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Study on the bioactive activities of fucoidan extracted from brown macroalgae (*Sargassum flavicans*) collected in Hon Son Island, Vietnam

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ABSTRACT

The pharmaceutical industry now recognizes the world's oceans as a crucial source for medical research, teeming with organisms possessing unique biological properties. Researchers have successfully extracted bioactive compounds from marine life, especially the extract of brown algae demonstrated various modes of action, including antiproliferative, antioxidant, and antimicrobial effects. This study aims to evaluate *in vitro* antioxidant, antibacterial, antifungal, cytotoxic, and enzymatic inhibitory properties of fucoidan extracted from *Sargassum flavicans* at Hon Son island, Vietnam. Specifically, the infrared spectroscopy spectra showed that the fucoidan, extracted using the HCl method, retained impurities that contained chlorine radicals in their molecular structure. The results of the 2,2-Diphenyl-1-Picrylhydrazyl assay indicated that the algal extract was capable of scavenging free radicals with the IC_{50} value of the brown algae extract was 206.21 ± 0.695 µg/ml. In addition, the algal extract displayed potential inhibition of α-amylase activity with an IC_{50} value of 10.87 ± 0.25 µg/ml. Moreover, the algal extract revealed weak activity against Hela cell line. Regarding antimicrobial activities, the results indicated that the extract had potential effects against *Escherchia coli*, *Bacillus cereus,* and *Cadida albicans*. Briefly, these present findings showed that fucoidan extract from *S. flavicans* can be considered as a new natural resource, and discovering and utilizing this resource can lead to improvements in both health and economic prosperity.

1. INTRODUCTION

Various sectors recognize the marine environment as a valuable source of naturally occurring organic compounds with a wide range of potential applications. Marine macroalgae, such as red algae (Rhodophyceae), green algae (Chlorophyceae), and brown algae (Pheophyceae), are the most abundant natural source of these non-animal and non-plant-based bioactive substances [1]. Brown seaweed, scientifically known as *Sargassum*, is a widespread marine plant with over 400 identified species globally [2]. In Vietnam, researchers have documented 143 brown seaweed species, including 22 and 13 *Sargassum* species in the North and South of the country, respectively [3,4]. *Sargassum flavicans* belongs to the class Phaeophyceae and *Sargassum* is the brown macroalgae in the order Fucales. It is normally yellowish to olive-brown and smooth algae, about 80 cm long or more, with small clinging discs. It is found along the coasts of Africa (Egypt, Ethiopia), South-west Asia (Bangladesh, India, Jordan, Yemen), South-east Asia (Vietnam, Indonesia), Australia, New Zealand, and Pacific islands [5].

In Vietnam, *S. flavicans* is distributing on many coasts including the coast of Kien Giang province, Hon Son is one of the island of this province. Research by Do and Do [6] showed that the Kien Giang coast has a rich diversity of seaweed species due to the large island area and diverse ecosystems such as coastal areas, tidal areas, and coral reefs. On the other hand, it may be due to the decline in corals, creating benefits for seaweed to grow on dead coral substrates [6].

Fucoidan is a compound exclusive to marine vegetation, not present in land plants [7]. Fucoidan is a complex, diverse polysaccharide found in seaweed. Its structure and composition vary depending on the seaweed species. In addition, fucoidan is determined by measuring the amount of sulfate, galactose, xylose, mannose, and uronic acid present [8]. This polysaccharide from seaweed has been shown to have many health benefits, including antioxidant [9], anticoagulant [10], antibacterial [11], immune-boosting [12], and gut-health-promoting effects [7]. However, the specific makeup and properties of fucoidan can differ based on the type of seaweed, where it is grown, and how mature it is when harvested [12]. Moreover, the specific biological activities of fucoidan can vary depending on its chemical composition and structure [13,14]. The effectiveness of fucoidan depends on its molecular structure, including the types of sugars, the amount of

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sulfate, and the location of sulfate groups. The method used to extract fucoidan significantly impacts its final properties, particularly the structure of its sugar and sulfate components [15].

