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Isolation, purification, partial characterisation and evaluation of the *in vitro* antioxidant and anti-inflammatory potential of fucoidan from *Sargassum fluitans* from Lagos Badagry coast

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Abstract

Sargassum fluitans is a species of free-floating brown macroalgae (seaweed) found in the Atlantic Ocean and Caribbean Sea, often forming massive mats that wash onto beaches. Its presence hinders fishing activities by locals and recreational activities around the beaches. Sargassum contains steroids, polyphenols and polysaccharides like alginate and fucoidan amongst many others. Fucoidan is a negatively charged and sulphated polysaccharide with known pharmacological activity. Thus, this study was intended to isolate, partially characterise and evaluate the *in vitro* antioxidant and anti-inflammatory activities of purified fucoidan from *Sargassum fluitans* from Badagry coast. The seaweed was collected and fucoidan was isolated using acid-extraction method for optimal yield. Purification was done using ion exchange chromatography, and partial characterisation was conducted with HPLC, FTIR and NMR. *In vitro* antioxidant (DPPH and H_2O_2 scavenging ability, reducing power, ferric reducing antioxidant power), and anti-inflammatory (NO, Protein inhibition and albumin denaturation assays) of the purified fucoidan were monitored and compared with standards (Ascorbic acid and Aspirin, respectively). The purified fucoidan had a higher fucose (67.96%) and sulfate (28.41%) content compared to the crude fucoidan. Compared to fucoidan from some tropical and subtropical species like *S. siliquosum*, and *S. hemiphyllum,* our isolate had higher sulfate but similar fucose content. *In vitro* antioxidant and anti-inflammation activities of the purified fucoidan was significantly higher ($p<0.05$) compared to the standard ascorbic acid and aspirin, respectively. These findings suggest potential application of this readily available bioresource currently regarded as an environmental nuisance.

Keywords: Brown seaweed, *Sargassum fluitans*, fucoidan, Antioxidant, Inflammation

1.Introduction

Seaweed is an important marine biological resource and plays an important role in the material cycle of the marine ecosystem and the maintenance of marine ecological balance. Recently, seaweed has been considered as a promising new biomass feedstock for the production of biochemicals and the development of commercial value-added products [1]. Sargassum is a type of brown algae that contains a variety of bioactive compounds, including fatty acids, polyphenols, peptides, and polysaccharides [2]. In many parts of Asia, Sargassum, as an edible brown algae, contains unique hydrocolloid polysaccharides, such as alginate and carrageenan, which is a potential source of bioactive polysaccharides [3]. *Sargassum* species are found throughout tropical and subtropical areas of the world and are reported to produce metabolites of structural classes such as terpenoids, polysaccharides (fucoidan and Alginate), polyphenols, sargaquinoic acids, sargachromenol, plastoquinones, steroids, glycerides etc., which possesses several therapeutic activities [4]. *Sargassum fluitans* is a species of free-floating brown macroalgae (seaweed) found in the Atlantic Ocean and Caribbean Sea, often forming massive mats that wash onto beaches [5]. This ecological versatility is largely the result of physiological and biochemical changes that Sargassum species undergo so that they can withstand and thrive in widely different environments while optimizing growth, reproduction, and longevity [6].

Previous studies by [7] on sargassum mainly focus on the structural analysis and biological activity evaluation of its main component, fucoidan. Fucoidan is a water-soluble heteropolysaccharide mainly compose of L-fucose and sulfate groups, containing uronic acid and other monosaccharides, such as glucose, xylose, galactose and mannose [8]. Its composition depends on the source of algae, extraction and purification methods, harvest time and location[9]. Fucoidan is a unique polysaccharide characterized by the presence of sulfate esters attached to specific carbon groups on the main α-L-fucopyranose chain [10]. This group of polysaccharides is predominant in the cell walls of brown seaweed, especially those of the genus Fucus and others. Research has shown different sources of fucoidan, such as Fucus vesiculosus, Ascophyllum nodosum, Laminaria japonica, Sargassum latissimi, and Ecklonia cava [10]. They are structurally complex polysaccharides made up of different monomers such as fucose (main sugar monomer), galactose, mannose, xylose, and uronic acids. Additionally, research has shown fucoidan to possess residues of proteins, minerals, and other phenolic compounds [11]. The skeletal structure of fucoidans is made up of either repeating units of (1→3) or alternating (1→3) and (1→4) linked α-fucopyranose units. Fucoidans are negatively charged due to the presence of their sulfate esters and the carboxylic moieties found in other species[10]. As part of their rich composition and structural heterogeneity, research has also proven fucoidan to possess different biological activities such as anticancer, anti-ageing, antioxidant, anti-inflammatory, anticoagulant, antimicrobial, antiatherogenic, gastroprotective, and cardioprotective activities.

General reports have indicated that the biological activities of fucoidans are dependent on certain factors, such as the molecular weight, the extraction method, the degree of sulphation, the source of fucoidan, and the type of sugar monomers [10]. In Nigeria, little is known about the properties and possible applications of fucoidan from *Sargassum fluitans*. In the recent time, the presence of *Sargassum fluitans* has been observed in many oceans in Nigeria, which poses a serious threat to fishing activities and the sustenance of the people. This research is an attempt to mitigate this problem by assessing the possible use of fucoidan isolated from this seaweed. This will in the future help in turning waste into wealth and create employment for the populace. Therefore, the study focused on the isolation, purification, partial characterisation and evaluation of the *in vitro* antioxidant and anti-inflammatory potential of fucoidan from *Sargassum fluitans* from the Lagos-Badagry coast.

2.MATERIALS AND METHODS

2.1 Collection of Seaweed and Identification

The fresh seaweed was collected at Suntan Beach Badagry, Lagos State (Longitude: 2° 57' 05. 1“E and Latitude: 6° 13' 27. 1“N). The seaweed was identified and authenticated in the botanical herbarium of the Department of Botany, Faculty of Science, Lagos State University with the voucher number **LSH 001177**.

2.2 Extraction of Crude Fucoidan

An acid extraction process was employed; the seaweed was thoroughly washed with freshwater to remove sand, salts, and other impurities. It was air-dried at room temperature and mechanically ground using a blender to obtain a fine powder suitable for extraction [12]. To remove lipids, the powdered seaweed was subjected to solvent extraction using a mixture of chloroform, methanol, and water in the ratio 4:2:1. The mixture was allowed to stand for 12–24 hours with occasional stirring. The chloroform phase will extract fats, while the methanol-water phase will retain the polysaccharides. The sample was sieved, washed with acetone to eliminate excess pigments, and air-dried. Acid pretreatment was done to further disrupt the algal cell wall and release intracellular compounds using 0.01 M HCl. The pretreated seaweed was soaked in this mixture and subjected to steaming for 6 hours. This process will enhance the release of fucoidan from the algal matrix. Following the extraction, the mixture was centrifuged at 10,000 rpm for 10 minutes. After centrifugation, the supernatant (containing fucoidan) was carefully decanted into a clean container for further analysis, while the pellet (containing insoluble debris) was discarded. The clarified extract was treated with ethanol to precipitate fucoidan. Equal volumes of cold ethanol were added to the supernatant and left to stand at low temperature until the polysaccharide precipitated. The precipitate was collected, washed with ethanol, and lyophilised to yield crude fucoidan.

