloroform ($CHCl_3$), and water (H_2O) with a solvent ratio of 4:2:1 [22]. After each extraction procedure described below, fractionated alginate and fucoidan were isolated from the algal biomass as described below. The supernatant was collected and treated with 1 % (*w/v*) $CaCl_2$ for alginate precipitation at 4 °C for 4 h. The alginate precipitate was collected by centrifugation at 8000*g* for 10 min. The supernatant was treated with absolute ethanol to a final alcohol concentration of 80 % (*v/v*). Fucoidan was precipitated at 4 °C for 24 h, then collected by centrifugation at 8000*g* for 10 min and then dried. The yield of extracted fucoidan was expressed as a percentage of algae dry weight (% dry weight). The extraction process was repeated thrice, and extracts were combined as a single batch for further analysis.

2.3.1. Hot water extraction

Fucoidan was extracted from dry-defatted *S. elegans* algal biomass using the hot water extraction method [23] with minor modifications. Briefly, 15 g of dry defatted algal powder was suspended in 450 ml of distilled water at a mass loading of 1:30 (*w/v*). The mixture was heated to 70 °C with stirring for approximately 16 h. Alginate was removed from the extract, and fucoidan precipitated using 80 % (*v/v*) absolute ethanol as described in Section 2.3.

2.3.2. EDTA-assisted extraction

A recently optimised ethylenedinitrilotetraacetic acid disodium salt dihydrate (EDTA) fucoidan extraction method [11] was used with minor modifications to extract fucoidan from non-defatted *S. elegans* algal biomass. An amount of 15 g of seaweed powder was dissolved in 450 ml of 0.5 % EDTA (*w/v*), and the mixture was heated with stirring at 70 °C for 3 h. The mixture was centrifuged at 5000*g* for 20 min at 4 °C. The supernatant was neutralised by adding 0.1 M NaOH and adjusting the pH to 7. Afterwards, alginate was removed from the supernatant by adding 1 % (*w/v*) $CaCl_2$ followed by centrifugation at 8000*g* for 10 min. Fucoidan was finally precipitated using 80 % (*v/v*) absolute ethanol, as described in Section 2.3.

2.3.3. Acid (HCl) assisted extraction

A 15 g quantity of defatted seaweed powder was dissolved in 450 ml of 0.15 M HCl in a 1:30 (w/v) ratio and heated to 70 °C with stirring. Then, the mixture was centrifuged at 5000*g* for 20 min at 4 °C. The supernatant was then neutralised to pH 7 with 2 M NaOH. Alginate was

removed by calcium chloride precipitation and centrifugation, as described in Section 2.3. Fucoidan was extracted from the supernatant by 80 % (*v*/v) ethanol precipitation.

2.3.4. Enzyme-assisted and citrate buffer extraction

Defatted algal biomass was suspended in 50 mM citrate buffer (pH 5). A total protein reaction concentration of 0.1 mg/ml (Celluclast® 1.5 L) mix was added to the enzyme-assisted reaction. The protein concentration of Celluclast® 1.5 L was determined using Bradford's method [24]. No enzyme was added to the buffer extraction reaction. The algal slurries were incubated incubator at 50 °C with agitation at 150 rpm for 48 h using a Biobase (BJPX 200B) shaking incubator.

2.4. Fucoidan structural analysis

2.4.1. FTIR analysis

A 100 mg quantity of ground fucoidan was scanned using a PerkinElmer Spectrum 100 Fourier-transform infrared spectroscopy (FTIR) spectrometer system (Perkin Elmer, Wellesley, MA, USA) equipped with a ZnSe (zinc selenide) ATR crystal. Signals were automatically recorded by averaging 4 scans over the 4000–650 cm^{-1} range, with a spectral resolution of 4 cm^{-1}. Baseline and ATR corrections for penetration depth and frequency variations were performed using the Spectrum One software (version 1.2.1) (Perkin Elmer, Wellesley, MA, USA).

2.4.2. NMR spectroscopy analysis

Fucoidan samples (10 mg each) were dissolved in 1 ml of deuterium oxide (D_2O), at 13000 *g* for 2 min, and the supernatant was filtered through 0.45 μm filters to remove insoluble material. The deuterium-exchanged samples were subjected to ^{1}H-nuclear magnetic resonance (NMR) analysis, and spectra were recorded at 23 °C using a Bruker Advance III 400 MHz spectrometer (Bruker, Karlsruhe, Germany). The spectra were processed and analysed using TopSpin NMR software, version 3.6.5 (Bruker, Karlsruhe, Germany), with chemical shifts expressed as δ ppm.

2.4.3. Thermogravimetric analysis

The thermogravimetric analysis of fucoidan extracts was performed on a thermogravimetric analyser (PerkinElmer, Pyris Diamond model) to determine the fucoidan ash content primarily. Approximately 4 mg of each fucoidan sample was placed in an aluminium pan for analysis. Pure nitrogen (purity 99.99 %) at a flow rate of 20 ml/min was used as a carrier gas to quench the mass transfer effect to a minimum. The fucoidan was heated from 30 °C to 900 °C at a heating rate of 30 °C/min. A separate blind experiment was performed for each test using an empty pan for baseline correction. Finally, the mass loss relative to temperature rise was automatically plotted, and the derived thermogram (DTG) was then plotted using GraphPad Prism v 9 (GraphPad Inc., San Diego, CA, USA).

2.5. Fucoidan chemical analysis

The total sugar content in the fucoidan was analysed by the phenol-sulfuric acid method using L-fucose as a suitable standard [25]. The amount of L-fucose was detected using a modified method [26]. Briefly, 200 μl of fucoidan sample was added to 900 μl of concentrated H_2SO_4. Acid hydrolysis was performed at 100 °C for 10 min, and then the samples were chilled on ice for 5 min. After the sample cooled to room temperature, 50 μl of 3 % (*w*/*v*) L-cysteine HCl solution was added. The absorbances were then read at wavelengths of 396 nm and 427 nm, respectively (the absorbance at 427 nm was then subtracted from the absorbance at 396 nm in each case to correct for the presence of hexoses) since methyl pentoses of fucose residues were the compounds of interest. The amount of L-fucose within the extracts was interpolated using an L-fucose standard curve.

Protein content was measured by Bradford's method using bovine serum albumin (BSA) as a standard [24]. Total phenolics were determined using a modified Folin–Ciocalteu method [27]. Phloroglucinol was used as a suitable standard. The fucoidan sulphate content was quantified using a modified barium chloride-gelatin method after fucoidan had been desulphated with 60 % (*v*/v) formic acid [28]. The sulphate content was quantified using sodium sulphate as a suitable standard.

Monosaccharides, including L-fucose, D-glucose, D-galactose, D-mannose, L-arabinose, and D-fructose, were quantified after 2 M trifluoroacetic acid (TFA) hydrolysis of fucoidan at 100 °C for 4 h [3]. These monosaccharides were quantified using a Shimadzu high-performance liquid chromatography (HPLC) instrument (Shimadzu, Kyoto, Japan) equipped with a refractive index (RID) detector. The neutral sugars were separated using a Fortis Amino analytical column (150 mm × 4.6 mm) (Fortis Technologies Ltd., Neston, UK) as previously described [10]. The uronic acid content in the extracts was determined calorimetrically according to a microplate uronic acid determination kit (K-URONIC) (Megazyme, Bray, WC, Ireland).

