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Screening of Antiviral Efficacy of Few Seaweeds of Tamil Nadu Coast

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Abstract: Lately there has been a lot of interest worldwide in studies on the antiviral activities of marine natural secondary metabolites, notably marine polysaccharides. It has been established that polysaccharides made from marine sources and their derivatives have antiviral effects against potent viruses. Agricultural, biological, food, and pharmaceutical industries all make extensive use of goods obtained from algae. The most well-known chemical components found in algae are polysaccharides, which have been the subject of a variety of studies because of their varied bioactivities. Polysaccharides made from algae have recently risen to the top of pharmaceutical research due to their fascinating antiviral potential. Currently, COVID-19 can be prevented with vaccination, but the brown alga *Sargassum wightii* has several bioactive compounds that have the following qualities and may be a better option. *S. wightii* is one of the marine algae species that is rich in sulfated polysaccharides, the secondary metabolites which have antiviral action and the capacity to prevent viral proliferation. Fucoidan, a long-chain sulfated polysaccharide found in various brown algae, has potent antiviral effects. Additionally, sulfated polysaccharides from green algae (such as ulvans) and red algae (such as carrageenan), and lectins from red algae (such as griffithsin) have antiviral therapeutic agents against coronaviruses and other viruses. This research focuses on screening seaweeds for possible antiviral compounds to treat viral infections notably COVID-19.

Keywords: Algae; Polysaccharides; Antiviral Efficacy; COVID-19.

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1. Introduction

Algae, a type of macrophytic eukaryote found in saline water, is a reliable source of numerous potent bioactive natural chemicals. Marine algae are separated into four categories based on their colors, morphology, anatomy, and reproductive structures: Chlorophyceae (green algae), Phaecophyceae (brown algae), Rhodophyceae (red algae), and Cyanophyceae (blue-green algae). Many people believe that seaweed beds are a very productive and active ecosystem [1]. In addition to other healthy compounds, brown algae, the most prevalent type of seaweed,

are rich in polyphenols, peptides, carotenoids, and polysaccharides [2]. These compounds have numerous pharmacological activity and therapeutic effects on various ailments.

1.1. Bioactive polysaccharides

With greater amounts of sulfate and polyphenols, brown algae polysaccharides were shown to have better antioxidant activity than green and red algae. Brown algae may be utilized as a functional component in medications due to its derivated polysaccharides which have been demonstrated to have antithrombotic and anticoagulant activities. Approximately 40%–80% of the dry-defatted algae biomass is made up of bioactive polysaccharides, particularly alginates, laminarins, and fucoidans, which indicates that brown algae are an abundant source of these useful polysaccharides [2,3].

1.2. Antiviral Drugs And Discovery

Viral infection is one of the threats to humans and a source of various ailments, hence, significant efforts are required to investigate and discover antiviral medications [3,4]. There were only approximately 10 drugs that were legally approved to treat viral infections up to the 21st century. Since then, new antiviral drugs have been developed as a result of countless investigations and a better knowledge of the viral life cycle. We are still far from being able to manage viral infections, despite some notable advancements [5,6]. This study describes a scientific inquiry into the algal potential as antiviral agents, as well as their potential and characterization.

2. Materials and methods

2.1. Sample collection and preparation

The starting material for this investigation was brown alga that was gathered in Rameswaram's study [7]. The name given to the brown alga utilized in the study and this experiment was *Sargassum wightii* [8]. Moisture content measurement was done every 20 minutes on the samples drying in the incubator to monitor the drying process. The dry material was pulverized and sieved as per standard laboratory procedures in order to get a fine output of alga powder [9,10].

2.2. Thin layer chromatography

To prepare a solvent solution for the thin layer chromatography (TLC), 1.6 mL of water was combined with 4.8 mL of n-butanol and 3.2 mL of ethanol. A pre-made TLC plate was used for the crude fucoidan, standard L-fucose, crude alginate, and standard alginic acid. They are then placed in a solvent system and instructed to use it. $AgNO_3$ and 10N sodium hydroxide are used as derivatizing agents. In a TLC visualizer, the TLC plates were observed in UV light at 254 nm and, following derivatization, observed at 366 nm. In addition to that, TLC plates were noted in white light. These studies help to find the secondary metabolite in the algae extract [11-14].

2.3. Characterization of the sample by FT-IR

Fourier transform infrared spectroscopy (FT-IR) was used to look into the vibrations of molecules and polar bonding between different atoms. Purified fucoidan was analyzed using FT-IR spectroscopy to determine its structural configuration. 2 mg of the solid substance was examined using a Nicolet 380 spectrophotometer (Thermo Scientific, USA). A Nicolet Impact 410 Fourier transform infrared spectrophotometer was used to capture the absorptive spectra in the 4000–400 cm^{-1} region [11,12,15].

2.4. Characterization of the sample by GC-MS

Investigation of the secondary metabolites in the algae was performed by glass chromatography-mass spectrometry (GC-MS) analysis. A temperature of 220°C was originally reached at the injector port. The shots were administered using spitless injections. The carrier gas was helium C-60, flowing at a steady rate of 1 mL/min. The oven was preheated to 40°C for one minute before being raised to 220°C for 30 minutes at a rate of 2°C per minute [16]. To identify molecules, the retention times of the chromatographic peaks were contrasted with those of actual substances that were examined under identical circumstances [12,17].

2.5. Composition analysis

2.5.1. Test for carbohydrates

The typical carbohydrate test was carried out by making various concentrations of glucose solution. Fifty µL of the standard was put on a 96-well plate, along with 150 µL each of 96% H_2SO_4 and 5% phenol, respectively, for the standard. Two of the samples in this study were conducted for the carbohydrate test. Fifty µL of the sample and 150 µL each of 96% H_2SO_4 and 5% phenol, respectively, were put on a 96-well plate. These solutions were exposed to 90°C for five minutes, followed by an immediate reading of absorbance at 490 nm [18-21].