2.3 Purification of Crude Fucoidan

Purification of the crude fucoidan was conducted using Diethylaminoethyl (DEAE) Sephadex A-25 (Sigma-Aldrich) and eluted by gradient NaCl stepwise elution [12]. Briefly, crude polysaccharides was dissolved in 0.1M buffer Tris-Cl pH 7.0 was loaded to a column of DEAE sephadex A-25 (sigma Aldrich) fast flow (4cm x 25cm), followed by step wise elution using 50ml of sodium chlorides solutions (0.5-2.5M) at the flow rate of 1ml/min. Eluents (5Ml/ tube) were separately collected, and ethanol (HPLC grade) was added at a 1:2. All fractions were filtered, freeze-dried and stored at 4°C until used. The carbohydrate content in each eluent was determined using a phenol-sulfuric acid method by [13] using fucose (Sigma-Aldrich) as the standard. Finally, all the fractions containing polysaccharides were dialysed with deionised water and lyophilised for further study.

2.4 Chemical Composition Analysis

The polysaccharide was determined using the phenol-sulfuric acid colourimetric technique, and L-fucose was used as the standard. The L-fucose content was analysed by the colourimetric method [13] using L-fucose as the standard. A modified carbazole colourimetry was used to calculate uronic acid. The sulphate concentration was measured by $BaCl_2$ gel method by [13]. The phenolic content was measured according to the method of Vázquez-Rodríguez *et al*., (2025): 250 µL of reconstituted fucoidans, 1500 µL of distilled water, and 125 µL of Folin–Ciocalteu reagent (F9252, Sigma-Aldrich, United States) were placed in a tube and allowed to react for 5 min at room temperature. Subsequently, 500 µL of distilled water and 375 µL of 7.5% Na_2CO_3 were added; the mixture was homogenised, and the reaction was incubated for 2 h in the dark. After this time, 200 µL of the reaction mixture was placed in triplicate, and the absorbance at 750 nm was measured.

2.5 Monosaccharide Composition Analysis

The monosaccharide composition was determined using HPLC using a method of [13]. Briefly, 5 mg of polysaccharides was hydrolysed with 1 mL of 2 M trifluoroacetic acid (TFA) at 110 °C for 6 h. After cooling to room temperature, TFA was eliminated using a rotary evaporator and repeated additions of methanol (2 mL). Thereafter, 1 mL of ultra-pure water was added to create the polysaccharide solution. Then, a 5 mL glass tube with a plug was filled with 400µL of polysaccharide solution, 5 mg/mL of monosaccharide standard solutions (Man, Rha, Glu, Gal, and Xyl), and mixed monosaccharide standard solution, respectively. After that, 400µL sodium hydroxide solution and PMP derivative reagent were added in turn and reacted at 70ºC for 100 min. After cooling to room temperature, 400µL hydrochloric acid was added to neutralise, and then extracted with 2 mL chloroform. The supernatant was filtered through a 0.22 µm aqueous membrane and loaded into a sample bottle for use. Analysis was conducted on an HPLC system (Waters E2695, Milford, MA, USA) with an analytical Kromasil 100-5-C 18 column

(150 x 4.6 mm, 5µm, Kromasil M05CLA15, Göteborg, Sweden). The mobile phases were 0.05 M phosphate buffer solution (pH 6.8) and acetonitrile (83: 17, v/v), with a flow rate of 1 mL/min, 30 °C. The UV detection wavelength was set at 245 nm.

2.6 Fourier Transform Infrared (FTIR) Spectrometric and Nuclear Magnetic Resonance ^{1}H NMR Analysis

According to Jia's procedure, the FTIR analysis was completed. The dried fucoidan sample was mixed with potassium bromide (KBr). The mixture was ground in an agate mortar for 5–10 min, and then pressed into tablets, which were scanned by FTIR (Bruker Tensor-27, Billerica, Massachusetts, Germany) in the range of 500–4000 cm^{-1} with KBr as background. For the ^{1}H NMR analysis, the dialysed polysaccharide was lyophilised and deuterium-exchanged with D2O. The samples were then dissolved in D2O, and a minute amount of deuterated dimethyl sulfoxide was added as an internal standard. NMR spectra were obtained using a JEOL JNMECX400, NMR spectrometer, Japan at 33 k.

2.7 *In vitro* Antioxidant Assay of Fucoidan

2.7.1 DPPH free radical scavenging activity

To create a radical solution (DPPH), 0.02g of DPPH was dissolved in 100ml of methanol. Then, 50 µL of the different concentrations of purified fucoidan (50-400µg/mL) was mixed with 2 mL of the methanolic DPPH solution. After thorough shaking, the mixture was left undisturbed at room temperature for a duration of 30 minutes, ensuring it was kept away from light. The absorbance of the resulting mixture was then measured using a spectrophotometer at a wavelength of 515 nm, a method by [14]. Ascorbic acid was used as the standard. The percentage of DPPH radical scavenging activity was determined using the following formula:

Formula 1: Percentage Inhibition = [(Control – Test) / (Control) * 100]

2.7.2 Hydrogen Peroxide scavenging activity

Different concentration (50-400µg/ml) of 0.6mL fucoidan was combined with 0.6 mL of phosphate buffer (50 mM, pH 7.4) and 0.6 mL of hydrogen peroxide solution (2 mM in phosphate buffer, 50 mM, pH 7.4). The mixture was thoroughly mixed using a vortexer, and after a duration of 10 minutes, the absorbance was measured at a wavelength of 230 nm using a UV-Visible spectrophotometer, a method by [15]. Ascorbic acid was employed as the standard, and a phosphate buffer (50 mM, pH 7.4) was utilised as the blank in the experiment. The percentage of hydrogen peroxide scavenging activity was determined using Formula 1 above.

2.7.3 Reducing Power Assay

The total reducing power assay of purified fucoidan was calculated by [16]. Briefly, 0.13 mL of different concentrations of fucoidan (50-400µg/mL) was prepared with phosphate buffer and potassium ferricyanide and incubated at 50 ◦C (20 min). The chemical reaction was then stopped by mixing 1.25 mL of TCA (10% w/v) with the test chemical. The chemical combination was centrifuged at 3000× g rpm (15 min), and then the supernatant was separated. Approximately 1.5 mL (0.1 % w/v) of ferric chloride solution was added, and the absorbance was measured at 700 nm. Ascorbic acid was employed as the standard.

2.7.4 Ferric Reducing Antioxidant Potential

FRAP reagent was prepared according to [16] by mixing in 25 mL acetate buffer (30 mM; pH 3.6), 2.5 mL TPTZ solution (10 mM) and 2.5 mL ferric chloride solution (20 mM). The mixture was incubated for 15 min at 37 °C before use. Ascorbic acid (vitamin C) was employed as a standard in this assay, and its calibration curve was obtained by using its concentrations ranging from 50 mg/L to 500 µg/mL in water. To 2.85 mL FRAP reagent in a test tube, 150 µL of different concentration of fucoidan (50-400µg/mL) was added. The mixture was incubated for 30 min in the dark, and its absorbance was measured at 593 nm. The blank will contain an equal volume of methanol instead of the test sample. The results were reported as µg of ascorbic acid equivalents (AAE) per mL.

2.8 *In vitro* ANTI-INFLAMMATORY ASSAY

2.8.1 Protein Inhibitory Action

A 2 mL reaction mixture was prepared, consisting of 1 mg trypsin, 1 mL of 20 mM Tris HCl buffer (pH 7.4), and 50 µL of the fucoidan extract at different concentrations (50-400 µg/ml). The mixture was then incubated at 37°C for 5 minutes, following which 1 mL of 0.8% (W/V) casein was introduced. The reaction was allowed to continue for an additional 20 minutes, after which 2 mL of 70% perchloric acid was added to stop the reaction. The cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank, and water was used as control, a method by [17]. Formula 1 was used to determine the percentage of inhibition of proteinase inhibitory activity.