2.6. Determination of fucoidan kinematic viscosities

The kinematic viscosities of 1 mg/ml solutions of the fucoidans dissolved in dH_2O were measured using a semi-micro glass viscometer of (size 50) (Cannon-Manning (State College, PA, USA) at ~25 °C. The averages of at least three readings were taken using three separately prepared replicates of the various fucoidan samples. Distilled water was used as a control and reference for the experiment. To obtain kinematic viscosity in mm^2/s (cSt), the efflux time in seconds was multiplied by the viscometer constant (K = 0.003992) supplied with the instrument using the following equation:

$$\text{Kinematic viscocity} = \text{viscometer constant (K)} \times \text{time (t)}$$

2.7. Determination of the molecular weight of fucoidan

The molecular weight of the fucoidans was determined using high-performance size exclusion chromatography (HPSEC) with a refractive index detector (HPLC-RID) (Shimadzu Corp, Kyoto, Japan). Following the manufacturer's recommendations, the fucoidan extracts were separated using a Shodex OHpak SB-806 M HQ (8.0 mm × 300 mm) column (Showa Denko, Tokyo, Japan). The mobile phase (0.1 M $NaNO_3$ aq) used was filtered through 0.22 μm nylon membranes (Membrane Solutions, Auburn, USA). The flow rate was 0.5 ml/min, the column temperature was at 30 °C, and the sample injection volume was 20 μl. Pullulan standards (Shodex, Tokyo, Japan) in the range (22–200 kDa) were used to construct the standard curve for interpolating fucoidan molecular weights.

2.8. Fucoidan bioactivity assays

2.8.1. α-glucosidase inhibition assays

The ability of the fucoidan extracts to inhibit the activity of α-glucosidase was investigated as described previous [10]. The α-glucosidase activity was measured by the release of *p*-nitrophenol (pNP) and enzyme % inhibition was calculated as:

$$\textit{Enzyme inhibition}\% = \frac{(p\text{NP released by control} - \textit{pNP released by test reaction})}{p\text{NP released by control}} \times 100$$

The inhibitor concentration resulting in 50 % inhibition of enzyme activity (IC_{50}) was determined graphically using GraphPad Prism software version 9 (GraphPad Inc.).

2.8.2. Inhibition kinetics of fucoidan against α-glucosidase

The α-glucosidase activity was measured in the presence of fucoidan extracts at fixed concentrations of 0, 0.1, 0.5 and 1 mg/ml with various concentrations of *p*-nitrophenyl-α-*D*-glucopyranoside (0.25 to 6.25 mM). The enzymatic reaction rates (v) were quantified using the released *p*-nitrophenol obtained from a standard curve prepared using *p*-nitrophenol. The Michaelis-Menten curve was constructed, and the K_M and V_{max} values were determined using GraphPad Prism 9.0 software (GraphPad Inc., San Diego, CA, USA).

2.8.3. Investigation of fucoidan-induced conformational changes of α-glucosidase using tryptophan fluorescence-based analysis

The α-glucosidase-fucoidan interaction was analysed through intrinsic tryptophan fluorescence [29]. Briefly, samples composed of α-glucosidase (0.02 mg/ml) and fucoidan extracts (0.0625–0.5 mg/ml) in 0.05 M sodium phosphate buffer (pH 7.4) were incubated for 20 min at 37 °C. After incubation, fluorescence was measured between 320 and 500 nm, in 5 nm increments, after initial excitation at 295 nm using a SpectraMax M3 microplate reader (Separations, Molecular Devices, USA) at 25 °C using black standard 96-well microplates. Relative fluorescence was calculated as the average value obtained from at least 4 spectra. A fucoidan buffer control was included to correct the raw data for the observed fluorescence.

2.8.4. Circular Dichroism (CD) analysis of secondary structural changes of α-glucosidase upon interaction with fucoidan

The secondary structural conformation of α-glucosidase was analysed using Far-UV circular dichroism (CD) as previously described [30]. Briefly, 0.2 μM α-glucosidase was suspended in 0.05 M phosphate buffer, pH 7.0. The analysis used a Chirascan v.4.4.1 Build spectrometer (Applied Photophysics Ltd., London, UK) equipped with a Peltier temperature controller at 19 °C, using a 0.1 cm path-length quartz cuvette (Hellma). The data were analysed and deconvoluted to α-helix, β-sheet, β-turns and unordered regions utilising the CONTIN program of the Dichroweb online server [31]. The procedure was repeated on α-glucosidase, which was incubated with varying concentrations of the extracted *S. elegans* fucoidans.

2.9. Statistical analysis

All experiments were conducted in triplicate (unless otherwise stated), and data were expressed as mean and standard deviations (SD). Any comparisons between data groups were determined by One-way analysis of variance (ANOVA). The ANOVA tests were performed using GraphPad Prism software version 9 (GraphPad Inc). Values were considered significantly different when $p < 0.05$.

3. Results and discussion

3.1. Fucoidan yield

Fucoidans were extracted from *S. elegans* seaweed using several methodologies, and the dry weight % yields of the product significantly varied (Fig. 1). The enzyme-assisted extraction method yielded the most fucoidan (about 28 % (*w*/w), followed by the citrate buffer extraction (20 % fucoidan yield). Also, the EDTA-assisted extraction produced about 15 % fucoidan yield, while the hot water and acid extraction protocols yielded the least fucoidan, 2 and 3.5 %, respectively. Although the acid extraction produced more fucoidan than the hot water extraction, this difference was not statistically significant (Fig. 1).

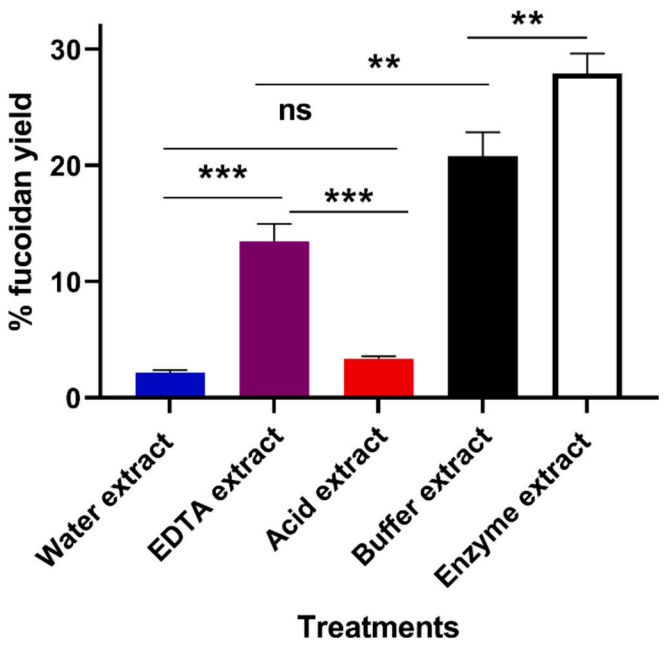


Fig. 1. Fucoidan yield from algal biomass in dry weight. The bars represent the fucoidan yield in terms of means ± SD from 3 biological replicates ($n = 3$). In the ANOVA analysis of fucoidan extracts, the (*) represents the strength of significance where the *p*-value in the range $0.001 < p < 0.05$, (***) represents a p-value <0.001 and (ns) represents data that is not significantly different at a 95 % confidence interval.

The enzyme-assisted extraction produced the highest fucoidan yield recorded in literature regardless of the extraction protocol used. Recently, literature has reported that fucoidans were successfully extracted through an enzyme-extracted approach and had about 4.75 % yield [32]. Our finding is intriguing as our enzyme protocol yielded over 7-fold fucoidan (28 %) to that reported in the literature. Although a few studies have used the enzyme-based approach to extract fucoidan, some did not report the yields attained [33,34]. The citrate buffer extraction method produced about 20 % (*w*/w) fucoidan, which was the second most effective extraction method after enzyme-assisted extraction.