Vietnam's coastal waters are abundant with brown seaweeds such as *Sargassum* sp. [16,17]. Although these seaweeds are known to contain valuable bioactive compounds, including polysaccharides, there is limited research on extracting and studying these compounds from Vietnamese species. This lack of information suggests a significant untapped potential and the promising health benefits reported for polysaccharides from similar seaweed species, therefore, this study chose to investigate *S. flavicans* for its potential bioactivities. Overall, this work was carried out to investigate the bioactivities of *S. flavicans* fucoidan extract including antioxidant, antibacterial, antifugal, α-amylase enzymatic inhibitory, and cytotoxic properties. This research could pave the way for a new functional ingredient derived from the seaweed. Our findings are likely to attract the attention of seaweed researchers who are exploring brown seaweed as a promising sustainable source of bioactive compounds that can contribute to Vietnam's economic growth and environmental health.

2. MATERIALS AND METHODS

2.1. Collection of Algal Material

Brown seaweed *S. favicans* freshly collected from the cost of Hon Son island belongs to Kien Giang province, Vietnam. The time collection of algae was in March and July 2023, the seaweed was collected with all parts of its organism by hand. The seaweeds were identified at the Department of Biology, Faculty of Natural Sciences, Can Tho University according to the Vietnamese Seaweed classification system [3,8] and then using the Algae Base website to confirm their identification. *Sargassum favicans* is smooth algae, about 80 cm long or more, small lobed disc. The main axis is short, the main branch is cylindrical, thread-like, 0.5 mm wide, smooth, lateral branches 5–10 cm long, 1–3 cm apart. Leaves are lanceolate, thin, 3 cm long, 4 mm wide. Leaf margins are deeply serrated, pointed, midrib clear, running through the top. The hairs are small but clear, and scattered. On the branches, the leaves are narrow, and the hairs are arranged in two rows.

The seaweed was cleaned with seawater, bagged, and then kept in a cool condition. The seaweed was dried in the shade and then in a 38°C ± 2°C oven. The dried seaweed was cut into small pieces (1–2 cm) and ground in an electric mixer. The ground seaweed was then placed in plastic bags and stored in the refrigerator.

2.1.1. Extract preparation

The algae (20 g) was soaked in 200 ml methanol solution (methanol: H_2O = 1:9) overnight at room temperature to separate the protein, colored matter, and fat in the algae. The sample solution was then centrifuged at 6,000 rpm for 10 minutes and the supernatant was removed. Then, the sample was dried and grinded into a fine powder by a blender. The dried seaweed powder was collected and put in a storage bag [18]. The purpose of pre-treating the algae with methanol solution is to remove lipids and colorants such as chlorophyll, fat, and protein.

2.1.2. Fucoidan extraction

Fucoidan was extracted using an acid solution according to the procedure as described [19]. Five grams of dried algal powder was mixed with 150 ml of 0.01M HCl. The mixture was stirred at 85°C for 1 hour and 30 minutes. Then, the mixture was refined to collect and store the first filtrate. The residue was continued to be extracted similarly by 150 ml of 0.01M HCl. After that, the second filtrate was collected. Both filtrates were mixed and then centrifuged at 6,000 rpm for 20 minutes to remove the residue in the extract to collect a cleaner and clearer extract. After the centrifugation, the extract was mixed with $CaCl_2$ 1%. The mixture then was centrifuged at 4°C, 6,000 rpm for 20 minutes to collect the algal extract containing fucoidan. The residue containing alginate was discarded. NaOH was added into the collected supernatant while being stirred to neutralize the extract solution (pH=7). 96° ethanol (30% volume of the extract) was added into the extract solution and was left to stay overnight at 4°C to precipitate the fucoidan. The mixture was centrifuged at 6,000 rpm for 20 minutes to collect the residue. This residue was the first raw fucoidan. The supernatant centrifugation then was added 96° ethanol (70% volume of the supernatant) and this mixture was left at 4°C overnight. After that, the mixture was centrifuged for 20 minutes at 6,000 rpm to collect the second residue. This second collected residue was the second raw fucoidan. The raw fucoidan obtained was dried at room temperature until constant weight was achieved, weighed, and kept in an air-tight sample bottle.

2.2. *In Vitro* Antioxidant Activity Assay

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity

A solution of DPPH with a concentration of 6×10^{-4} M was prepared in methanol. Determination of DPPH radical scavenging activities of fucoidan extracted from *S. favicans* used a variation of the method of Sharma and Bhat [20] with some modifications. Briefly, the extract of *S. favicans* was diluted by methanol to have a different concentration of algal extract solution (0–600 mg/ml), and then 0.1 ml of the extract solution (0–600 mg/ml) was mixed with 0.1 ml prepared DPPH solution in the test tubes. The reaction tubes, in triplicates, were wrapped in aluminum foil and kept at room temperature for 30 minutes in the dark. Spectrophotometric measurements were calculated at 517 nm using a Libra S60PC spectrophotometer. The data are mean ± SD.