2.8.2 Inhibition of albumin denaturation

A 1% stock solution of bovine serum albumin (BSA) was prepared using double distilled water. Additionally, a stock solution of fucoidan extract was prepared at a concentration of 10 mg/mL. The reaction mixture consisted of 0.5 mL of BSA and 50 µl of the fucoidan extract (50-400µg/mL). This mixture was then incubated at room temperature for a duration of 15 minutes. To induce denaturation, the reaction mixture was subjected to heating at 60°C for a period of 10 minutes. Subsequently, the absorbance of the mixture was measured at a wavelength of 660 nm using a UV-visible spectrophotometer, a method

by [18]. Only BSA was used as a negative control. The formula shown below was used to determine the percentage inhibition of denaturation:

Percentage Inhibition = [(Negative control – Test) / (Negative control) * 100

2.8.3 Inhibition of Lipoxygenase

The 15-LOX (Sigma) will make up to a working solution of 200 units/mL and kept on ice according to the method of Soares, et al. [19]. A volume of 12.5 µL of the fucoidan (50-400µg/ml) or control (dissolved in DMSO) was added to 487.5 µL of 15-LOX and incubated at room temperature for 5 min. After incubation, 500 µL substrate solutions (10 µL linoleic acid dissolved in 30 µL ethanol, made up to 120 mL with 2 M borate buffer at pH 9.0) were added to **the** solution. After 5 min incubation at room temperature, the absorbance was measured at 234 nm. Aspirin (1 mg/mL) was used as a positive control, while DMSO was used as the negative control (100 % enzyme activity or no enzyme inhibition). The percentage enzyme inhibition of each test sample was compared with the positive control.

2.8.4 Nitric oxide (NO) Radical Scavenging Assay

Nitric oxide inhibitory assay was analysed according to the methods of Soares, et al. [19]. The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate-buffered saline (0.5 mL), and 0.5 mL of fucoidan extracts (50-400µg/ml) was incubated at 25 ◦C for 150 mins. After incubation, 0.5 mL of the nitrite reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulfonic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 mins to complete diazotisation. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was added, mixed, and allowed to stand for 30 min at 25 ◦C. A pink-coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions using a UV-Vis spectrophotometer. Aspirin was used as a standard. The percentage (%) inhibition activity was calculated from the following equation: [(A0 – A1)/A0] x 100. Where A0 is the absorbance of the control, and A1 is the absorbance of the extract or standard.

2.9 STATISTICAL ANALYSIS

Data were expressed as mean ± SEM using GraphPad Prism 9.0 statistical package. Values were considered significant at ($p<0.05$).

3. RESULTS

3.1 Purification and partial characterisation

Figure 1 shows the chromatographic elution profile of the polysaccharide fractions**.** The elution profile of the fucoidan extract at 490 nm revealed three major sub-fractions: F1 (tubes 4–7), F2 (tubes 18– 20), and F3 (tubes 26–28). Among them F1 exhibited the highest absorbance.

Table 1 shows **t**he chemical and monosaccharide compositions of the crude fucoidan and the three purified fractions (pF1, pF2, pF3). The crude fucoidan had a significant increase in polysaccharide content of (84.25 ± 0.09%) and a decrease in sulphate content of (15.31 ± 0.01%) compared to the other fractions. Progressive purification through the column fractions resulted in systematic changes in composition. The pF3 had a significant increase ($p<0.05$) in the sulphate content 28.41 ± 0.14% compared to the 15.31 ± 0.01% in the crude fucoidan, 18.20 ± 0.31% in pF1 and 20.33 ± 0.01% in pF2. Similarly, uronic acid content significantly ($p<0.05$) increased progressively from 5.10 ± 0.03% in crude preparation to 11.10 ± 0.06% in pF3. Polyphenol content significantly ($p<0.05$) decreased progressively across fractions, from 0.42 ± 0.01% in crude fucoidan to 0.15 ± 0.01% in pF3. Galactose was the second most abundant monosaccharide, significantly ($p<0.05$) decreasing from 28.72% in crude fucoidan to 17.22% in pF3 as purification enriched the fucose-rich fraction. Minor sugars including rhamnose, mannose, xylose, and glucose were present in smaller proportions and showed significant ($p<0.05$) decreasing trends with progressive purification.

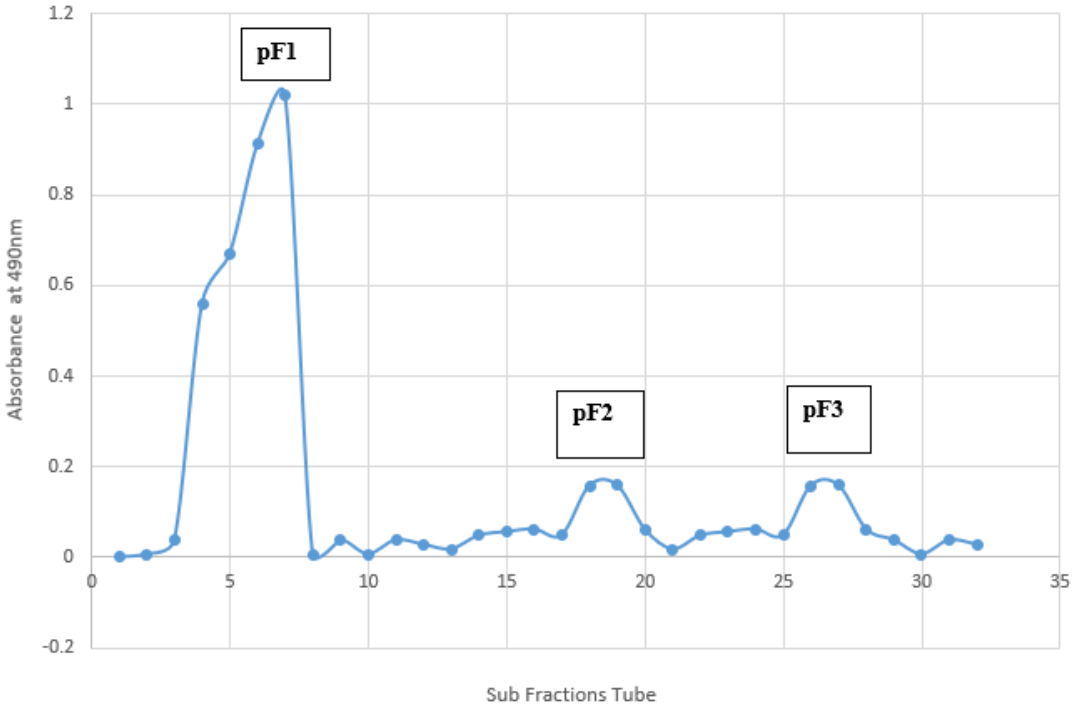


Figure 1: Chromatographic elution profile of Polysaccharide fractions showing absorbance at 490 nm

Table 1: Chemical and monosaccharide composition of crude Fucoidan and column fractions from *Sargassum fluitans* from Lagos Badagry coast