The increase in fucoidan yield using enzyme extraction compared to the citrate buffer extraction is statistically significant ($p < 0.05$), suggesting that Celluclast® 1.5 L played a substantial role in the extraction process. Our study provides the first report on fucoidan extracted using citrate buffer. Perhaps the most significant finding is that enzyme-assisted extraction outperformed most known extraction methods, some of which have been explored in this study (Fig. 1). This high fucoidan yield is due to the effectiveness of the selected enzyme Celluclast® 1.5 L, which is a concoction of endo and exoglucanases, β glucosidases and hemicellulases. These enzymes, in combination, can break through the complex brown seaweed cell wall to release fucoidan into solution for extraction. The EDTA-assisted extraction was the third most effective, with approximately 15 % (*w*/w) fucoidan yield. Previously, it was reported that EDTA yielded 16 % (w/w) of fucoidan compared to the routinely used mineral acid extraction procedures [11]. Our study validated the findings of Zhao and colleagues by demonstrating that EDTA extraction increases fucoidan yield, particularly when contrasted with the conventional extraction methods, including water and mineral acid extraction. The impressive yield by the EDTA was not a surprise as it is known to chelate divalent and trivalent metal ions that stabilise the structural integrity of the cell wall matrix, disrupting the structural cohesion and thereby releasing fucoidan into the extraction medium. Furthermore, the EDTA process eliminates the depigmentation step as the EDTA managed to depigment algal biomass in solution in a single step. This method is more efficient as it saves time and reduces the use of

organic solvents during depigmentation. The process is environmentally friendly and could be technically and economically feasible on an industrial scale. The water extraction protocol yielded the least fucoidan (Fig. 1). This result is unsurprising as these low yields are typical. Hot water breaks hydrogen bonds and disrupts the seaweed cell wall matrix composed of polysaccharides, proteins, and other structural components. The heat causes partial hydrolysis of cell wall polysaccharides, loosening the network and allowing fucoidan, which is highly water-soluble, to diffuse out of the matrix. The low fucoidan yields may be because of this partial cell wall hydrolysis. However, the literature demonstrates that water extracted fucoidan exhibits superior bioactivity [10,35]. This could be attributed to the fact that the optimised hot water extraction preserves important structural and biochemical features, including sulphate content, molecular size and monosaccharide distribution. Other methods, such as mineral acid (HCl) extraction, are known to degrade the structural features of fucoidan, such as sulphate content, reducing their bioactivity [6]. Additionally, it has been proposed that enzyme-assisted extraction provides a more regulated method, maintaining the structural integrity of fucoidan and improving bioactivity through the customization of sulphate group distribution. Additionally, this approach helps clarify structural details, which are essential for comprehending bioactivity [1]. Nevertheless, our novel citrate buffer and enzyme-assisted techniques outperform the conventional fucoidan extraction approaches regarding fucoidan yield. This could be explained by the combined effect of the slightly acidic citrate buffer conditions and hydrolase activity of Celluclast 1.5 L on the seaweed structure to release fucoidan into the solution for extraction,

3.2. Structural characterisation

3.2.1. FTIR analysis

FTIR was used in the structural analysis of the algal polysaccharide extracts. All fucoidan extracts showed a spectral band between 3500 cm^{-1} and 3200 cm^{-1}, characteristic of polysaccharides (Fig. 2). This peak is associated with the stretching vibrations of the OH groups within the carbohydrate. The smaller bands observed in the 2900 to 3000 cm^{-1} region assigned to the CH stretching vibration in the pyranose ring and the methyl groups associated with fucose [11]. The peaks observed around 1650 cm^{-1} represent the carbonyl groups and stretching of *O*-acetyl groups [36]. The peaks between the 1210 cm^{-1} and 1270 cm^{-1} range are associated with the S═O bond linked with sulphate groups, a characteristic of fucoidans [37]. Our extracts showed these sulphate peaks (Fig. 2) in varying intensities. The fucoidan extracted with water showed the most prominent peak of all other extracts. Remarkably, in the acid-extracted fucoidan, the peak almost disappeared. This may indicate that acidic environments cleave the sulphate groups during fucoidan extraction as sulphate ester bonds (between sugars and sulphates) are prone to protonation in acid. Furthermore, the fucoidan extracts showed bands around 1100 cm^{-1}, illustrating stretching vibrations of the glycosidic C—O bonds within the fucoidan structure [38]. This peak at 1100 cm^{-1} was more visible in the water-extracted fucoidan than in any other extract. The *S. elegans* fucoidan extracts also displayed small peaks in the 850 cm^{-1} regions. The peaks at approximately 850 cm^{-1} represent sulphate groups attached to the carbonyl clusters of sidechains such as galactose [36]. Also, the sulphate absorption band, around associated with the C–O–S "the secondary axial sulphate", indicating that the sulphate group is located at position C-4 in fucopyranosyl residues [39]. It has been suggested that absorptions around 820–825 cm^{-1} are associated with sulphate groups located at the equatorial C-2 and C-3 positions of fucose [39]. Our extracted fucoidan appears to have secondary axial sulphates linked to the C-4 of the fucopyranosyl residues [40]. The FTIR analysis of the extracts generally revealed characteristics of fucoidan.

3.2.2. Proton NMR analysis of extracted fucoidans

To further elucidate the structural composition of the extracted fucoidan, proton NMR was performed. In general, chemical shifts in all extracts appearing as peaks at 1.28 ppm and 1.45 ppm (Fig. 3) indicate the presence of alternating α-(1–3) and α-(1–4) linkages of fucose residues, which are occasionally sulphated (α-L-Fuc, α-L-Fuc (2-SO_3^-) and α-L-Fuc (2,3-diSO_3^-) [41]. Our results showed that the water extract had the most prominent peaks in this region. The enzyme and buffer extracts showed smaller peaks than the water extracts, although their peaks were more intensified than the EDTA and acid extracts (Fig. 3). This observation may suggest that the water-extracted fucoidan was the purest among the extracts. In addition, the peaks at 1.45 ppm are also assigned to symmetric CH_3 deformations originating from methyl protons at C6 of fucose [42].

The peaks in our fucoidan extracts around 2 ppm are assigned to H-6 methylated protons of L-fucopyranosides, as asserted by the literature [43]. In addition, the observed peaks in the range of 3.5–4.5 ppm (Fig. 3) are characteristic of the (H2 to H5) ring protons of L-fucopyranosides. The peaks in this ring proton region also indicated variable sulphates linked to fucose at variable glycosidic linkages within varying monosaccharide patterns of the different extracts (Fig. 3). These observed peaks are coherent with previously characterised fucoidans [3,43]. Furthermore, the definitive peaks seen at 3.3 ppm and 3.7 ppm in all extracts indicate the presence of hexoses, including glucose, galactose, and mannose [43]. Although the monosaccharides have overlapping peaks, chemical characterisation assists in interpreting NMR spectral data. Our fucoidan extracts contained sugars other than fucose in their structure, as shown by peaks in the 3.3 ppm and 3.7 ppm range. These sugars were identified by HPLC and colourimetric analysis, which are discussed in Section 3.3.