2.5.2. Test for phenol

The extract was generated in triplicate samples at a concentration of 10 mg/mL. Twenty µL of material and 100 µL of 10% Folin Ciocalteau (FC) reagent were combined in the wells of a 24-well plate. Eighty µL of 75% Na_2CO_3 was added after an incubation period of 5 minutes at room temperature. It was then maintained at 50°C for 5 minutes, followed by an immediate reading of absorbance at 700 nm.

2.5.3. Test for protein

A total of 250 µL of 1% $CuSO_4$ and 250 µL of 2% NaK were combined with 5 µL of 2% NaOH to create Reagent A. A total of 2.5 µL of FC reagent were mixed with 2.5 µL of water to create Reagent B. A total of 100 µL each of reagent A and the sample (BSA standard as the benchmark for control, algae extracts as the sample) were added to a 24-well plate. After being left alone for 10 minutes, 300 µL of reagent B was added, followed by an immediate reading of absorbance at 750 nm.

2.6. Antioxidant assay (DPPH)

For the total antioxidant test, two of the samples were separately prepared in various amounts of 10, 25, 50, 100, and 140 µL, and then brought up to 140 µL using a solution of H_2SO_4 and water. The stock solutions were supplemented with 140 mL of extract and DPPH reagent, which was created using methanol as the solvent. A sterile 96-well plate with 140 µL of methanol and 60 µL of DPPH solution in the control wells and 200 µL of methanol as the blank was used to hold the standard (sodium ascorbate) in varied concentrations. After 30 minutes of incubation at 37°C, the absorbance was measured at 517 nm using a Thermo Scientific Multiskan FC Micro-plate reader [17,22,23].

2.7. Antibacterial assay

The antibacterial assay was carried out by preparing the media by adding 1.3 g of Muller-Hington agar in 100 mL of distilled water, 0.085 g of sodium chloride in 10 mL of water, and the McFarland standard gradually for neutralization until it reaches an optical density (OD) value between 0.08–0.1, and the 10 mL of NaCl solution is divided evenly into two Petri plates that are filled with the prepared media and allowed to set. *Staphylococcus aureus* saline solution is added to one of the Petri dishes and disseminated with an L-rod, followed by

Escherichia coli saline solution being poured and spread. Another Petri dish started with pouring and spreading *E. coli saline* solution. Four discs containing the conventional medication (standard), crude extract, crude alginate, and crude fucoidan were deposited on each agar plate. The zone of inhibition is measured after 24 hours [24].

3. Results and discussions

3.1. Extraction of sample

3.1.1. Sample preparation

The analysis of moisture content is shown in **Table 1**. They were then powdered after being chopped into little bits. The total yield of moisture content is finalized at 29%. **Figure 1** revealed the reduction of moisture content in the algae with respect to the time during the drying process [12].

Table 1. Moisture content analysis

Content	Quantity
Soxhlet sample	32.00 g
HCl residue	27.26 g
Crude fucoidan	0.54 g
Crude alginate	1.53 g
Final residue	50.14 g

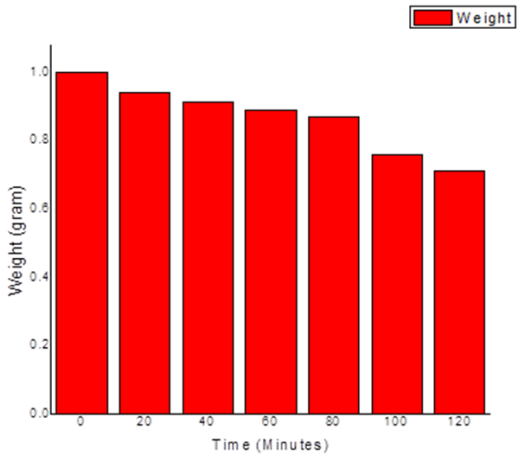

Figure 1. Plot between algae weight and time (min) during the drying process

3.1.2. Soxhlet extraction

Two muslin cloths, each weighing 32 g, are used to weigh and store the powdered sample. They are knotted firmly and kept in a thimble. The Soxhlet extraction is carried out for 6 hours while using methanol as the solvent [12,25].

For the extraction using hydrochloric acid, 0.1 M of HCl is prepared with distilled water and then adjusted to pH 3.2. The Soxhlet residue is then combined with 150 mL of an aqueous, pre-heated HCl solution, and shaken overnight [5,12].

For the extraction using sodium carbonate, after adding 2M of NaOH for neutralization to the residue and extract combination made in the previous stage, the mixture is separated using a funnel and filter paper. Following the addition of ethanol for precipitation, the resulting extract is then placed in storage containers

and kept at 4°C overnight [5,26]. The leftover material is then allowed to dry before being mixed with 150 cc of 0.2 M Na_2CO_3 solution and stored in a shaking incubator at 45°C for two hours [12]. Precipitated ethanol is then centrifuged, with the pellet and supernatant being separated and kept at 4°C. The Na_2CO_3 residue is held in a shaker and added with distilled water up to 600 mL. After that, it is kept in a low centrifuge. The pellet is then dried and kept in an incubator. The supernatant is then precipitated with ethanol and kept overnight at 4°C.

3.1.3. Characterization of the sample by TLC

Two separate methods were used to visualize the TLC plate, the first of which is the iodination method. A chamber with a few iodine crystals was used to enclose the dry plate. Different compounds inside the spots are oxidized by the iodine vapor in the chamber, making them visible. Before the iodine color vanishes and the image was taken, the visible spots are noted with a pencil. The plate was visualized using the TLC Visualizer under white light illumination (**Figure 2A**), long wavelength UV light at 366 nm (**Figure 2B**), and short wavelength UV light at 254 nm (**Figure 2C**), and the findings were reported.