		Crude Fucoidan	pF1	pF2	pF3
Yield (%)		23.40 ±0.19[a]	13.14±0.15[b]	11.04±0.03[b]	23.14±0.07[a]
Chemical Composition (%)	Polysaccharides	84.25 ±0.09[a]	64.25 ±0.21[b]	64.75 ±0.08[b]	68.25 ±0.22[c]
	Sulphate	15.31±0.01[a]	18.20±0.31[b]	20.33±0.01[b]	28.41±0.14[c]
	Uronic acid	5.10±0.03[a]	8.10±0.07[b]	7.10±0.11[c]	11.10±0.06[d]
	Polyphenols	0.42 ±0.01[a]	0.28±0.01[b]	0.21±0.01[c]	0.15±0.01[d]
Monosaccharide Composition (%)	Fucose	43.21	45.21	52.83	69.57
	Galactose	28.72	25.36	21.47	17.22
	Rhannose	1.85	1.70	1.20	0.7
	Mannose	7.29	6.82	5.72	1.1
	Xylose	4.30	3.91	2.85	1.9
	Glucose	8.51	7.30	4.22	2.1

Chemical composition was calculated based on triplicate determinations. Results are given as the mean ± SD. Values with different alphabets are significantly different from each other across each row ($p<0.05$).

pF- purified Fucoidan

Table 2 shows the absorption band at 1245.34 cm^{-1}, attributed to the asymmetric S=O stretching vibration of sulphate ester groups. The C–O–S bending vibrations observed at 882.96 and 816.06 cm^{-1} are indicative of sulphate substituents at the axial C-2 and C-4 positions on the fucopyranose ring. The absorption at 1029.00 cm^{-1} corresponds to C–O–SO_3 vibrations associated with the sulphate group linkage.

Figure 2 shows the FT-IR spectra of fucoidan fraction isolated from the brown seaweed *Sargassum fluitans from* Lagos Badagry coast. The FTIR spectrum of the fucoidan extract displayed characteristic absorption bands consistent with a sulphated polysaccharide structure. A broad absorption band at 3422.91–3271.02 cm^{-1} corresponded to O–H stretching vibrations, while peaks at 2930.59 and 2361.89 cm^{-1} were attributed to C–H stretching and bending vibrations of alkyl groups. The band at 1597.70 cm^{-1} indicated the presence of carboxyl groups, likely from uronic acids, whereas the peak at 1412.35 cm^{-1} was assigned to C–H bending vibrations. A strong absorption at 1245.34 cm^{-1} was characteristic of S=O stretching of sulphate esters, and the peak at 1029.00 cm^{-1} was attributed to C–O–S stretching vibrations. Distinct bands at 882.96 cm^{-1} and 816.06 cm^{-1} were assigned to C–O–S bending vibrations, suggesting sulphate substitution at the axial C2 and C4 positions of the fucose residues. Low-frequency peaks (661.13– 629.69 cm^{-1}) corresponded to skeletal vibrations of the sugar ring and sulphate groups.

Figure 3 shows the ^{1}NMR spectra of fucoidan (pF3) fraction isolated from the brown seaweed *Sargassum fluitans*. The figure depicts the characteristic anomeric proton signals in the region of 4.5–5.5 ppm, consistent with α-configured L-fucopyranose residues. The methyl doublet signal of the C-6 methyl group of fucose, typically appearing around 1.2 ppm, was also detected.

TABLE 2: FTIR absorption bands of pF3 fucoidan fraction and their corresponding functional groups

S/No	Wavenumber cm-1	Functional Groups
1	2930.59, 2361.89	Stretching vibrations and bending vibrations of Alkyl groups (-CH_2- and -CH_3-)
2	1245.34	S=O Stretching vibration
3	1029.00	C-O-SO_3 vibration
4	882.96, 816.06	C-O-S bending vibration of the Sulphate Substitute at the axial C2, C4 position

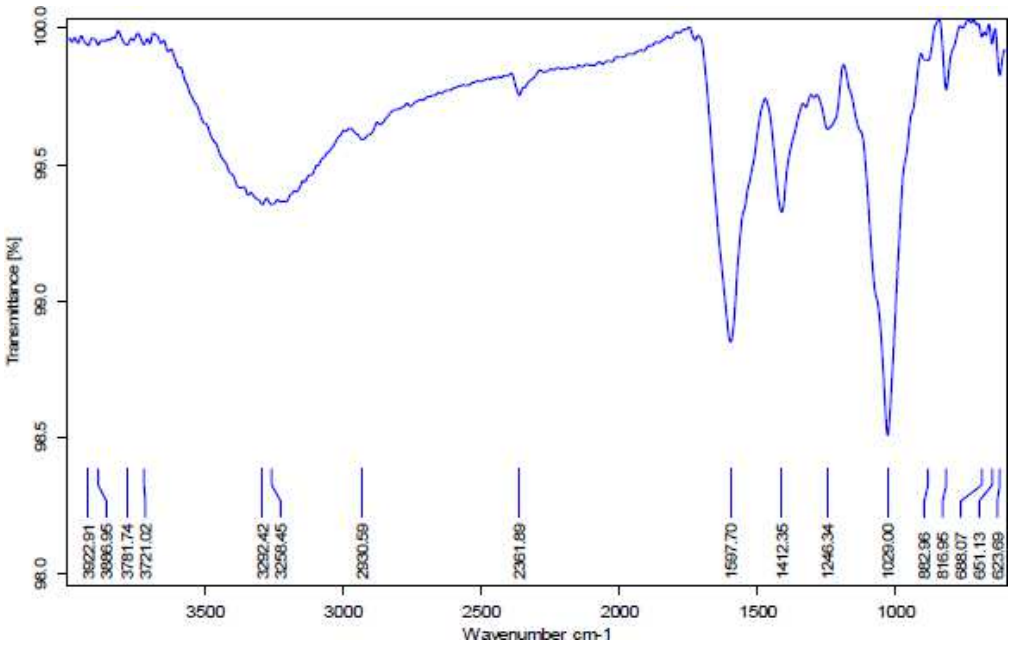


Figure 2. FT-IR spectra of pF3 fucoidan fraction isolated from the brown seaweed *Sargassum fluitans* from Lagos Badagry coast.

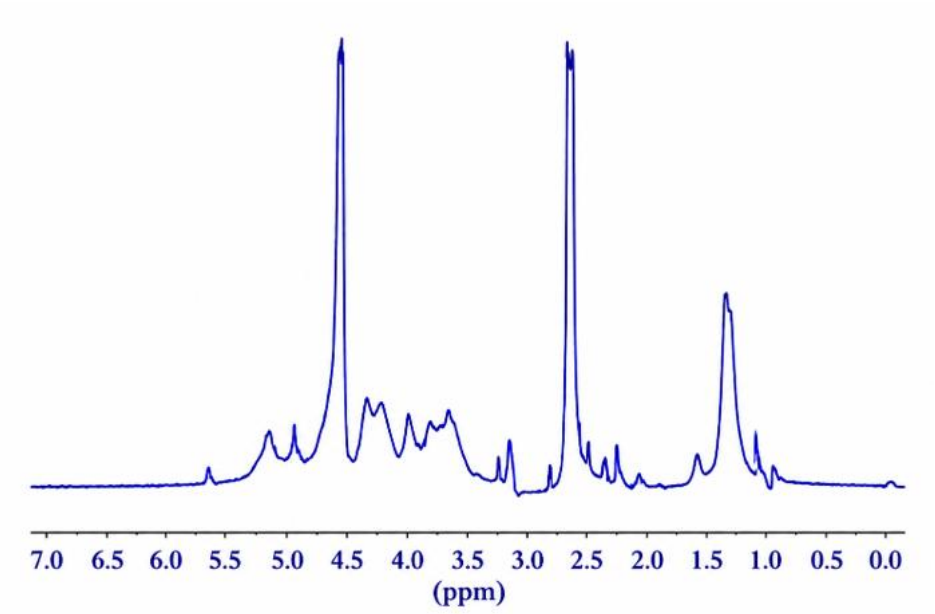


Figure 3: ^{1}NMR spectra of fucoidan (pF3) fraction isolated from the brown seaweed *Sargassum fluitans* from Lagos Badagry coast.