Ascorbic acid (0–15 µg/ml) was used as standard for the experiment.

The measurement of activity to scavenge the free radicals was loosely based on a decrease in the absorbance of DPPH and calculated as follows:

$$\text{Scavenging effect (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The values of IC_{50} in µg/ml were calculated by analyzing the linear regression to represent the concentration of the test samples causing 50% inhibition (IC_{50}).

2.3. α – Amylase Inhibitory Activity

The test samples were estimated α – amylase inhibitory activity, according to the method of Truong *et al.* [21], with adjustments. The experiment involved mixing a phosphate buffer solution (50 µl, pH = 7), 50 µl sample solution (25–400 mg/ml), and 3U α-amylase enzyme (50 µl). This mixture was incubated at 37°C for 5 minutes. Then, 50 µl of starch (2 mg/ml) was added and incubated at 37°C for 15 minutes. To stop the reaction, 200 µl of concentrated HCl was added. Finally, 1% iodine reagent (300 µl) was used to determine the starch left after the reaction. The amount of remaining starch was measured by its ability to react with iodine and create a blue color. This blue color was

measured using a spectrophotometer at 660 nm wavelength. Acarbose was used as a positive control.

The activity of α-amylase was calculated as follows:

% Inhibition = 100 – ((Ao–A1)/Ao×100)

where Ao: Absorbance of control solution.

A1: Absorbance of the solution after reaction.

2.4. Antibacterial Assay

Escherichia coli ATTCC® 25922TM, *Listeria innocua* ATCC 33090, *Salmonella typhimunum* ATCC® 13311TM, *Bacillus cereus* ATCC® 10876TM were provided by the Biology Department, College of Natural Sciences, Can Tho University, Vietnam. The turbidity of the suspension was standardized against 0.5 McFarland using a spectrophotometer at 600 nm wavelength. The bacterial inoculum was 10^8 cfu ml^{-1}. Antibacterial activity tests included positive control, negative control, and antibacterial activity tests for the algal extract. The positive control test was carried out using Tetracycline (300 µg/ml), and the negative control test used solvents [10% dimethyl sulfoxide (DMSO)]. The method applied in this test was the Well diffusion test with modification [22]. 25 ml of Lactose Broth agar were melted, cooled to 55°C, and poured into the assay plate (9 cm in diameter). Then, the mixture was allowed to cool down on a leveled surface. Once the medium had solidified, spread 100 µl of the inoculum evenly over the entire agar surface, ensuring no gaps between streaks. Subsequently, four wells, each 9 mm in diameter, were cut out of the agar, and 50 ±l of fucoidan extracts with concentrations of 37.5; 75; 150; and 300 mg/ml were placed into each well. The samples were incubated for 24 hours at 37°C. The distinct zone around the well was a sign of the inhibition zone of bacterial activity. Every experiment was conducted three times. Inhibition zones ≥ 20 mm were declared as strong, 15–19 mm as moderate, and ≤14 mm as weak activities [23].

2.5. Cytotoxic Activities

The toxicity test of the extract was performed by determining the cell density using the CCK8 kit on HeLa and HepG2 cell lines (cell density was 10^5 cells/well) in a 96-well plate [24]. The cell survival rate was determined indirectly through CCK-8 with the mechanism that WST-8 (highly water-soluble tetrazolium salt) in CCK-8 was reduced by the activity of dehydrogenase present in living cells to produce orange formazan. The cells were incubated with the extract at concentrations ranging from 400 to 3,125 µg/ml, or 5% DMSO in the corresponding cell culture medium for 48 hours. After the treatment period, the culture medium was replaced with fresh medium containing CCK-8 solution (Dojindo Laboratories Kumamoto, Japan) at a ratio of 10:1 (v/v) and incubated at 37°C for 3 hours. The absorbance was measured at 450 nm using a spectrophotometer (SH-1200, Corona Electric, Japan). The toxicity of the extract was expressed as the percentage of viable cells in each treatment.