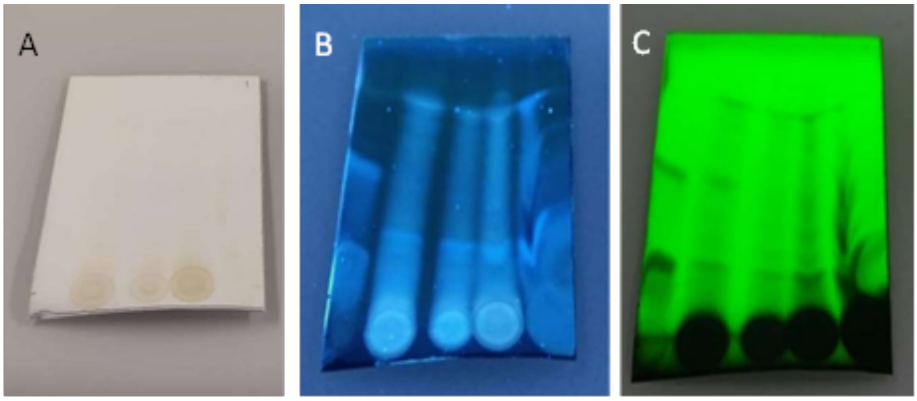

Figure 2. Thin layer chromatography visualization. (A) Visualization under white light; (B) Visualization under long wavelength UV light at 366 nm; and (C) Visualization under short wavelength UV light at 254 nm.

Figure 3 showed the UV absorbance of fucoidan in TLC plates and these compounds give the peak under the UV region. Three peaks were raised due to the presence of functional groups in fucoidan. Fucoidan is one of the important secondary metabolites and a long-chain sulfated polysaccharide that is present in brown algae.

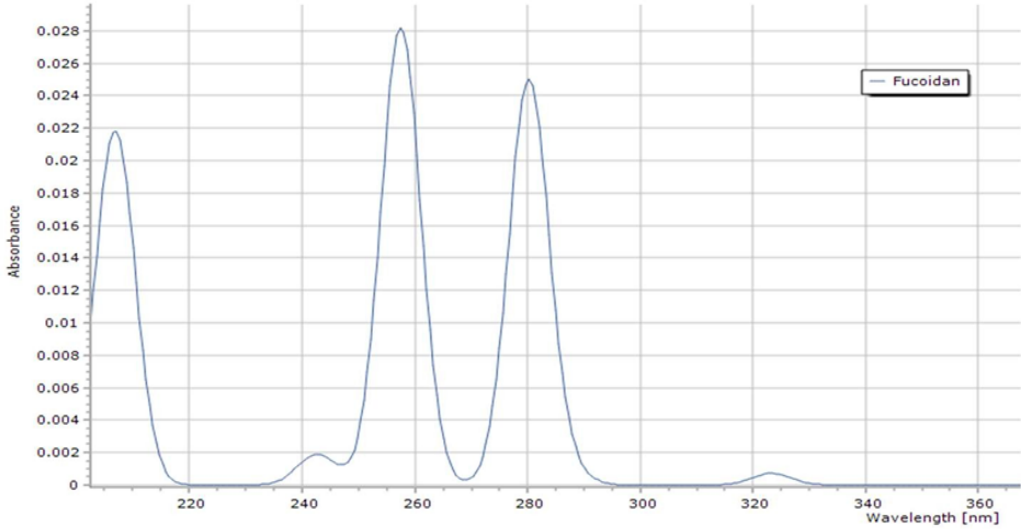

Figure 3. UV absorbance in TLC plates

3.1.4. Characterization of the sample by GC-MS

According to the Duke's database [27], the phytocomponents found in the ethanolic extracts of algae demonstrated that they are highly active components that are responsible for the diverse activities, such as antibacterial, antimicrobial, and antiviral, attributed to them [28]. **Figure 4A** displayed the fucoidan components that were isolated from the algae ethanol extract. Additionally, the compound alginate is shown in **Figure 4B**.