As observed in Fig. 3, peaks at 2.5 and 2.7 ppm are present solely in the buffer and enzyme extract spectra and appear to stem from the citrate buffer components. These peaks were also detected in the extraction of pectin using Celluclast [44], supporting their association with citrate buffer constituents. Furthermore, the peaks at 3.5–4.5 ppm from the EDTA showed a slightly different pattern to the water, buffer, and enzyme extracts. This could be due to the different monosaccharide distributions on the fucopyranoside rings. Notably, these peaks are quite diminished in the acid extracts. Additionally, two chemical shifts at 2.2 ppm and 2.8 ppm were observed only in the EDTA extract (Fig. 3). The chemical shift around 2 ppm could be EDTA-Na ions. Our deduction is supported by the findings of Mónico and colleagues, who reported similar proton NMR spectra for EDTA [45]. The NMR spectra showed that extraction technology influences the fucoidans' structural nature. These structural differences may influence the potential bioactivities of these fucoidans.

3.2.3. Thermogravimetric analysis of fucoidan extracts

The thermogravimetric analysis (TGA) of the *S. elegans* fucoidan extracts was mainly performed to determine the ash content of the compounds. Three distinct stages of mass degradation were observed during the heating of the fucoidan samples. The first stage involves the loss of volatiles; the second may be due to polymer degradation, the third to soot, and the remaining mass to ash content. These characteristic mass degradation phenomena have been observed in the thermograms of all our fucoidan extracts (Fig. 4).

The TGA thermograms for all fucoidan extracts showed similar moisture levels (~20 %) as seen in the TGA thermograms in the first drop stage between 0 and 240 °C. This stage, denoted as (x) in Fig. 4, is associated with the loss in moisture content through evaporation [46] and some volatile matter [47]. The differential thermogravimetry (DTG) shows that the rate of mass loss was similar in all the extracts (Fig. 4B). The greatest mass loss in weight (~45 %) was between 240 °C and 500 °C denoted as (y), where most depolymerisation and decomposition of organic components, including polysaccharides, took place [48]. The TGA and DTG experiments show that the water and acid extract decomposed between 200 and 300 °C (Fig. 4). However, it was noted that fucoidans extracted with EDTA, buffer and enzyme degraded much

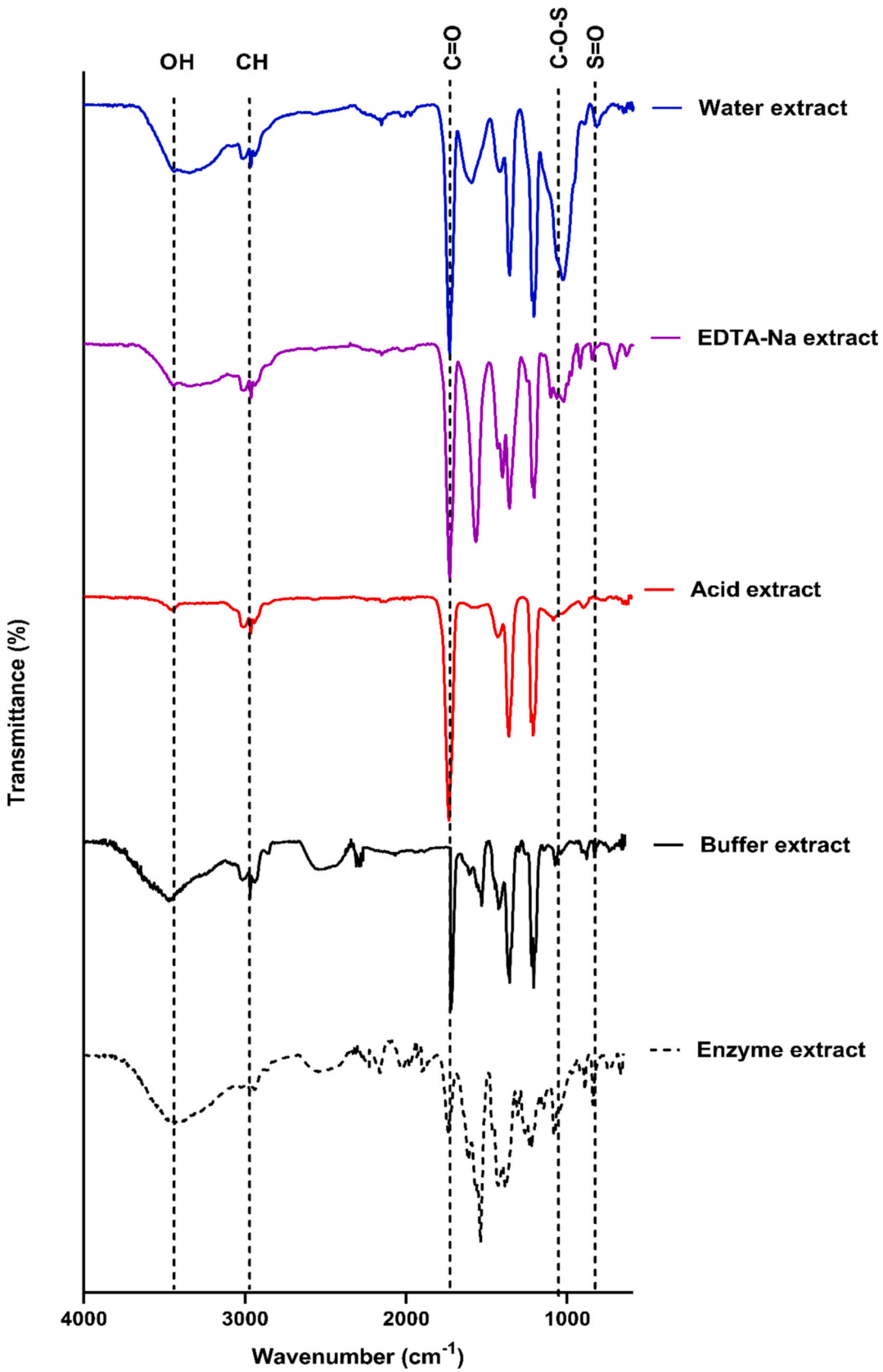


Fig. 2. FTIR spectra for the extracted fucoidan. The diagram shows the overlaid FTIR spectra of the *S. elegans* fucoidan extracted by the technologies mentioned.