2.6. Antifungal Activity Assay

Potato D-glucose Agar (PDA) medium was autoclaved and kept warm at 75°C. Fungi *Cadida albicans* were cultured in liquid form by placing 1/4 to 1/2 of the fungal plate in 40 ml of PDA medium in a clean test tube with a lid and shaking for 24 hours. The activity test plate was prepared by placing 10 ml of PDA medium in a petri dish, allowing the medium to solidify. After that, spread 100 µl of fungal suspension on the surface of the petri dish and punch 5 wells on the agar plate, each well having a diameter of 9 mm [25].

The algal extract (0.6 g) was dissolved in 1 ml of 10% DMSO, and we had an extract solution at the concentration of 600 mg/ml, then continued to dilute the extract (600 mg/ml) with 10% DMSO to reach the extract with different concentration of 37.5, 75, 150, and 300 mg/ml, respectively. After that, 50 µl extract of the different concentrations was added to the well on the petri dish and incubated at room temperature for 24 hours. The negative control was 10% DMSO and the positive control was Nystatin fungicide (1 mg/ml) dissolved in methanol. Each treatment was repeated 3 times. The antifungal ability was evaluated by the diameter of the antifungal zone (minus the diameter of the agar well), the unit was mm.

2.7. Statistical Analysis

All experiments were done in triplicate (n = 3). Data are expressed as mean ± standard deviation (± SD). The data were analyzed using Microsoft Excel 2013 and Minitab 17 software used to analyze oneway ANOVA with a 95% confidence level.

3. RESULTS AND DISCUSSIONS

3.1. Determination of Fucoidan

Infrared spectroscopy (IR) is a fast and convenient analytical method to study molecular structure through the arrangement of vibrational spectral lines corresponding to certain groups of atoms in the molecule. In the analysis of polysaccharide structure, IR spectroscopy provides information about sulfate groups and glycoside bonds existing in fucoidan molecules, thereby revealing their structural characteristics [26]. Specifically, from Figure 1 the infrared spectrum of fucoidan gives the following characteristic signals: 3,200–3,550 cm^{-1} is characteristic of the valence vibration of the O-H group, 1,020–1,080 cm^{-1} is characteristic of the C-O glycoside bond, 1,110–1,260 cm^{-1} is characteristic of the vibration of the S=O bond of the sulfate group and the deformation vibration signal of the C-O-S bond of the sulfate ester, in which the sulfate groups in the equatorial position are characterized by the signal 820–828 cm^{-1} and the ester groups in the axial position are characterized by the signal 840–848 cm^{-1}.

The results of IR spectra of the fucoidan extracted by the HCl method are presented in Figure 1 and Table 1. Accordingly, it can be observed that the IR spectral signals of this method fully demonstrated the characteristic IR spectral signals of fucoidan, specifically, the samples gave characteristic signals of the vibrational region of the S=O bond of the sulfate group at 1,256.40 cm^1, and the characteristic signal of the valence vibrational region of the C=O bond at 1,615.09 cm^{-1}. In addition, the IR spectrum of the sample also showed signals in the 802–810 cm^{-1} vibrational region, which is characteristic of the folding vibration of the C-O-S bond of the sulfate group, and the 840–848 cm^{-1} vibrational region, characteristic of the sulfate group in the axial position in the sugar moieties [27]. Thus, based on the actual measured spectral data of the extracted sample, combined with the comparison with the characteristic nasal signal of fucoidan, it can be concluded that the study successfully extracted fucoidan.

Moreover, the IR spectra showed that for the HCl extraction method, the signals had low-intensity absorption peaks, especially for the characteristic vibration signal of the S=O bond of the sulfate group. This can confirm that the sulfate radical content in fucoidan extracted by the HCl method was not too high. Sulfate radicals are one of the most important factors determining the biological activity of fucoidan, important activities such as anticoagulation and anticancer of fucoidan depend on the density and position of sulfate groups on the sugar groups. In addition, the spectral data showed that there was