3.2 *In-vitro* Antioxidant Activity fucoidan from *Sargassum fluitans* from Lagos Badagry coast

Figure 4A depicts the DPPH free radical scavenging activity of purified fucoidan (pF3) from *S. fluitans* at concentrations of 50–400 µg/mL. The purified fucoidan demonstrated concentration-dependent DPPH radical scavenging activity, with activity significantly ($p<0.05$) increasing as concentration increased from 50 to 400 µg/mL. Although, at 100 and 150 µg/mL, ascorbic acid had a significant higher DPPH scavenging activity compared to fucoidan. The DPPH scavenging activity of fucoidan was significantly higher than that of the standard (ascorbic acid) at the highest concentration of fucoidan.

Figure 4B shows the hydrogen peroxide (H_2O_2) scavenging activity of purified fucoidan (pF3) at concentrations of 50–400 µg/mL. The result showed that as the fucoidan concentration increases, there was a significant ($p<0.05$) increase in the H_2O_2 scavenging activity. The purified fucoidan exhibited significant concentration-dependent across the tested concentration range. The pF3 significantly ($p<0.05$) exhibited a greater H_2O_2 scavenging activity compared to the standard (ascorbic acid) at corresponding concentrations.

Figure 4C shows the ferric reducing antioxidant potential (FRAP) of purified fucoidan (pF3) from *S. fluitans* at concentrations of 50–400 µg/mL. FRAP values, expressed as µg of ascorbic acid equivalents (AAE) per mL, significantly ($p<0.05$) increased in a concentration-dependent manner across all tested concentrations of purified fucoidan. Although, at 100 and 150 µg/mL, ascorbic acid had a significant higher ferric reducing antioxidant potential compared to fucoidan. Fucoidan significantly ($p<0.05$) exhibited a higher ferric reducing antioxidant potential compared to the standard (ascorbic acid) at the highest concentrations.

Figure 4D shows the reducing power of purified fucoidan (pF3) from *S. fluitans*, at concentrations of 50–400 µg/mL. The result showed that the reducing power of fucoidan significantly ($p<0.05$) increased in a concentration-dependent manner from 50 to 400 µg/mL. Although, at 100 and 150 µg/mL, ascorbic acid had a significant higher reducing power compared to fucoidan. The reducing power of fucoidan was significantly ($p<0.05$) higher than that of the standard (ascorbic acid) at the highest concentrations.

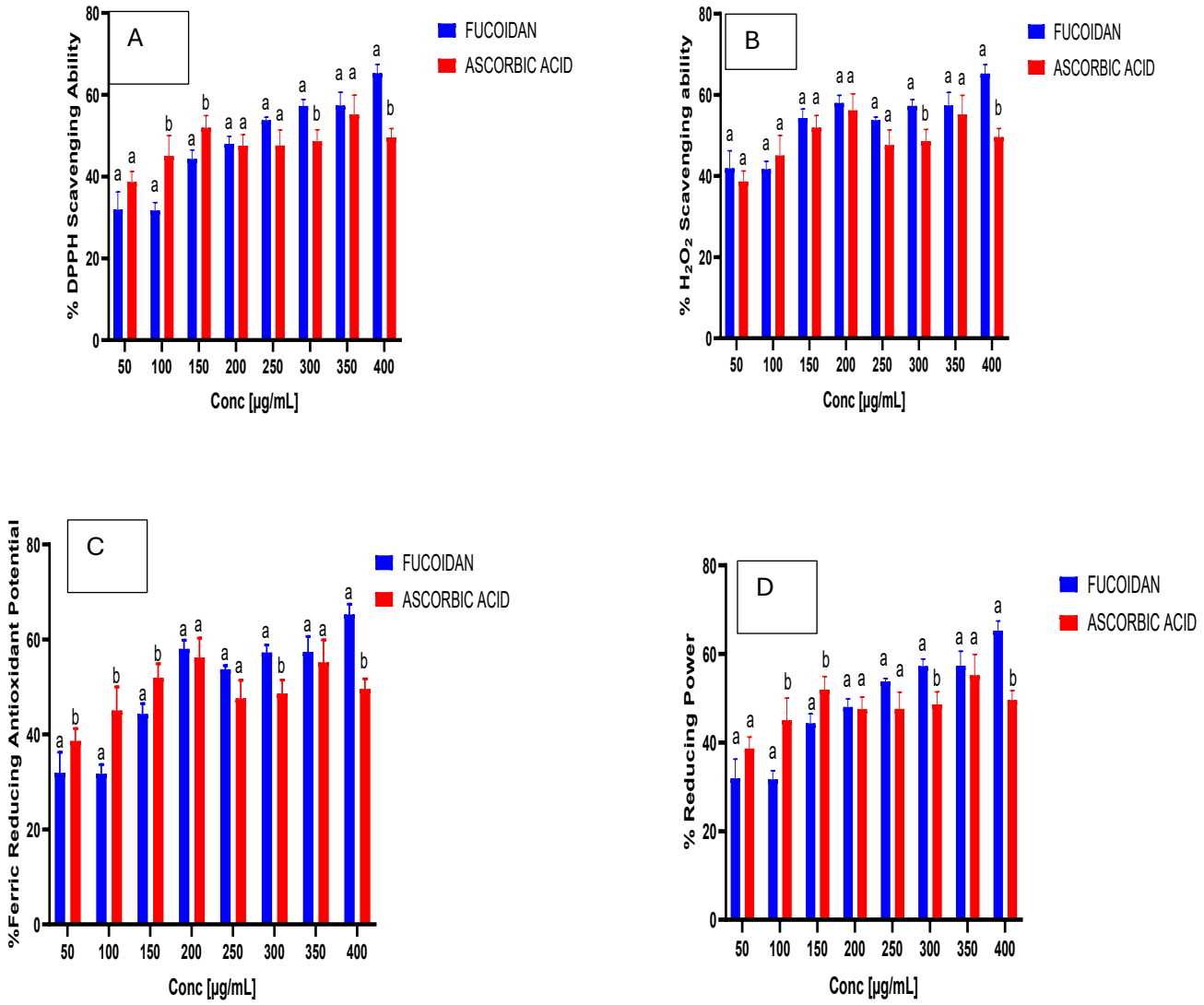


Figure 4: (A)DPPH Scavenging Activity (B) H_2O_2 Scavenging Ability (C) Ferric Reducing Antioxidant Potential, (D) Reducing Power of fucoidan from *Sargassum fluitans* from Lagos Badagry coast. All values are represented as mean ± SEM. Bars with different alphabets are significantly different from each other ($p<0.05$).

3.3 *In-vitro* Anti-inflammatory Activity of fucoidan from *Sargassum fluitans* from Lagos Badagry coast

Figure 5A shows the protein inhibitory activity of purified fucoidan (pF3) from *S. fluitans* at different concentrations. Purified fucoidan demonstrated concentration-dependent inhibition of trypsin-mediated proteolysis across the tested concentration range of 50–400 µg/mL. The percentage inhibition of proteinase activity significantly ($p<0.05$) increased with increasing fucoidan concentration, indicating a positive dose-response relationship. The protein inhibitory potential of fucoidan was significantly ($p<0.05$) higher than that of the standard (aspirin) at the highest concentrations.