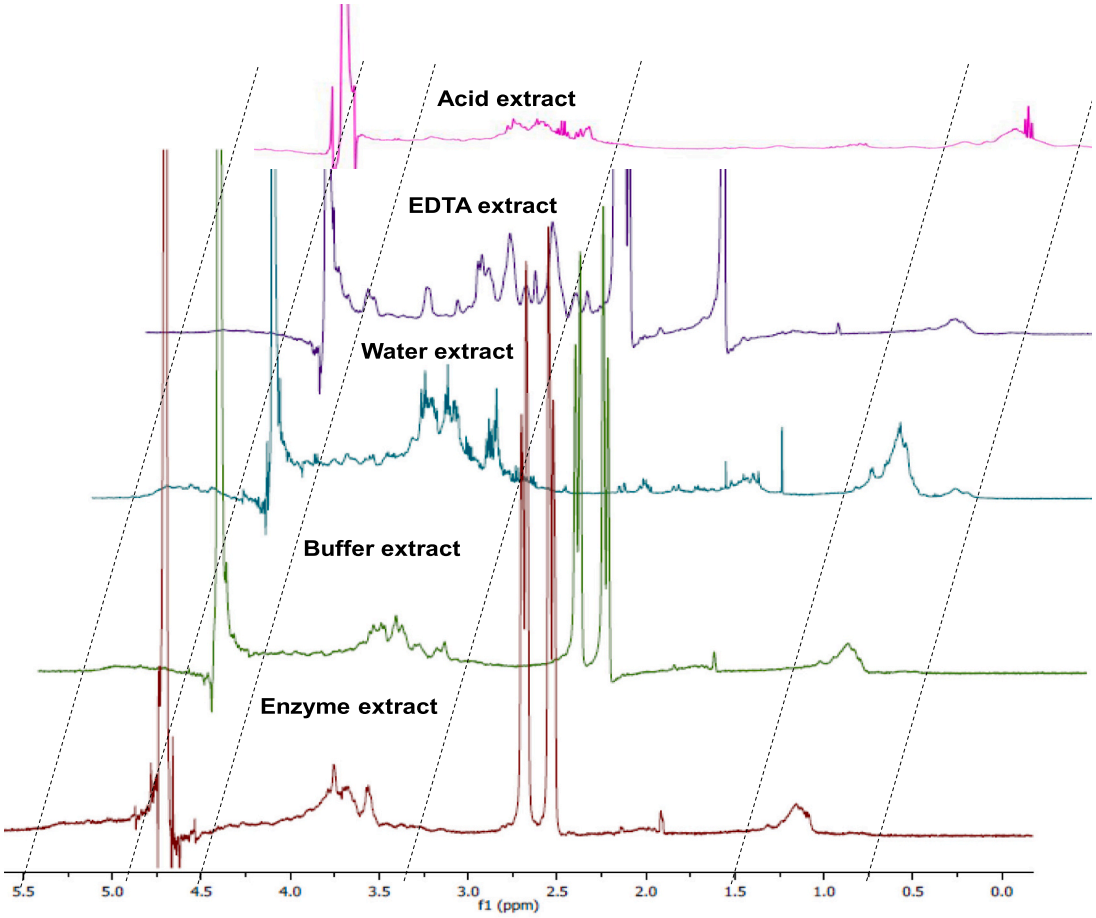


Fig. 3. ^{1}H NMR spectra of overlaid *S. elegans* fucoidan extracts. Overlaid ^{1}H NMR spectra of *S. elegans* fucoidan extracts, featuring dotted lines that mark chemical shift regions corresponding to characteristic functional groups.

more slowly than the water and acid extracts (Fig. 4). Although there is considerable uncertainty this observation is likely due to the different amorphous (glycosidic linkages degrading at lower temperature) and even crystalline conformations (glycosidic linkages degrading at higher temperature) within these fucoidan extracts. The ash content was between 18 and 24 % within all extracts.

3.3. Chemical characterisation of *S. elegans* fucoidan extracts

The total carbohydrate content of the fucoidan extracts was analysed using the phenol-sulfuric acid method described in Section 2.5. The water, enzyme, and acid extracts had the most carbohydrates (~50 %) compared to the other extracts (~30 %) (Table 1). Our extracts had comparable total sugar content to a recently characterised Chinese *Sargassum* species fucoidan extracts, which was about 33 % [49]. However, these quantities are much smaller than the total carbohydrates reported in some fucoidans (~90 %), specifically those extracted from *Ecklonia* species [3,10]. From our analysis, extraction protocols affect the carbohydrate nature of the resulting fucoidan to some degree (Table 1).

The presence of several monosaccharides was confirmed and quantified to further ascertain the fucoidan extracts' composition. Fucose, the fucoidans' major monosaccharide, was quantified in our extracts using the *L*-fucose assay [26]. The hot water-extracted fucoidan had the highest *L*-fucose content of about 15 % compared to the other extracts, which had about 5 % (Table 1). The other prominent monosaccharides detected in most extracts were glucose, galactose, xylose and mannose. The distribution of monosaccharides in our extracts indicates that the extracts are heterofucans a recent revelation in fucoidan definition [4].

More recent studies recognise the chemical and structural diversity of fucoidans and recognise that fucoidans can contain substantial amounts of 'other' monosaccharides such as D-galactose, D-glucose, D-mannose, D-xylose [42,50]. As such, the term fucoidan now describes fucose-containing sulphated polysaccharides, including those of heterogeneous compositions (i.e., fucogalactans, fucoglucuronans and xylofucoglucuromannans) [51]. The heterogeneous composition and resultant complex structure of fucoidans, despite the efforts of researchers, still make it extremely difficult to elucidate the structural features of fucoidans fully [52].

Nevertheless, the routine characteristics of fucoidan, the total sulphate, phenolics and protein content of the fucoidan extracts were quantified. The water-extracted fucoidan had the highest sulphate level (~9.7 %) of all extracts (Table 1). The acid extract had the lowest sulphate content (~2.3 %), followed by the EDTA extract. The acidic nature of the extraction solvent is attributed to the low sulphate content of fucoidan. The extracts had a similar sulphate content to fucoidans from other *Sargassum* species reported in the literature, ranging from 5 to 12 % sulphation [49]. However, a sulphate content of around 17 % has been reported from *S. wightii* fucoidan [53], almost twice the amount of our highest extract. Our characterisation results are comparable with literature which ascertains that our extracts are fucoidans classified under heterofucans.

Protein levels in all extracts were found to be low, below 4 %. This is an improvement compared to the literature, where protein content can reach up to 8 % in fucoidan extracts [53]. Minimal levels of phenolics were also detected in the extracts (Table 1). In addition, total uronic acid (glucuronic acid) content was also quantified, as these sugar acids are normally associated with alginate contamination in fucoidan extracts.

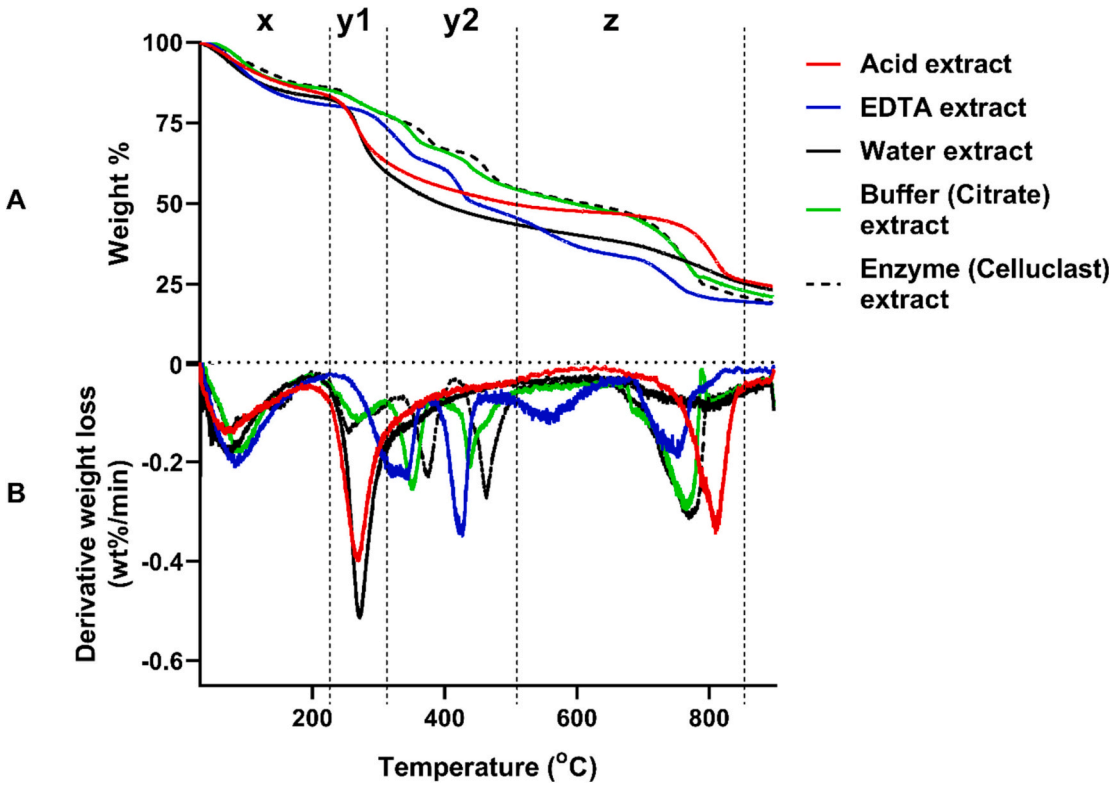


Fig. 4. Thermal gravimetric analysis (TGA) of *S. elegans* fucoidan extracts. A: TGA and B: derivative thermogram (DTG) plots of the fucoidan extracts.