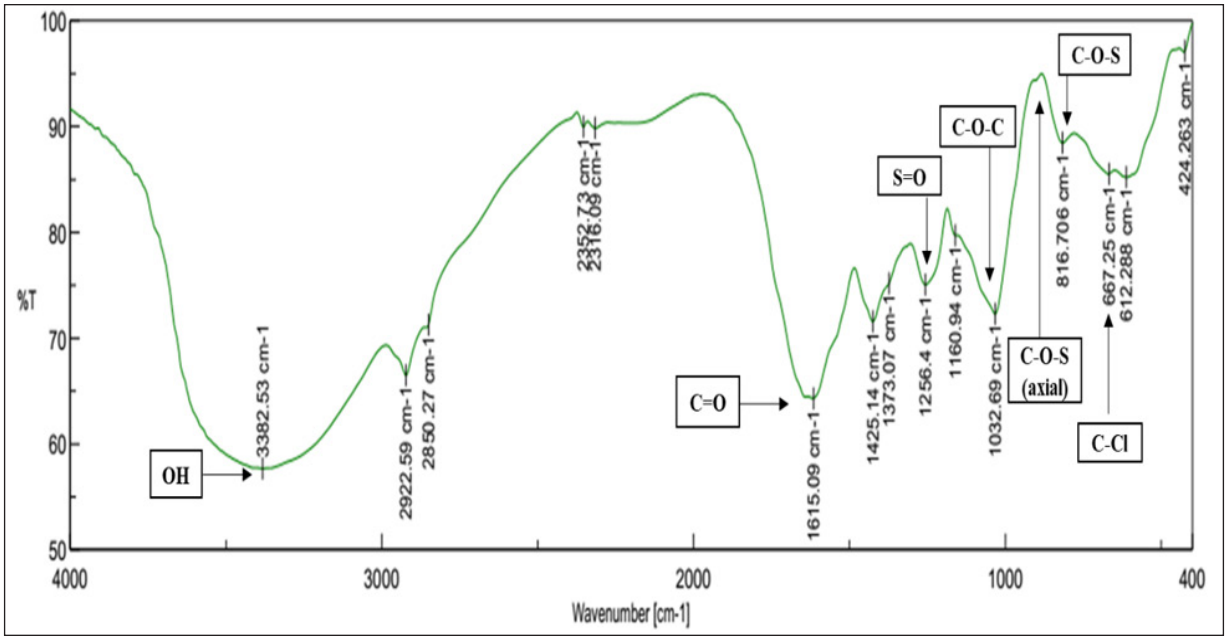

Figure 1. Infrared analysis of the *S. favicans* fucoidan extracted by chlohydrid acid extraction method.

Table 1. A list of infrared (IR) vibrational modes characteristic to the algal extract.

Vibrational modes (ν, cm^{-1})	ν_{OH} 3,200–3,550	$\nu_{C=O}$	$\nu_{S=O}$ 1,255–1,264	ν_{C-O} glycoside 1,020–1,080	δ_{C-O-S} (axial) 840–848	δ_{C-O-S} (C2 or C3 of pyranose sugar) 802–810
Samples	3,417.24	1,622.80	1,255.43	1,032.69	840–848	816.71

Table 2. DPPH scavenging activity (%) of the *S. flavicans* extract,

The concentration of *S. flavicans* extract (mg/ml)	DPPH scavenging activity (%) of the *S. flavicans* extract (%)
0	$0^a \pm 0$
37.5	$7.802^b \pm 0.003$
75	$13.926^c \pm 0.001$
150	$22.795^d \pm 0.002$
300	$36.793^e \pm 0.001$
600	$70.590^f \pm 0.001$

Note: Same letters in the same column indicate 5% nonsignificant difference by Tukey's test.

Table 3. IC_{50} value of ascorbic acid and the *S. flavicans* extract.

Samples	Curve equation	IC_{50} (µg/ml)
Ascorbic acid	$y = 3.0302x + 1.6539$ ($R^2 = 0.9924$)	15.95 ± 0
S. flavicans extract	$y = 0.2258x + 3.4386$ ($R^2 = 0.9931$)	206.21 ± 0.695

Data are expressed as three replicates' mean ± standard deviation (SD).

a peak signal at 667.25 cm^{-1}, which was a characteristic peak signal for the vibration of the C-Cl bond. Thus, it can be concluded that the structural composition of fucoidan extracted by the HCl method still contained impurities containing Cl^- radicals.