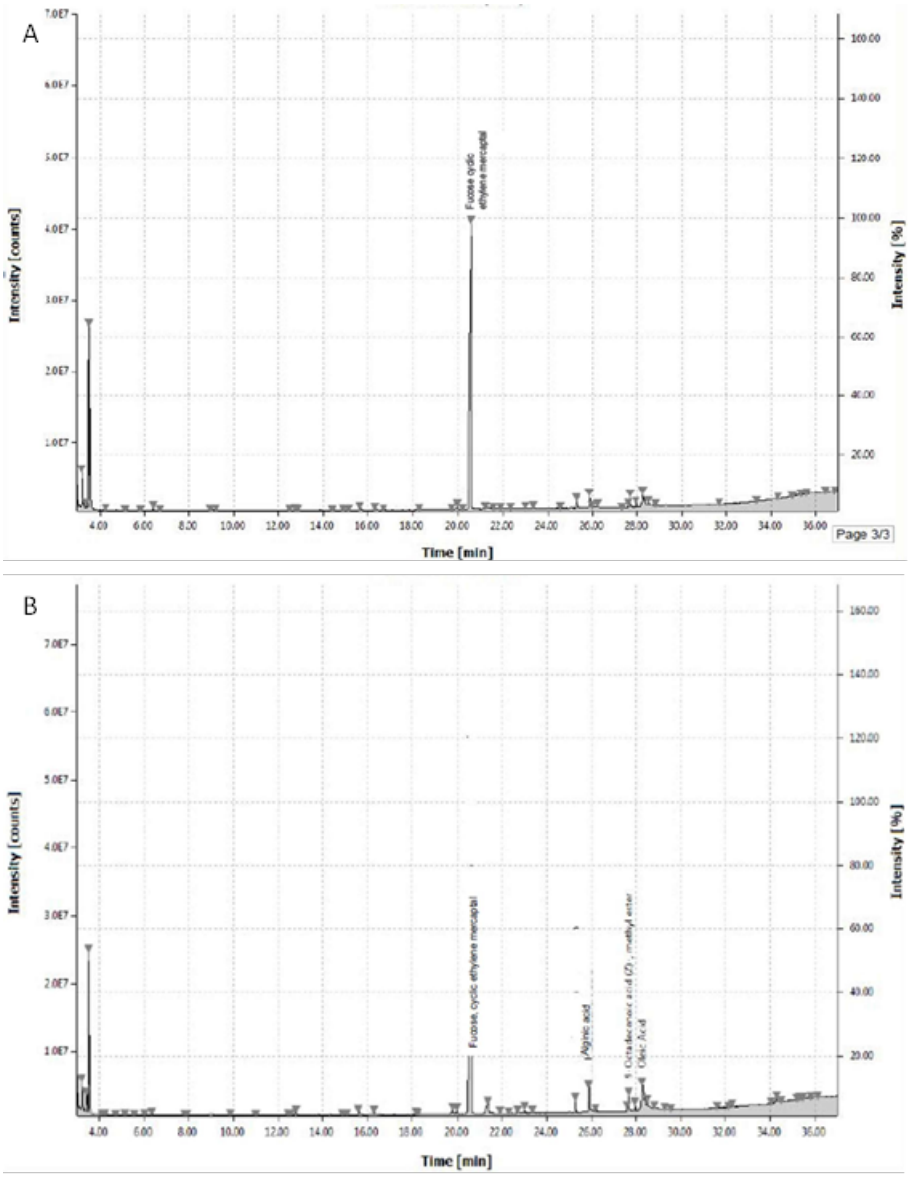

Figure 4. GC-MS analyses. (A) GCMS analysis results for fucoidan; (B) GCMS analysis results for alginate with fucoidan.

3.1.5. Characterization of the sample by FTIR

Figure 5 showed the FTIR analysis of a pure extract from *S. wightii*. The FTIR analysis's spectrum revealed recognizable absorbance bands. Five peaks are present in each of the absorption bands from the purified extract. The symmetric CO vibration and asymmetric S=O stretching vibration of the CO-SO_3 group are responsible for the peaks at wavelength numbers close to 1250 cm^{-1}. More sulfation at the equatorial at the point in C3 and C2 is the cause of the wave near 820 cm^{-1} that was found in the fucoidan standard [8]. Another study also showed that fucoidan demonstrated vibrations between 820 cm^{-1} and 1000 cm^{-1} [28]. As shown in **Figure 6**, alginate peaks were formed between 1500 cm^{-1} and 2000 cm^{-1}.