Table 1
Chemical composition, molecular weight and kinematic viscosity of the extracted fucoidans.

Fucoidan extracts	Water	EDTA	Acid	Buffer	Enzyme
Total carbohydrate	49.4 ± 6.2	34.3 ± 5.4	46.8 ± 6.7	29.3 ± 3.2	27.1 ± 5.9
Sulphate content	9.7 ± 1.8	3.4 ± 0.7	2.3 ± 0.6	4.5 ± 1.4	4.1 ± 0.8
Total phenolics	2.83 ± 0.8	1.08 ± 0.7	1.5 ± 0.7	1.1 ± 0.3	1.4 ± 0.2
Total proteins	3.1 ± 0.4	2.0 ± 0.3	2.2 ± 0.6	1.9 ± 0.2	2.4 ± 0.5
Uronic acids	4.8 ± 0.6	2.9 ± 0.7	4.4 ± 0.7	2.1 ± 0.4	1.9 ± 0.3
L-fucose	15.2 ± 2.6	3.76 ± 0.8	4.85 ± 1.2	5.1 ± 1.7	5.7 ± 1.3
D-glucose	5.7 ± 1.7	4.9.0 ± 3.3	5.8 ± 2.1	3.1 ± 0.3	2.9 ± 0.4
D-galactose	5.68 ± 0.1	ND	5.4 ± 0.5	ND	ND
D-mannose	7.14 ± 1.8	4.34 ± 0.8	3.73 ± 0.4	4.4 ± 0.8	4.1 ± 0.5
D-xylose	5 ± 1.9	2.4 ± 0.7	4.2 ± 1.6	2.3 ± 0.6	2.1 ± 0.5
L-arabinose	ND	ND	ND	ND	ND
Ash content	23 ± 3.1	19 ± 3.2	24 ± 2.8	21 ± 3.1	19 ± 2.5
Mw (kDa)	84	<22	<22	<22	<22
Kinematic viscosity (cSt/s) of 0.1 g/dl fucoidan solution	1.12 ± 0.01	1.06 ± 0.01	1.1 ± 0.01	1.14 ± 0.01	1.13 ± 0.02

The values are represented as means ± SD ($n = 3$). ND: Not detected. Chemical composition is reported on a dry mass basis.

All extracts contained low levels of uronic acids, with the water extract having the highest level (~4.8 %). In addition, the ash content of the fucoidan extracts was profiled using TGA, and most extracts contained approximately 20 % ash (Fig. 4; Table 1). The ash content of these extracts was consistent with the fucoidan ash content reported in the literature [3,49], although higher contents (~40 %) are possible [53]. The relatively low protein, uronic acid and polyphenolic content also suggest that our extracts are relatively pure. This was expected as all the extraction methods included steps which removed phenolics (defatting) and the major contaminant alginate through calcium chloride treatment.

The molecular sizes of the fucoidan extracts were determined through HPSEC, with all of them exhibiting medium molecular weight fucoidan according to size classification in the literature [52]. The water-extracted fucoidan showed polydispersity with three major fractions, 84 kDa, and two fractions that were < 22 kDa. All other extracts had a molecular weight below 22 kDa (Table 1). The absence of the larger molecular weight fraction found in the water extract suggests that the other methods examined cleave the polysaccharides during extraction. Furthermore, the kinetic viscosity of the fucoidans was measured, and the results revealed that the extracts had a similar viscosity (Table 1) to water, which had a kinematic viscosity of about 1.06 cSt. Although there was no significant difference in the viscosities of these extracts, the buffer and enzyme extracts were less soluble than the acid and EDTA extracts, most soluble in water. The chemical characteristics of our fucoidan extracts prove that extraction methodologies affect the nature of the fucoidan product.

3.4. Potency of extracts as α glucosidase inhibitors

The potential of fucoidan extracts from *S. elegans* to inhibit the activity of α-glucosidase, an enzyme required to break down maltodextrins (mainly maltose) produced during starch digestion by α-amylase, was investigated. All extracts inhibited the activity of α-glucosidase in a dose-dependent manner except for the acid extract (Fig. 5). The water extract and the buffer extract showed approximately 95 % maximal inhibition of the enzyme under the assay conditions. The enzyme and EDTA extract showed approximately 80 % and 60 % maximal inhibition, respectively. Remarkably, all our fucoidans except the acid extract were

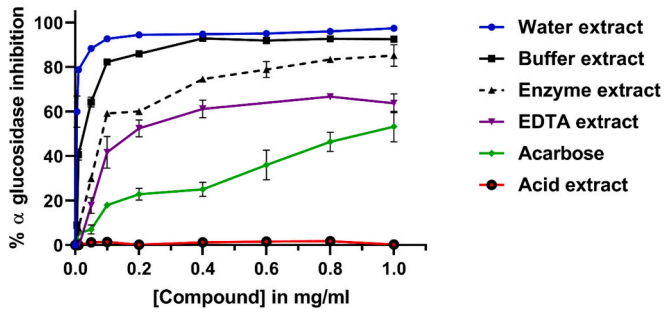


Fig. 5. Inhibition of α-glucosidase activity by *S. elegans* fucoidan. The figure shows the inhibitory effect of various *S. elegans* fucoidan extracts on α-glucosidase activity, with a comparison to the known inhibitor acarbose. The data values are presented as mean ± standard deviation ($n \geq 3$), where n represents the number of independent assays performed.

Table 2
IC_{50} (μg/ml) of *S. elegans* fucoidan extracts against α-glucosidase.

Inhibitor	IC_{50} ± SD
Water extract	25.1 ± 4.1***
Buffer extract	88.6 ± 7.9***
Enzyme	186.4 ± 10.3 **
EDTA	273.4 ± 21.2*
Acarbose	332.5 ± 23.4

Data are presented as means ± standard deviation from at least three independent experiments. The statistical significance of the results was tested using ANOVA employing the Tukey-Kramer's test between the control (acarbose) and different groups and between groups. The results were considered significant at (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$ and (ns) when not significant.

better inhibitors of the α-glucosidase enzyme than the commercial α-glucosidase inhibitor, acarbose (Fig. 5).