3.2. *In Vitro* Antioxidant Activity

DPPH is a substance with free electrons, purple color, and has the highest absorption at 517 nm wavelength. In the presence of antioxidants, the free electrons of DPPH are able to combine with the hydrogen of the antioxidant to form DPPH-H form which is yellow. Therefore, the higher the ability of the antioxidant to neutralize DPPH free radicals, the lower the Abs value measured at 517 nm wavelength [20]. The antioxidant efficiency of the fucoidan extracted from 0.01M HCl was determined based on the ability to neutralize DPPH free radicals. The DPPH free radical neutralization efficiency was determined based on the ratio of the decrease in spectral absorption of DPPH with and without the presence of the extract. The antioxidant content in brown algae extract was calculated as equivalent to the ascorbic acid standard through the standard curve equation $y = 0.2258x + 3.4386$, with $R^2 = 0.99$.

The results presented in Table 2 show that the *S. flavicans* extract had a DPPH free radical scavenging efficiency proportional to the extract concentration significantly ($p < 0.05$). Specifically, when the extract concentration increased (from 37.5 to 600 mg/ml), the free radical neutralization ability also increased (from 7.80% ± 0.003% to 70.59% ± 0.001%). In addition, the IC_{50} value is the value indicating the concentration at which the extracted sample is capable of scavenging 50% of DPPH free radicals. The smaller the IC_{50} value, the stronger the free radical scavenging ability of the sample being examined. Table 3 illustrates that the IC_{50} value of the brown algae extract was 206.21 ± 0.69 µg/ml. The results showed that the algal extract had the ability to neutralize DPPH free radicals about 12.92 times lower

than that of standard ascorbic acid (IC_{50} value = 15.95 µg/ml). A study by El-Sheekh *et al.* [28] indicated fucoidan obtained from *D. dichotoma var. dichotoma* reached the highest scavenging activity against DPPH radicals as 50.51% ± 1.9% at 5.0 mg/ml, with an IC_{50} of 4.59 mg/ml [28]. The ability to neutralize DPPH free radicals of *S. flavicans* fucoidan with IC_{50} of 206.21 ± 0.69 µg/ml is higher than that of fucoidan obtained from *D. dichotoma var. dichotoma* (IC_{50} = 4.59 mg/ml) about 21.86 times.

According to Moroney *et al.* [29], acidic polysaccharides such as fucoidan likely neutralize radicals through electron transfer. The negative charge of sulfate groups is thought to be a major contributor to this radical scavenging ability. As far as we are aware, the antioxidant properties of polysaccharides are mainly associated with glycosidic linkages, functional groups, molecular weight, and sugar composition [30]. In comparison to previous studies, the DPPH free radical scavenging ability of the *S. flavicans* extract (IC_{50} = 206.21 ± 0.69 µg/ml) was stronger than that of the chloroform, methanol, and H_2O extracts from green algae (*Caulerpa racemosa*) with IC_{50} of 0.65 ± 0.03; 2.51 ± 0.09; 7.46 ± 0.20 mg/ml, respectively [31], the 90% ethanol extract and H_2O extract from Spirulina powder (*Anthrospira platensis*) with IC_{50} of 0.66 and 67.1 mg/ml, respectively [32] and dichloromethal extract from brown algae (*S. siliquastrum*) with IC_{50} value of 1.58 mg/ml [33]. The choice of solvent significantly influences the types of antioxidants extracted from brown algae. Polar solvents are ideal for extracting phenolic compounds, while semi-polar solvents can extract a broader range of compounds, including phenolics, terpenoids, alkaloids, and glycosides. Non-polar solvents, however, are most effective for extracting non-polar substances like waxes, lipids, and volatile oils [34,35].

3.3. α – Amylase Inhibitory Activity

Studies have found that fucoidan, a compound extracted from different types of seaweed, can vary in its ability to inhibit enzymes involved in the breakdown of starch. These enzymes include α-amylase, α-glucosidase, and amyloglucosidase [36–38]. In a 2014 study by Kim *et al.* [38], they found that fucoidan extracted from the seaweed *Ascophyllum nodosum* could block the activity of both α-amylase and α-glucosidase enzymes. However, fucoidan from another seaweed, *Fucus vesiculosus*, only inhibited alpha-amylase. Furthermore, the research suggests that the effectiveness of fucoidan in blocking these enzymes is linked to the amount of sulfate it contains. These findings point to the conclusion that fucoidan's inhibitory action on starch-hydrolyzing enzymes is not uniform. Its effectiveness varies depending on both the particular enzyme involved and the unique chemical structure of the fucoidan itself.