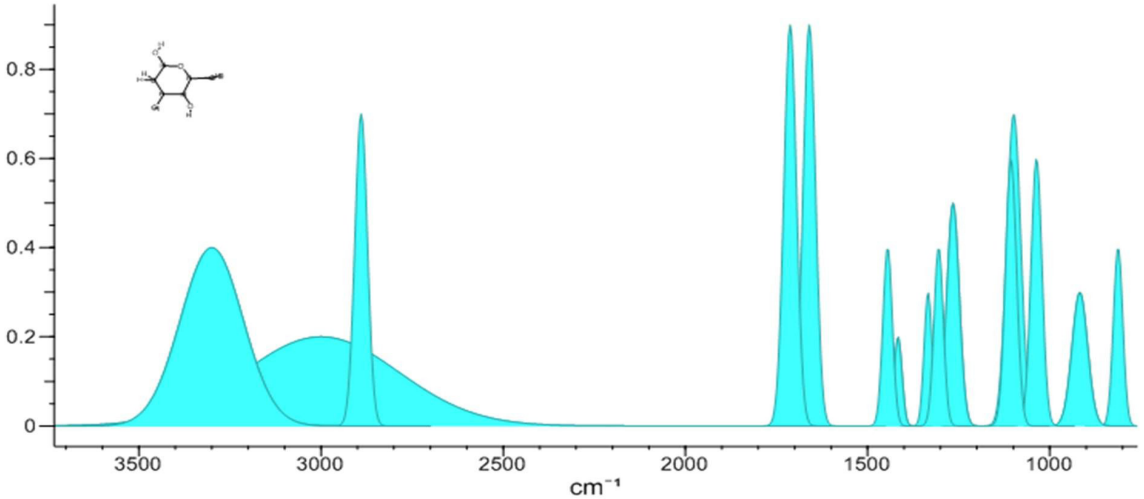

Figure 5. FTIR data for the compound isolated from *S. wightii*

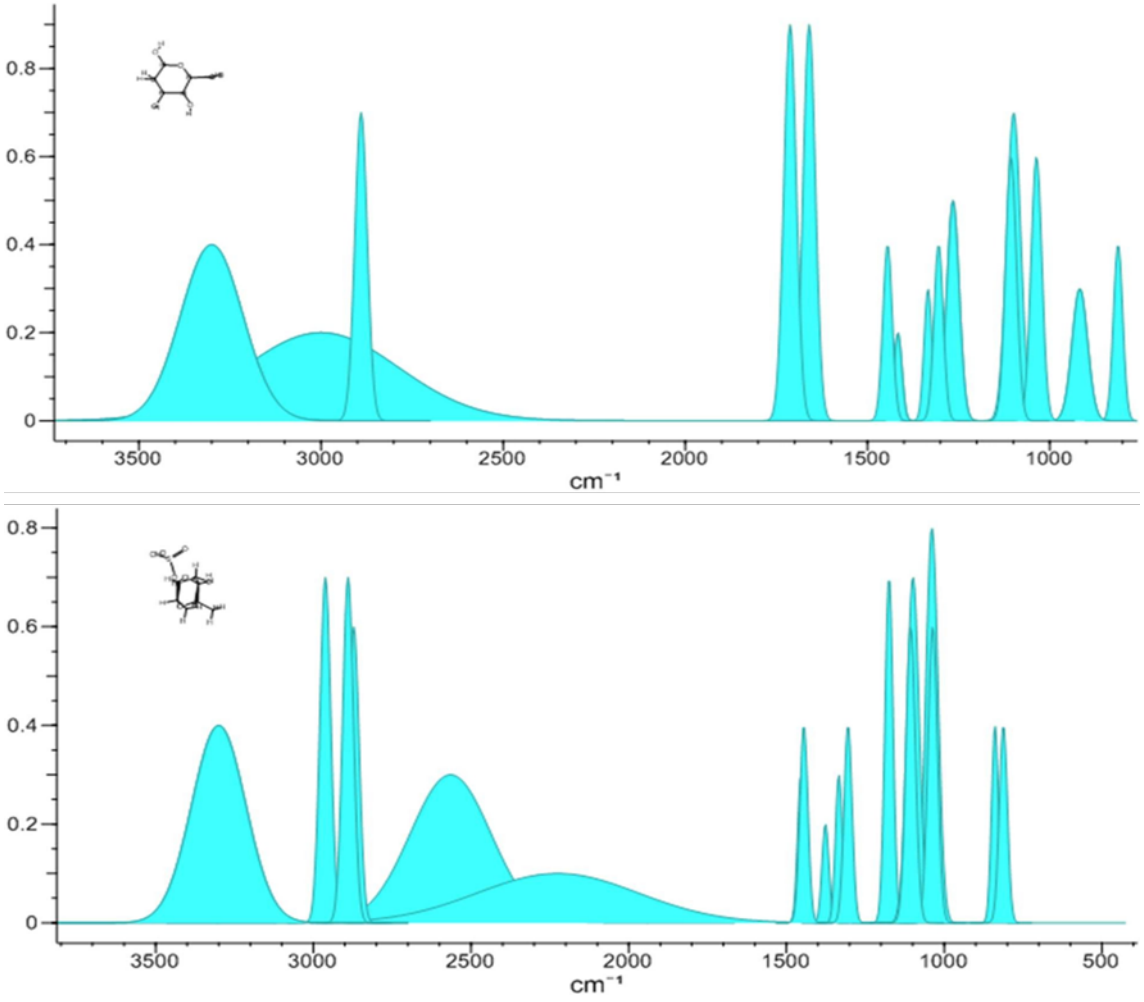

Figure 6. FTIR data for isolated compounds

3.1.6. UV Spectrophotometer

The peak for fucoidan was found between the wavelengths of 250 and 260 nm in **Figure 3**. The peak for alginate was found between 290 and 300 nm, as seen in **Figure 7**.

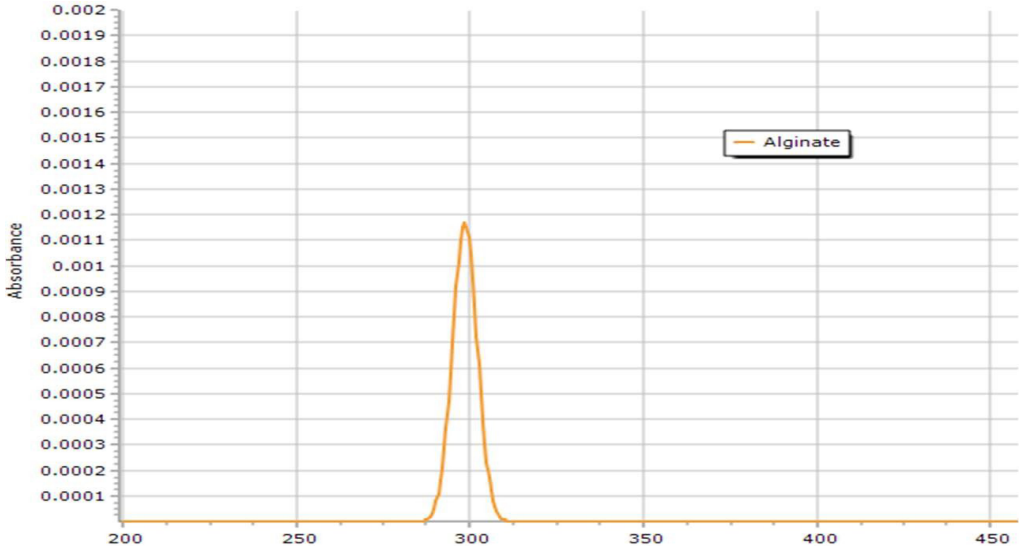

Figure 7. UV spectroscopy analysis of alginate from *S. wightii*

3.2. Composition analysis

3.2.1. Total carbohydrate content

The brown tint in **Figure 2A** denotes the presence of fucose. In a test for carbohydrates, hexose sugar produces a brown hue. It is possible to infer that the extracted material includes fucose by evaluating the color change that was present. The standard carbohydrate graph and the equation to determine the total carbohydrate content of the sample are shown in **Figure 8**, and the data for the standard graph are shown in **Table 2**. When the acquired sample OD values are substituted in the calculation, 74.50 g of carbohydrates (74.5% of the total yield) are present in 100 g of crude fucoidan, whereas 68.47 g of carbohydrates (68.4% of the total yield) are present in 100 g of crude alginate [29].