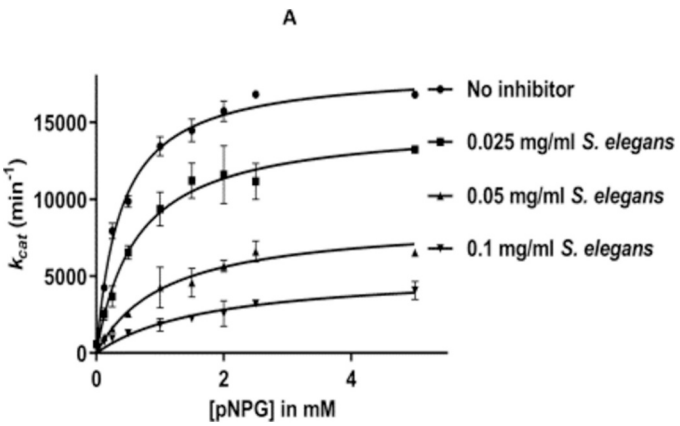
The inhibitors' half-maximal inhibitory concentrations (IC_{50}) were determined to measure the efficacies of the fucoidan extracts at inhibiting α-glucosidase. The water and buffer-extracted fucoidans were the most potent α-glucosidase inhibitors, having the lowest IC_{50} values. These were followed by the enzyme extract, EDTA extract and acarbose (Table 2). All the extracts significantly had lower IC_{50} values than the acarbose, thus the deduction that our extracts were comparably better inhibitors of α-glucosidase than the commercially available acarbose.

Acarbose is a currently used drug, among others, for T2DM management and has well-known side effects, including flatulence, meteorism, abdominal distension and even diarrhoea [54]. Our findings show that extracted fucoidans are significantly more potent inhibitors of α-glucosidase than acarbose (Fig. 5; Table 2). Acarbose IC_{50} values in an approximate range of 280–415 μg/mL are also reported in the literature [41]. Notably, the extracted fucoidans have IC_{50} values close to those of the most potent known fucoidans, including *Ascophyllus nodusum* with an IC_{50} of about 30 μg/ml [8] and *Undaria pinnatifida* with an IC_{50} of 137 μg/ml [55]. In summary, the *S. elegans* fucoidan extracts can be used as a therapeutic agent in the targeted amylolytic enzyme inhibition treatment of T2DM. The fucoidan slows down glucose production, thereby reducing its absorption into the bloodstream and reducing potential diabetic manifestations.

Fucoidan's biological activity has been linked to three main attributes: (1) molecular size, (2) sulphate content, and (3) total carbohydrate content and monosaccharide composition [8]. Our study showed that these characteristics are dependent on the extraction protocol used. Structural analysis techniques (FTIR and NMR analysis) demonstrated varying sulphate distributions among extracts. The water and enzyme extracts showed relatively more intense FTIR sulphate stretching bands between 1220 and 1260 cm^{-1} regions (Fig. 2) than the other extracts. This band is almost non-existent in the acid extract. The water extract has another distinct band at 850 cm^{-1}, also associated with sulphates linked to sugars [36]. The sulphate content may be attributed to the water extract's superior bioactivity (α-glucosidase inhibition) and relatively good bioactivity of the enzyme and citrate buffer extract (Fig. 5; Table 2). Similar to the FTIR analysis, NMR showed prominent peaks in the water extract's 1.28–1.45 ppm region. Although the enzyme and buffer extracts showed less pronounced peaks in this region than the water extract, these were significantly more intense than those of the EDTA and acid extracts. Since the peaks between 1.25 and 1.45 ppm depict the presence of sulphates linked with fucose [41]; thus, these sulphates are essential for improving fucoidan bioactivity. Moreover, colourimetry analysis quantified the highest sulphate content (~9.7 %) in the water extract compared to the ~4.5 % sulphates within the buffer and enzyme-extracted fucoidan (Table 2). These data together fortify that sulphate content is important for α-glucosidase inhibition. Our results and argument are consistent with a study that linked fucoidan bioactivity to a higher sulphate constitution [56]. Sulphates are known to increase the overall negative charge of fucoidan, helping the compounds to interact with α glucosidase [8] . It is thought that fucoidan's sulphated groups are important because they bind to α-glucosidase's active site, blocking substrate access and lowering enzymatic activity. Additionally, fucoidan may reduce the catalytic efficiency of an enzyme by changing its conformation through hydrogen bonding and electrostatic interactions [55]. These mechanisms point to its potential as a treatment for diabetic conditions that involve postprandial hyperglycaemia.

Carbohydrate content is another fucoidan component implicated in their biological activities. Quantification of total sugars demonstrated that the water and acid extract had the most carbohydrate content than all the other extracts. Although their carbohydrate content is comparable, the acid extract has low sulphates, rendering it inactive. Also, interestingly, the enzyme and citrate buffer extract had half the carbohydrate content of the acid extract; these showed remarkable activity. This observation may suggest that sulphates are more critical than carbohydrate content for α glucosidase inhibition. Furthermore, fucose content seems critical in enhancing the bioactivity as this monosaccharide is more abundant (Table 2) in the most bioactive water extract than any other extract. These quantifications are in agreement with our FTIR data (Fig. 2) (band at ~850 cm^{-1}) and NMR data (Fig. 3) (1.28–148 ppm) which showed prominent peaks known to be fucose-sulphate links. Thus, this monosaccharide is important for the fucoidans' bioactivities.

Molecular weight is another determining characteristic associated with the biological activity of fucoidans. Low molecular weight fucoidans are mostly associated with high biological activity due to their improved bioavailability [57]. Our fucoidans were classified as medium molecular weight fucoidans. Interestingly, our water-extracted fucoidan showed variable molecular weight fractions, with 84 kDa being the largest and two other fractions <22 kDa. In addition, the water extract was the most inhibitory for α-glucosidase activity. The low molecular weight fractions of the fucoidan may be associated with increased bioactivity [57,58]. However, all other technologies examined extracted low Mw (<22 kDa) heterofucans. Given this fact, they still showed lower bioactivity than the water extract with a larger molecular weight fraction. This shows that molecular weight is not the only bioactivity-determining factor in fucoidans bioactivities although it is an important factor. Taken together, we suggest that extraction technologies influence many aspects, including the structural and biochemical heterofucan properties essential for their bioactivity. These properties should be in a complex balance to enhance fucoidans' α-glucosidase inhibition.



B

Experiment	K_M (mM)	k_{cat} (min^{-1})
No inhibitor	0.4	14 812
0.025 mg/ml	0.63	10 956
0.05 mg/ml	1.08	4 618
0.1 mg/ml	1.77	1 321

Fig. 6. Kinetic parameters for α-glucosidase inhibition by *S. elegans* fucoidan.
(A) Michaelis-Menten curves showing α-glucosidase activity in the presence of increasing concentrations of *S. elegans* fucoidan, illustrating its inhibitory effect. (B) Derived kinetic parameters, including turnover number (k_{at}) and Michaelis constant (K_m), of α-glucosidase inhibitory capacity by *S. elgans* fucoidan.

3.5. Mode of inhibition of S. elegans fucoidan

The hot water extract of *S. elegans* (the most active *S. elegans* fucoidan) was further studied to determine its mode of inhibition against α-glucosidase. The fucoidan displayed a mixed-type mode of inhibition with an increase of K_M and a decrease of V_{max} (which was represented as k_{cat}) as the inhibitor concentrations were increased (Fig. 6) [59]. This finding was consistent with our previous work [3,10]. In addition, as a mixed inhibitor, fucoidan can limit competition with the natural substrate of enzymes. This will be significant because the fucoidan drug would still work after a carbohydrate-rich meal. Otherwise, the carbohydrate would outcompete the therapeutic agent if it were a competitive inhibitor.