Figure 5B shows the ability of purified fucoidan (pF3) to inhibit heat-induced albumin denaturation at concentrations of 50–400 µg/mL. The percentage inhibition of Albumin denaturation significantly ($p<0.05$) increased as the fucoidan concentration increased from 50 to 400 µg/mL, demonstrating a clear dose-response relationship. The albumin denaturation inhibition potential of fucoidan was significantly ($p<0.05$) higher than that of the standard (aspirin) at the highest concentrations.

Figure 5C depicts the nitric oxide radical scavenging activity of purified fucoidan (pF3) from *S. fluitans* at concentrations of 50–400 µg/mL. The percentage inhibition of NO significantly ($p<0.05$) increased as the concentration of the purified fucoidan concentration increased from 50 to 400 µg/mL, demonstrating a clear dose-response relationship. The NO inhibitory potential of fucoidan was significantly ($p<0.05$) higher than that of the standard (aspirin) at the highest concentrations.

Figure 5D shows the lipoxygenase (LPO) inhibitory activity of purified fucoidan (pF3) from *S. fluitans*. The purified fucoidan demonstrated concentration-dependent inhibition of 15-lipoxygenase activity across the tested concentration range of 50–400 µg/mL. The LPO inhibitory potential of fucoidan was significantly ($p<0.05$) higher than that of the standard (aspirin) at the highest concentrations.

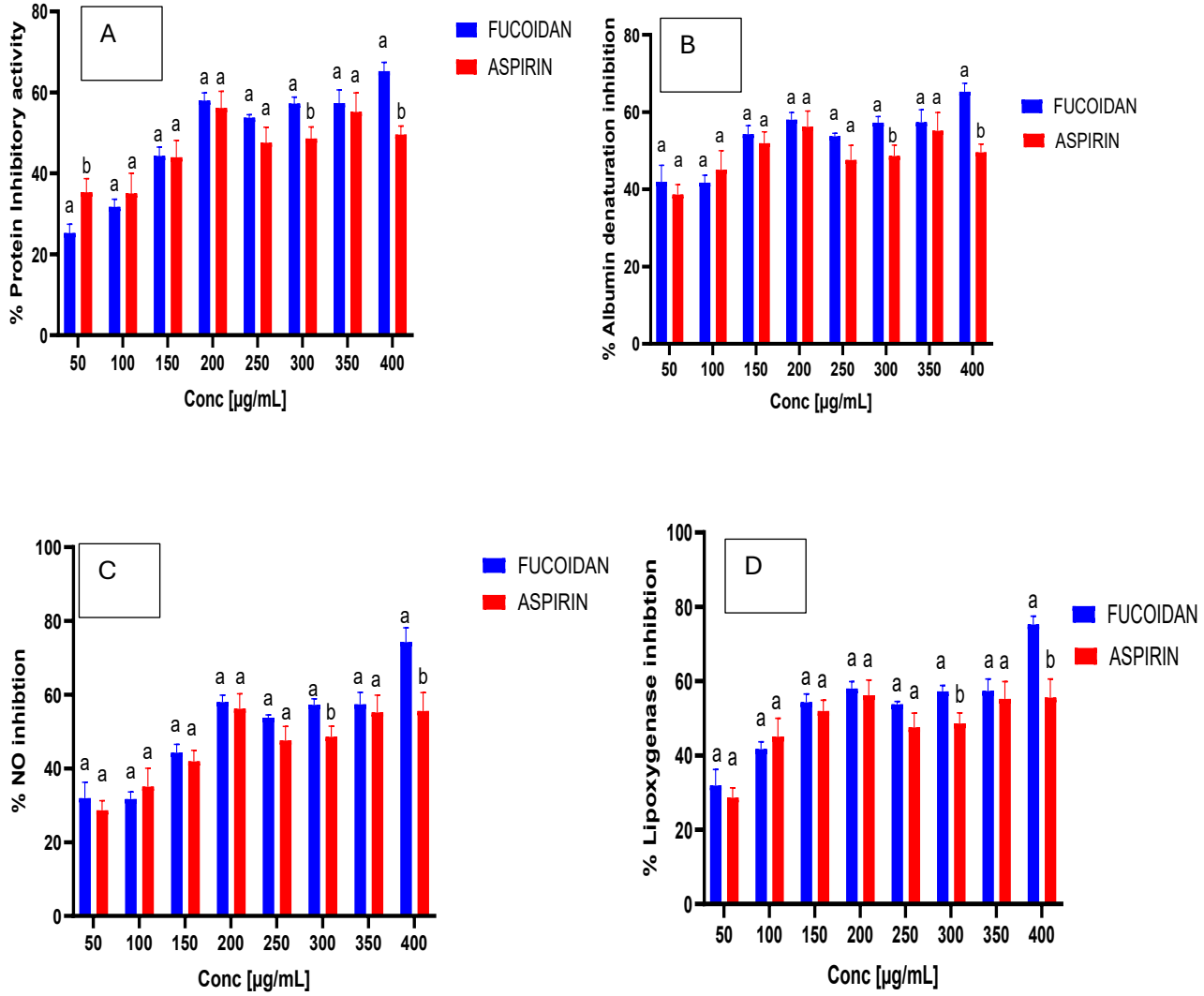


Figure 5: (A) Inhibition of Albumin denaturation ability, (B) Protein inhibitory ability, (**C**) Nitric Oxide inhibition, (D) Lipoxygenase inhibiton ability of fucoidan from *Sargassum fluitans*. All values are represented as mean ± SEM. Bars with different alphabets are significantly different from each other ($p<0.05$).

3.4: The EC_{50} values of the *invitro* antioxidant and anti-inflammatory activity of fucoidan from *Sargassum fluitans* from Lagos Badagry coast

Table 3 shows the EC_{50} antioxidant value for the fucoidan and ascorbic acid. The result depicts that fucoidan exhibited significant ($p<0.05$) lower EC_{50} values than ascorbic acid in the FRAP, and DPPH scavenging assays indicating stronger activity in the FRAP and DPPH assays. However, the hydrogen peroxide scavenging and reducing-power assay, fucoidan showed a significant ($p<0.05$) higher EC_{50} compared to ascorbic acid

Table 4 shows the EC_{50} anti-inflammatory value for the fucoidan and aspirin. The result depicts that fucoidan exhibited significant ($p<0.05$) lower EC_{50} values compared to aspirin in inhibiting albumin denaturation, protein inhibition, and Lipoxygenase activity. However, the Nitric oxide scavenging ability of fucoidan showed a significant ($p<0.05$) higher EC_{50} compared to aspirin.

Table 3: EC_{50} Antioxidant Values (µg/mL) for fucoidan from *Sargassum fluitans* and Ascorbic acid

	Hydrogen Peroxide Scavenging Activity (µg/mL)	Ferric Reducing Antioxidant Power (µg/mL)	Reducing Power (µg/mL)	DPPH Scavenging Activity (µg/mL)
Fucoidan	216 ± 0.01^{a}	115 ± 0.03^{a}	129 ± 0.01^{a}	148 ± 0.03^{a}
Ascorbic Acid	192 ± 0.01^{b}	173 ± 0.01^{b}	103 ± 0.01^{b}	181 ± 0.01^{b}

Values are represented as Mean ± SD in triplicates. Values with different alphabets are statistically different from each other at $p \leq 0.05$ within each column.

Table 4: EC_{50} Anti-inflammatory activity values (µg/mL) for fucoidan from *Sargassum fluitans* and Ascorbic acid

	Protein Inhibition (µg/mL)	Albumin denaturation (µg/mL)	Nitric Oxide (NO) Inhibition (µg/mL)	Lipoxygenase activity (µg/mL)
Fucoidan	116 ± 0.11^{a}	125 ± 0.13^{a}	192 ± 0.16^{a}	136 ± 0.03^{a}
Aspirin	122 ± 0.21^{a}	164 ± 0.22^{b}	133 ± 0.05^{b}	141 ± 0.08^{b}

Values are represented as Mean ± SD in triplicates. Values with different alphabets are statistically different from each other at $p \leq 0.05$ within each column.