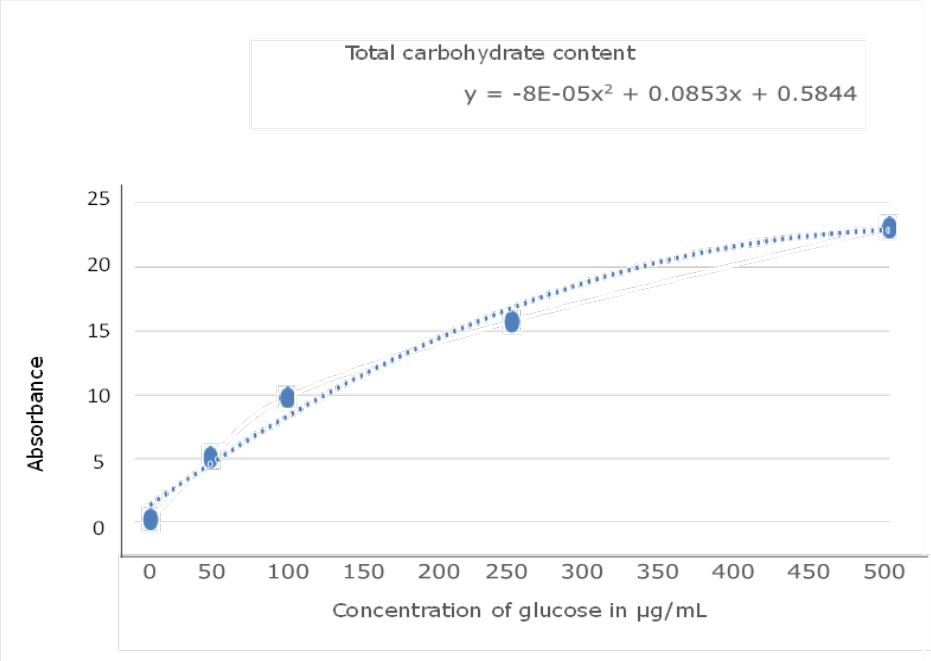

Figure 8. Standard graph for carbohydrate content estimation

Table 2. Data for standard graph and test samples for total carbohydrate content

	Sample	Concentration (µg/mL)	OD value
1	Glucose	10	0.29
2	Glucose	50	5.13
3	Glucose	100	9.81
4	Glucose	250	15.78
5	Glucose	500	23.11
6	Fucoidan	100	6.59
7	Alginate	100	6.05

3.2.2. Total phenol content

The blue color shift in **Figure 2B** denoted the presence of phenol in the extracted material. After determining the equation for total phenol content, the substituted sample OD values showed that 13 g of phenol (13% of the total yield) are found in 100 g of crude fucoidan, whereas 0.4 g of phenol (0.4% of the total yield) are found in 100 g of crude alginate (**Figure 9** and **Table 3**).

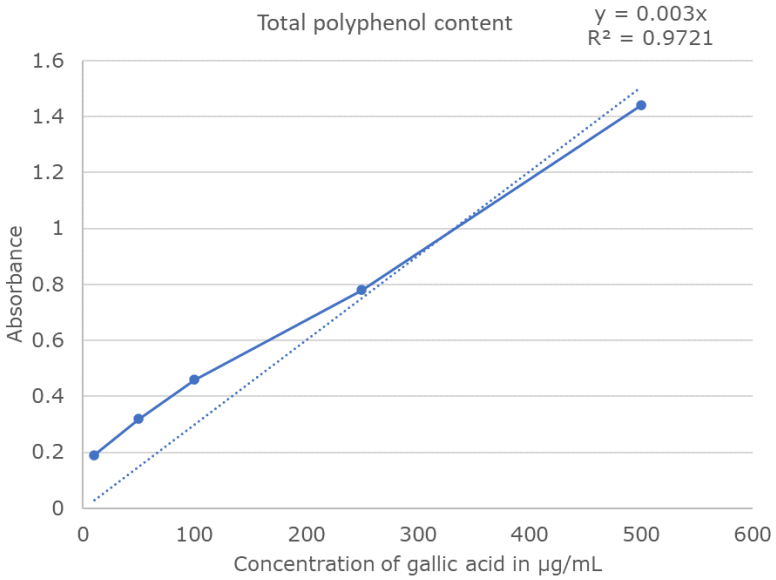

Figure 9. Standard graph for polyphenol content estimation

Table 3. Total phenol content

	Sample	Concentration (µg/mL)	OD value
1	Gallic acid	10	0.19
2	Gallic acid	50	0.32
3	Gallic acid	100	0.46
4	Gallic acid	250	0.78
5	Gallic acid	500	1.44
6	Fucoidan	100	0.21
7	Alginate	100	0.19

3.2.3. Total protein content

The green color shift in **Figure 2C** denoted the presence of protein in the extracted material [30]. It is possible to infer that the extracted material includes protein by evaluating the color change. **Figure 10** provided the equation to determine the total protein content of the samples in addition to displaying the standard protein graph. The substituted sample OD values into the equation found that 2.7 g of protein (2.7% of the total yield) are present in 100 g of crude fucoidan, whereas 22.1 g of protein content (22.1% of the total yield) are present in 100 g of crude alginate (**Table 4**).