3.6. Fucoidan directly interacts with α-glucosidase

Perturbations caused by the inhibitor generally lead to conformational changes in the enzyme's tertiary structure, which can be represented by dose-dependent shifts in relative tryptophan fluorescence with increasing inhibitor concentration. This implies that fucoidan interacts with α-glucosidase in a way that changes the enzyme conformation, obscuring its tryptophan residues and quenching the fluorescence (Fig. 7A). The modified Stern-Volmer plot's *n* value of approximately 1, indicates that a direct interaction of fucoidan and the enzyme occurs at 1 site. This interaction may occur in the active site region or elsewhere since fucoidan is a mixed inhibitor. Also, the binding constant (*K*) between fucoidan and α-glucosidase was calculated to be 133.3 μg/ml.

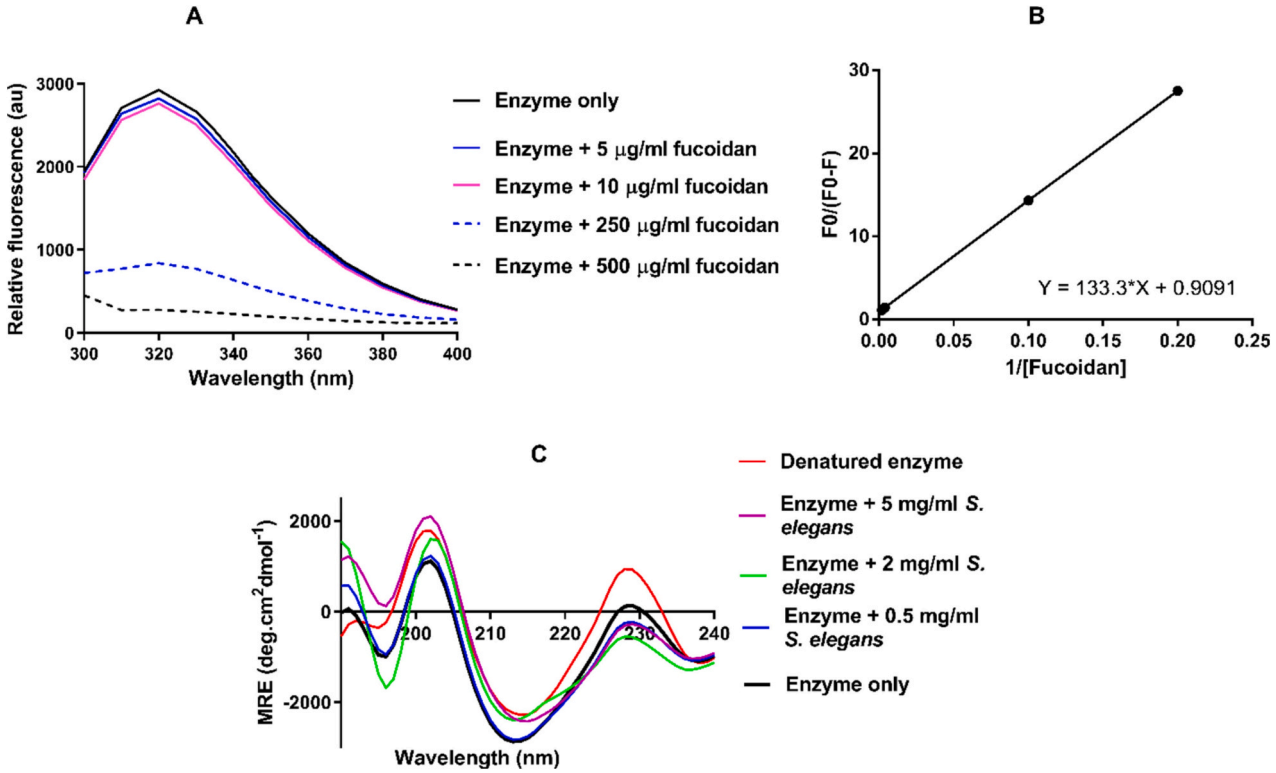


Fig. 7. Fucoidan directly interacts with α glucosidase. A: Fluorescence emission spectra of intrinsic fluorescence quenching of α-glucosidase in the presence of *S. elegans* fucoidan. B: Modified Stern-Volmer plot of the α-glucosidase-fucoidan complex C: Circular dichroism spectra showing changes of α-glucosidase secondary structure in the presence of the *S. elegans* fucoidan. Values are presented as means ± SD ($n = 3$).

Table 3
S. elegans fucoidan effect on α-glucosidase secondary conformation.

Fractions of deconvoluted secondary structure	α-helix	β-strands	β-turns	Unordered
α-glucosidase	0.036	0.396	0.189	0.379
boiled enzyme	0.026	0.425	0.204	0.344
0.5 mg/ml *S. elegans*	0.033	0.397	0.190	0.380
2 mg/ml *S. elegans*	0.035	0.392	0.183	0.388
5 mg/ml *S elegans*	0.037	0.43	0.213	0.319

Although these types of experiments on digestive enzymes are limited, similar observations have been reported in the literature [10,60]. In addition, *S. elegans* fucoidan interfered with the secondary structure conformation of α-glucosidase. The incremental presence of the inhibitor shifted the deconvoluted spectrum towards the positive horizontal in a dose-dependent fashion (Fig. 7B). The deconvoluted CD spectra α-glucosidase showed that the enzyme's secondary structure primarily consists of β-sheets (Table 3).

The protein's secondary structure constituted 39.6 % β-strands, 37.9 % unordered regions, 18.9 % β-turns and 3.6 % α-helices. When the fucoidan is introduced, there is a redistribution of conformations, which is also true for the boiled/denatured enzyme used as a control (Table 3). Along with the tryptophan fluorescence experiment, it can be suggested that fucoidan directly interacts with α-glucosidase. Although limited studies are available on the interaction of fucoidan with α-glucosidase, several other inhibitors, including gallotannins and bis (2.3 dibromo-4,5-dihydroxybenzyl) ether interact directly with the enzyme [61,62]. Our results are consistent with reports that have indicated that slight shifts in the secondary and tertiary structure of α-glucosidase were observed in the presence of inhibitors [61,62]. Furthermore, our findings in these experiments are consistent with our previous work using different fucoidan extracts [3,10].

4. Conclusions

The extraction technologies yielded different amounts of structurally and biochemically unique fucoidan fractions. In addition, two novel fucoidan extraction methods (citrate buffer and enzyme-assisted) improved the fucoidan yield 4-fold compared to the conventional extraction methods using water and mineral acid. However, the purity of fucoidan was reduced within the EDTA, citrate buffer and enzyme extracts, which yielded heteropolymeric extracts. Regarding the bioactivity of our fucoidan extracts, the degree of sulphation increased the AGI potency of the fucoidans. However, other factors, including carbohydrate content, monosaccharide composition, molecular weight, and purity of the fucoidan, may also be critical for its bioactivity. Overall, this study has shown that *S. elegans* fucoidan extracted using most of these technologies, except for mineral acid-derived extracts, can potentially serve as a biotherapeutic agent for the targeted inhibition of amylolytic enzymes to treat T2DM. In addition, we believe that polysaccharide extractions may illustrate the commercial feasibility of algal products in the nutraceutical and food industries by providing a higher extraction yield while maintaining adequate bioactivity levels in the extracts. Moreover, the impressive AGI activity of the fucoidans makes them plausible candidates in the alleviation of the burden of type 2 diabetes. Our work also probes for further research in fucoidan structure bioactivity studies.

CRediT authorship contribution statement

Blessing Mabate: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chantal Désirée Daub:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Samkelo Malgas:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Brett Ivan Pletschke:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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