4. DISCUSSION

The purification and partial characterisation of fucoidan resulted in fractions with distinct sulphate and carbohydrate contents. The chromatographic elution profile showed that the crude fucoidan extract was successfully separated into three major sub-fractions (pF1–pF3). Among these, pF1 exhibited the highest absorbance at 490 nm, indicating that it contained the largest proportion of polysaccharides. The presence of multiple peaks suggests heterogeneity within the crude fucoidan extract, which is consistent with earlier studies reporting that fucoidan from brown seaweeds often consists of distinct sub-fractions differing in molecular weight, charge density, and sulphate content [20, 21]. The purification step therefore not only enriched fucoidan but also allowed the separation of its structural variants, which could contribute effectively to its antioxidant and anti-inflammatory activities.

The compositional differences between crude fucoidan and the purified fractions obtained by DEAE-Sephadex A-25 chromatography. Although Carbohydrate, fucose, uronic acid and sulfate ester group contents can vary slightly due to extraction techniques, harvest location, harvest season and alga species [1, 22]. Variability in components has been documented for other seaweeds such as *S. polycystum, S. graminifolium* and *S. wightii* [23, 24]. In the present study, the crude extract of *Sargassum fluitans* from Lagos Badagry coast showed a high polysaccharide content (84.25%), pF3 exhibited a substantially higher sulphate content (28.41%) and uronic acid content (11.10%), together with a significant increase in the fucose content from 43.21% in the crude extract to 69.57% in pF3, which is higher compared to the fucoidan isolated from *Sargassum fluitans* Borgesen from Mexico which was reported to have a fucose and sulphate content of 11.67% and 15.6% respectively [25].

The high fucose and sulphate contents of pF3 of *Sargassum fluitans* from Lagos Badagry coast are characteristic of a highly sulphated fucoidan and are biochemically relevant because sulphate substitution and monosaccharide composition influence molecular charge, structural conformation and interactions with biological targets [26]. Therefore, the high fucose and sulphate content observed in pF3 supports its selection as the purified fucoidan fraction for subsequent antioxidant, anti-inflammatory and anticancer investigations [27].

Conversely, galactose, mannose, xylose, glucose and rhamnose significantly ($p<0.05$) decreased following purification, indicating selective enrichment of the fucose-rich sulphated polysaccharide fraction (pF3). The reduction in polyphenol content from 0.42% to 0.15% further suggests removal of non-polysaccharide co-extractives during purification [28]. This compositional pattern agrees with previous reports showing that ion-exchange fractionation of *Sargassum henslowianum Sargassum fusiforme* (Fucales)-derived fucoidans can produce fractions with progressively higher fucose and sulphate contents [29, 30].

The FTIR spectrum obtained in this study is consistent with the typical structural features of fucoidan reported in previous literature where the broad absorption band between 3422.91–3271.02 cm^{-1} reflects O– H stretching vibrations, a characteristic feature of polysaccharides [31]. Peaks at 2930.59 and 2361.89 cm^{-1} correspond to C–H stretching vibrations of $–CH_2–$ and $–CH_3–$ groups was also observed in fucoidan extracted from *Sargassum hemiphyllum* and *Turbinaria* species [32]. The band at 1597.70 cm^{-1} indicates the presence of uronic acids, which has similarly been reported in fucoidan from *Fucus vesiculosus* and other brown algae [33]. The strong absorption peak at 1245.34 cm^{-1} is characteristic of the asymmetric stretching of S=O bonds, confirming the presence of sulphate ester groups, which is a key functional group responsible for the biological activity of fucoidan [34, 35]. The peak observed at 1029.00 cm^{-1} corresponds to C–O–S stretching vibrations and glycosidic linkages, further supporting the sulphated polysaccharide structure. Notably, the absorption bands at 882.96 cm^{-1} and 816.06 cm^{-1} represent C–O–S bending vibrations, which indicate that the sulphate groups are substituted at the axial C2 and C4 positions of the fucose residues. Similar assignments have been described in fucoidan from *Sargassum binderi* and *Undaria pinnatifida* [36, 37]. Thus, the FTIR analysis confirms that the fucoidan extracted from *Sargassum fluitans* possesses the characteristic sulphate ester groups, uronic acid residues, and sugar backbone vibrations typical of bioactive fucoidan polysaccharides. These findings agree with previous studies by Wang *et al*., (2020), thereby validating the structural identity of the isolated compound. The presence of uronic acids may further contribute to the biological activities observed. These structural features are consistent with previous reports by [38] on *Sargassum muticum* and *S. henslowianum*, thus confirming that *S. fluitans* from the Badagry coast is a viable source of bioactive fucoidan

The ^{1}H-NMR spectrum of the isolated fucoidan from *Sargassum fluitans* exhibited characteristic resonances associated with sulphated polysaccharides. Signals in the δ 3.5–4.5 ppm region were attributable mainly to the ring protons of fucose and other sugar residues, while resonances in the δ 4.5–5.5 ppm region corresponded predominantly to anomeric protons of pyranose residues, supporting the presence of α-linked sugar units, similar to the findings of Ale *et al*., (2011). The signal observed around δ 1.2–1.5 ppm was consistent with the methyl protons of L-fucopyranose residues. The broad and overlapping nature of the resonances reflected the structural heterogeneity of fucoidan, which arises from variations in monosaccharide composition, glycosidic linkages and sulphate substitution (Ale *et al*., 2011). Overall, the ^{1}H-NMR profile supported the identity of the isolated material as a fucose-containing sulphated polysaccharide, consistent with fucoidan reported from brown seaweeds, including *Sargassum* species (Zayed, A. *et al*. 2020).

Naturally synthesised antioxidants from seaweed and other natural products are eco-friendly and less toxic than synthetic antioxidants. Many studies reported that seaweed possesses potential antioxidant properties [39]. Fucoidan antioxidant activity was determined by measuring its hydrogen peroxide, DPPH free radical scavenging, ferric reducing potential and reducing power activity. The hydrogen peroxide assay measures the ability of an antioxidant to reduce hydrogen peroxide, which is a reactive oxygen species [40-42]. Fucoidan showed a dose-dependent reduction of hydrogen peroxide. The lower the EC_{50}, the greater the potency of a bioactive compound or drug. The hydrogen peroxide scavenging activity of fucoidan was comparable to that of the ascorbic acid. A similar report was documented by Chang *et al*., (2023) who investigated the antioxidant activities of crude polysaccharides obtained from *H. fusiforme* by boiling water extraction and the H_2O_2-degraded polysaccharides.