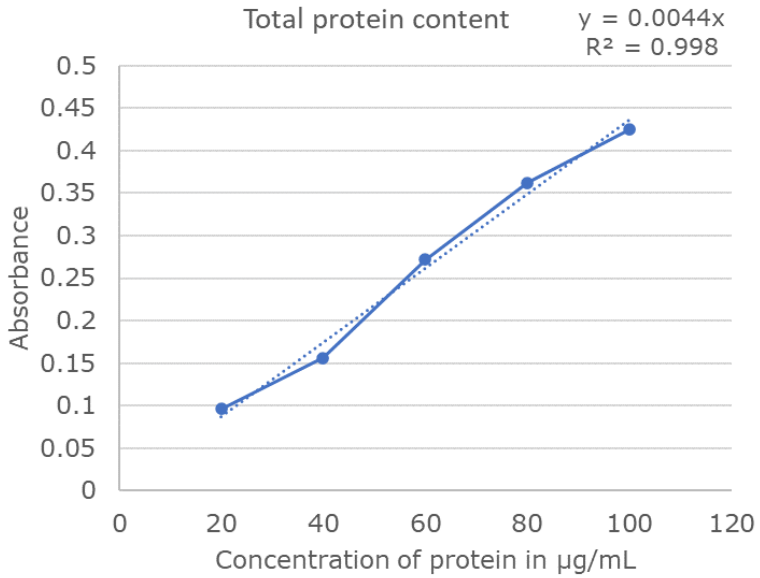

Figure 10. Standard graph for protein content estimation

Table 4. Total protein content

	Sample	Concentration (µg/mL)	OD value
1	Gallic acid	10	0.096
2	Gallic acid	50	0.156
3	Gallic acid	100	0.272
4	Gallic acid	250	0.362
5	Gallic acid	500	0.425
6	Fucoidan	100	0.012
7	Alginate	100	0.097

3.3. Antibacterial assay

The antibacterial activity of the crude alginate and fucose extracts was evaluated using the disc diffusion technique. *E. coli* and *S. aureus* bacterial suspensions were produced, and different amounts of alginate extract and fucose extract were put in the corresponding sterile discs. Positive control was loaded with streptomycin. Additionally, the inhibition zone (ZOI) was estimated and tabulated after the plates were incubated at 37°C for 24 hours in the incubator. At a dose of 50 g, both *E. coli* and *S. aureus* had inhibition zones that ranged from 17 to 18 mm. Furthermore, it is extremely effective against *E. coli* with an inhibitory zone of 27 mm at 100 g

concentrations. As indicated in **Table 4**, the conventional medication (E) had a modest impact on *E. coli* and *S. aureus*, with inhibition zones of 13 mm and 17 mm, respectively [24].

Table 4. Data for antibacterial activity

	Isolates	Concentration (µg)	Zone of inhibition (mm)	
			E. coli	*S. aureus*
1	A (fucoidan)	50	-	17
2	B (alginate)	50	17	18
3	C (fucoidan)	100	27	25
4	D (alginate)	100	23	21
5	E (standard)	30	13	17

3.4. Antioxidant activity by DPPH:

Scavenging DPPH, a stable free radical, has been used to investigate the antioxidant activity of the samples. The reduction in DPPH radical absorbance brought on by antioxidants is due to the interaction between antioxidant molecules and radicals, which scavenges the radical by donating hydrogen. The standard sample used for the determination of the antioxidant activity by the DPPH technique is ascorbic acid. The scavenging % of DPPH was calculated and tabulated as given in **Table 5**. Absorption was measured at 520 nm. At a concentration of 140 µL, the crude fucoidan showed substantial percentage inhibition (64.46%) [16,23].

Table 5. Data for antioxidant activity

Concentration	OD of alginate at 520 nm	Percentage inhibition	OD of fucoidan at 520 nm	Percentage inhibition
10	1.96	18.18	1.39	33.88
25	1.74	24.24	1.23	38.29
50	1.62	27.54	0.76	51.23
100	1.55	29.47	0.44	60.05
140	1.43	32.78	0.28	64.46

4. Conclusion

The present investigation showed the screening of algae for anti-viral applications. Marine polysaccharides present in the algae possess various pharmacological activities such as antimicrobial, antiviral, and antioxidant activities. The antioxidant potential of algae extracts confirmed there is a potential for scavenging free radicals generated by cells through the reaction between secondary metabolites and free radicals molecules. Given this correspondence, marine algae can be a potential feedstock for isolating secondary metabolites as lead molecules for antiviral therapy to combat viral infections.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Kolanjinathan K, Ganesh P, Saranraj P, 2014, Pharmacological Importance of Seaweeds: A Review. WJFMS, 6(1): 1–15.

[2] Fauziee NAM, Chang LS, Wan Mustapha WA, et al., 2021, Functional Polysaccharides of Fucoidan, Laminaran and Alginate from Malaysian Brown Seaweeds (*Sargassum polycystum*, *Turbinaria ornata* and *Padina boryana*). Int J Biol Macromol, 167: 1135–1145.

[3] Shi Q, Wang A, Lu Z, et al., 2017 Overview on the Antiviral Activities and Mechanisms of Marine Polysaccharides from Seaweeds. Carbohydr Res, 453–454: 1–9.

[4] Domettila C, Joselin J, Jeeva S, 2013, Phytochemical Analysis on Some South Indian Seaweeds. JOCPR, 5(4):275-278.

[5] Ahmadi A, Zorofchian Moghadamtousi S, Abubakar S, et al., 2017, Antiviral Potential of Algae Polysaccharides Isolated from Marine Sources: A Review. BioMed Res Int, 2015: 825203.

[6] Premnathan M, Chandra K, Bajpai SK, et al., 1992, A Survey of Some Indian Marine Plants for Antiviral Activity. Botanica Marina, 35: 321–324.

[7] Roy S, 2020, Seaweed Diversity Within Intertidal Zone of Olaikuda and Vadakkadu, Rameswaram, Southeast Coast of India. Heliyon, 6(7): e04585.

[8] Artemisia R, Nugroho AK, Setyowati EP, et al., 2019, The Properties of Brown Marine Algae *Sargassum turbinarioides* and *Sargassum ilicifolium* Collected from Yogyakarta, Indonesia. Indonesia Journal of Pharmacy, 30(1): 43–51

[9] Wang S, Wang W, Hou L, et al., 2020, A Sulfated Glucuronorhamnan From the Green Seaweed *Monostroma nitidium*: Characteristics of Its Structure and Antiviral Activity. Carbohydrate Polymers, 227: 115280.

[10] Fitton JH, Park AY, Karpiniec SS, et al., 2021, Fucoidan and Lung Function: Value in Viral Infection. Mar Drugs, 19(1): 4.

[11] Zayed A, El-Aasr M, Ibrahim ARS, et al., 2020, Fucoidan Characterization: Determination of Purity and Physicochemical and Chemical Properties. Mar Drugs, 18(11): 571.

[12] Lorbeer AJ, Lahnstein J, Bulone V, et al., 2015, Multiple-Response Optimization of the Acidic Treatment of the Brown Algae *Ecklonia radiata* for the Sequential Extraction of Fucoidan and Alginate. Bioresour Technol, 197: 302–309.

[13] Zhang H, Row KH, 2015, Extraction and Separation of Polysaccharides from *Laminaria japonica* by Size-Exclusion Chromatography. Journal of Chromatographic Science, 53: 498–502.

[14] Bouhlal R, Haslin C, Chermann J-C, et al., 2011, Antiviral Activities of Sulfated Polysaccharides Isolated from *Sphaerococcus coronopifolius* (Rhodophytha, Gigartinales) and *Boergeseniella thuyoides* (Rhodophyta, Ceramiales). Mar Drugs, 9(7): 1187–1209.

[15] Yu P, Sun H, 2014, Purification of a Fucoidan from Kelp Polysaccharide and Its Inhibitory Kinetics for Tyrosinase. Carbohydr Polym, 99: 278–283.

[16] Vijayabaskar P, Shiyamala V, 2012, Antioxidant Properties of Seaweed Polyphenol from *Turbinaria ornate* (Turner) J. Agardh, 1848. Asian Pacific Journal of Tropical Biomedicine, 2(1): S90–S98.

[17] Li H, Liu J, Zhang L, et al., 2016, Antioxidant Responses and Photosynthetic Behaviors of *Kappaphycus alvarezii* and *Kappaphycus striatum* (Rhodophyta, Solieriaceae) During Low Temperature Stress. Botanical Studies, 57: 21.

[18] Morita A, Tanino Y, Ojima T, et al., 2019, Influence of Temperature on the Anti-Allergic Activity of Fucoidan Extracted from *Saccharinn japonica*. Food Science and Technology Research, 25(4): 607–611.

[19] Bedoux G, Caamal-Fuentes E, Boulho R, et al., 2016, Antiviral and Cytotoxic Activities of Polysaccharides Extracted from Four Tropical Seaweed Species. Natural Product Communications, 12(6): 807–811.

[20] Rioux L-E, Turgeon SL, Beaulieu M, 2007, Characterization of Polysaccharides Extracted from Brown Seaweeds. Carbohydrate Polymer, 69(3): 530–537.

[21] Babahan I, Kirim B, Mehr H. Major Natural Vegetation in Coastal and Marine Wetlands: Edible Seaweeds. In Plant Communities and Their Environment. 2019, Intech Open, 1–17.

[22] Sharma OP, Bhat TK, 2009, DPPH Antioxidant Assay Revisited. Food Chemistry, 113(4): 1202–1205.

[23] Lee YH, Choo C, Waisundara VY, 2014, Determination of the Total Antioxidant Capacity and Quantification of Phenolic Compounds of Different Solvent Extracts of Black Mustard Seeds (*Brassica nigra*). International Journal of Food Properties, 18(11): 2500–2507.

[24] Baker CN, Thornsberry C, Hawkinson RW, 1983, Inoculum Standardization in Antimicrobial Susceptibility Testing: Evaluation of Overnight Agar Cultures and the Rapid Inoculum Standardization System. J Clin Microbiol, 17(3): 450–457.

[25] Lim SN, Cheung PCK, Ooi VEC, et al., 2002, Evaluation of Antioxidative Activity of Extracts from a Brown Seaweed, *Sargassum siliquastrum*. J Agri Food Chem, 50(13): 3862–3866.

[26] Wang C-Y, Wu T-C, Hsieh S-L, et al., 2015, Antioxidant Activity and Growth Inhibition of Human Colon Cancer Cells by Crude and Purified Fucoidan Preparations Extracted from *Sargassum cristaefolium*. J Food Drug Anal, 23(4): 766–777.

[27] Thanigaivel S, Vidhya Hindu S, Vijayakumar S, et al., 2015, Differential Solvent Extraction of Two Seaweeds and Their Efficacy in Controlling *Aeromonas salmonicida* infection in *Oreochromis mossambicus*: A Novel Therapeutic Approach. Aquaculture, 443: 56–64.

[28] Huang C-Y, Wu S-J, Yang W-N, et al., 2016, Antioxidant Activities of Crude Extracts of Fucoidan Extracted from *Sargassum glaucescens* by a Compressional-Puffing-Hydrothermal Extraction Process. Food Chem, 197 Pt B: 1121–1129.

[29] Agrawal N, Minj DK, Rani K, 2015, Estimation of Total Carbohydrate Present in Dry Fruits. IOSR JESTFT, 1(6): 24–27.

[30] Redmile-Gordon MA, Armenise E, White RP, et al., 2013, A Comparison of Two Colorimetric Assays, Based Upon Lowry and Bradford Techniques, to Estimate Total Protein in Soil Extracts. Soil Biol Biochem, 67(100): 166–173.