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is effectively scavenged by antioxidants, and it is characterised by a stable nitrogen-centered free radical with an unpaired electron [41]. The purple alcoholic DPPH solution turned yellow due to the reduction of DPPH to DPPH-H, which confirms the presence of an antioxidant [43]. The DPPH radical has been extensively used as a free radical to test extracts for their reductive ability as free radical scavengers or hydrogen donors, as well as to evaluate plant extract antioxidant capacity [41, 44]. Fucoidan showed dose-dependent scavenging activity of the DPPH radical comparable to the natural antioxidant ascorbic acid. However, fucoidan had a significantly lower EC_{50} value compared to Ascorbic acid. This activity may be attributed to the high sulphate content of the purified fucoidan. This is in accordance with the previous study of [45] which correlated the antioxidant activity and sulphate content of the fractions of sulphated polysaccharides from *Porphyra haitanesis*

The FRAP assay is a preferred method for the measurement of the antioxidant capacity of a particular compound due to the fast nature of the reaction [46, 47]. The assay is based on the reduction of a ferric tripyridiltriazine complex (Fe^{3+}–TPTZ) to its ferrous form (Fe^{2+}–TPTZ). This study depicted that fucoidan from *Sargassum fluitans* from Lagos Badagry coast had a dose-dependent ferric reducing potential with higher potency compared to L-ascorbic acid. The above findings is in tandem with earlier reports where fucoidan from *Sargassum polycystum* showed a dose-dependent ferric reducing potential [43].

Reducing power assay depends on the reduction of potassium ferricyanide by the bioactive compounds and demonstrates the ability to give up electrons at a pH close to neutral. The principle of the assay explains it reduces ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}). The purified fucoidan from *Sargassum fluitans* displayed a greater reducing power with increasing concentration but the EC_{50} value was significantly higher than L-ascorbic acid indicating lower potency compared to the standard. The above findings coincide with earlier reports where fucoidan of *S. tenerrimum* showed dose-dependent antioxidant activity in reducing Fe^{3+} to Fe^{2+} [48].

The consistent results from FRAP, DPPH, reducing power and hydrogen peroxide scavenging assays highlight fucoidan's broad-spectrum antioxidant potential. It's ability to donate electrons and neutralize oxidants underscore its role as a natural antioxidant that could contribute to the prevention of oxidative damage. It can be suggested that sulphate content and concentration of the fucoidan played a vital role in the hydrogen peroxide scavenging activity. In agreement with the present study, Zhang *et al*. (2011) earlier reported that the sulphated derivatives of polysaccharides obtained from fresh persimmon (*Diospyros kaki L*.) fruit, showed dose-dependent reducing power and free radical scavenging effect of 1,1-dipheny-l-2-picrylhydrazyl, they further stated that the sulphated modification of polysaccharides significantly increased their antioxidant activities.

Protein denaturation explains inflammation status in disease-associated conditions such as cancer, rheumatoid arthritis [49, 50]. The prevention of protein denaturation is the main mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs) [49]. Proteinases of leukocytes play a major role in the development of tissue damage during inflammation [51]. A significant level of protection is provided by proteinase inhibitors [52]. Several studies report that fucoidans show radical-scavenging and anti-inflammatory effects [53]. From the result, there was a significant increase in the protein inhibitory activity of the fucoidan from *Sargassum fluitans* from Lagos Badagry compared to the standard (Aspirin). The presence of fucose and sulphate present in fucoidan may contribute to its anti-inflammatory activity [53]. The fucoidan inhibited protein denaturation, and its percentage inhibition was significantly ($p<0.05$)

higher when compared to the standard aspirin. Fucoidan from *Sargassum wightii* (27.5% of sulphate and 31.8% of fucose) also prevented the protein denaturation effect on bovine albumin solution (BSA) [53]. Sulphated polysaccharide from *S. swartzii* (in which fucose is the main sugar) similarly showed inhibition of BSA denaturation by 49.1% at a concentration of 10 µg/mL [54]. The findings from this study suggests that fucoidans can inhibit protein denaturation and may modify autoantigen synthesis, making them a promising agent for the treatment of inflammation-related diseases.

Nitric oxide (NO) is an important signalling molecule but becomes potentially damaging when produced excessively during inflammation [55]. Excess NO can react rapidly with superoxide ($O_2^{\bullet -}$) to generate peroxynitrite ($ONOO^-$), a highly reactive nitrogen species capable of oxidising proteins, lipids and DNA and thereby contributing to inflammatory and oxidative tissue injury [56]. From the result of this study, the nitric oxide radical-scavenging assay revealed that purified fucoidan from *Sargassum fluitans* exhibited significant concentration-dependent antioxidant activity at concentrations ranging from 50 to 400 µg/mL. The progressive increase in NO inhibition with increasing fucoidan concentration indicates an enhanced capacity of the purified polysaccharide to neutralise reactive nitrogen species. Therefore, the observed NO-scavenging activity suggests that fucoidan may attenuate reactive nitrogen species-mediated cellular damage. This activity may be associated with structural characteristics of fucoidan, including its sulphated fucose-rich polysaccharide structure, molecular weight and degree of sulfation. Previous studies have demonstrated that fucoidans from brown seaweeds can inhibit NO production and inflammatory responses through both direct antioxidant mechanisms and modulation of iNOS, COX-2, NF-κB and MAPK signalling pathways [57]. Similarly, purified fucoidan fractions from *Sargassum fusiforme* have been shown to inhibit NO production and inflammatory mediators, while suppressing iNOS and COX-2 [58]. The above findings is in agreement with previous studies reporting NO-scavenging and anti-inflammatory activities of brown-seaweed fucoidans [59].

Lipoxygenases (LPO) are non-heme iron-containing dioxygenases involved in the oxidation of polyunsaturated fatty acids, particularly arachidonic acid (AA) and linoleic acid [60]. The lipoxygenase pathway generates hydroperoxyeicosatetraenoic acids (HPETEs), which can subsequently serve as precursors for several bioactive lipid mediators [61]. The LPO pathway therefore represents an important alternative inflammatory pathway to cyclooxygenase (COX)-mediated prostaglandin synthesis [62]. From the present report, the fucoidan isolated from *Sargassum fluitans* from Lagos Badagry coast demonstrated significant ($p<0.05$) increased concentration-dependent inhibition of lipoxygenase activity at concentrations ranging from 50 to 400 µg/mL ($p < 0.05$). The progressive increase in inhibitory activity with increasing fucoidan concentration indicates that the purified fucoidan possesses appreciable anti-lipoxygenase activity, with the effect at higher concentrations approaching that observed for the aspirin standard. Inhibition of lipoxygenase by fucoidan from *Sargassum fluitans* may therefore reduce the

formation of selected oxygenated lipid metabolites and modulate downstream inflammatory signalling. This finding is particularly relevant because the lipoxygenase pathway represents a complementary branch of arachidonic acid metabolism to the cyclooxygenase pathway [63], suggesting that fucoidan may exert *in vitro* anti-inflammatory activity through multiple biochemical mechanisms. The observed activity may be influenced by the structural characteristics of fucoidan, including molecular mass, sulphate content and degree of sulfation, which are known to affect the biological activities of brown-seaweed fucoidans [64]. Furthermore, the lipoxygenase inhibitory activity complements the NO-scavenging activity observed for fucoidan, collectively suggesting that the purified fucoidan possesses both antioxidant and enzyme-modulating properties. Overall, the concentration-dependent inhibition of lipoxygenase provides biochemical evidence supporting the anti-inflammatory potential of purified *S. fluitans* fucoidan, which is similar to the research of [65], who reported the *in vitro* anti-inflammatory properties of fucoidan from *Sargassum fusiforme*.

5. CONCLUSION

In conclusion, the analysis conducted in this research showed that the fucoidan obtained from *S. fluitans* collected from the Badagry coast is indeed a potent bioactive compound which exhibits strong antioxidant and anti-inflammatory activities. However, more research is needed to understand the mechanisms of action and to evaluate the safety and efficacy of fucoidan before they can be used in clinical settings. Overall, this study contributes to the growing body of research on natural products as potential sources of therapeutic agents for various diseases.

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Declarations

Ethics approval and consent to participate -Not applicable. No animal and human participants were involved in this research.

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