As indicated in Table 4, the results showed that the extract *of S. flavicans* showed the ability to inhibit α-amylase enzyme at different concentrations. The inhibitory efficiency of α-amylase enzyme increased gradually with the concentration of extract (from 12.39% ± 0.001% to 99.18% ± 0.001%). Table 5 shows that the IC_{50} value of the *S. flavicans* extract was 10.86 ± 0.25 µg/ml. In previous research, the α-amylase inhibitory ability of the 90% methanol extract of the *Dictyopteris polypodioides* brown algae had an IC_{50} of 52.95 ± 0.28 mgl^{-1} [21] and the methanol extract of the *Sargassum glaucescens* had an IC_{50} of 8.9 ± 2.4 mg/ml [39]. It was shown that the extract of the brown algae *S. flavicans* had a higher of α-amylase inhibitory ability compared to the extracts of *D. polypodioides* and *S. glaucescens*. The effectiveness of fucoidan in blocking α-amylase activity differed significantly based on the seaweed source. The extent of alpha amylase inhibition was affected by both the harvest season and the amount of fucoidan present [39].

Table 4. The α-amylase enzyme inhibition efficiency of *S. flavicans* extract at different concentrations.

Concentrations (mg/ml)	The α-amylase enzyme inhibition efficiency (%)
0	$0^{a} \pm 0$
25	$12.392^{e} \pm 0.001$
50	$18.907^{d} \pm 0.003$
100	$33.677^{c} \pm 0.002$
200	$56.036^{b} \pm 0.002$
400	$99.180^{a} \pm 0.001$

Note: Same letters in the same column indicate 5% nonsignificant difference by Tukey's test.

Table 5. The curve equation and IC_{50} value of the extract.

Sample	The curve equation	IC_{50} (µg/ml)
S. flavicans	y = 4.0704 + 5.7703 (R^2 =0.9907)	10.87 ± 0.25

Data are expressed as three replicates' mean ± standard deviation (SD).

3.4. Antibacterial Activities

Ponce *et al.* [40] identified fucoidan as the primary bioactive compound in *Sargassum*. Fucoidan is a water-soluble polysaccharide primarily composed of L-fucose and sulfate, with smaller amounts of glucuronic acid and xylose. It is a high molecular weight (around 20,000 Da) water-soluble dietary fiber or polysaccharide. Fucoidan belongs to a family of sulfated homo- and heteropolysaccharides primarily consisting of α-(1/2)-, α-(1/3)-, and/or α-(1/4)-linked α-L-fucose pyranose residues [33]. The primary monomer for bioactive components is fucose, one of the eight essential biological sugars. Additionally, fucoidan contains galactose, mannose, xylose, and glucuronic acid residues [41,42]. The chemical makeup of fucoidan, a substance derived from brown seaweed, varies depending on factors such as the seaweed species, its age, where it was grown, and the local climate [43]. These factors also influence fucoidan's wide range of biological effects. Fucoidan's biological properties are largely determined by its level of sulfation, unique molecular structure, and size [44]. Research proved that fucoidans, when broken down into smaller pieces (depolymerized), also possess antibacterial properties. This is believed to happen because these modified sugars interact with proteins on the bacterial cell surface, causing the cell membrane to break and ultimately killing the bacteria [44].

In the present study, 10% DMSO was used as a solvent to dilute the extract, therefore, its antibacterial activity was also tested. The results showed that 10% DMSO did not affect the growth of bacterial strains such as *B. cereus, L. innocua, E. coli, and S. typhimunum*. In addition, the results of the antibacterial activity of the *S. flavicans* extract and positive control Tetracycline (300 µg/ml) on bacterial strains were determined based on the ability to inhibit bacterial growth shown through the diameter of the antibacterial zone created on a petri dish (mm). Inhibition zones ≥ 20 mm were declared as strong, 15–19 mm as moderate, and ≤ 14 mm as weak activities [23]. Results indicated the *S. flavicans* extract (150 and 300 mg/ml) and Tetracycline (300 µg/ml) had similar inhibitory activity to *E. coli* and *L. innocua* while 300 mg/ml algal extract had higher antimicrobial ability than 300 µg/ml Tetracyline toward *S. typhimunum* and *B. cereus* (*p*-value 0.05).

As it can be observed from Table 6, the *S. flavicans* had a weak ability to inhibit the growth of four bacterial strains at the extract concentration ranging from 37.5 to 300 mg/ml. The resistance level depended on

Table 6. Antibacterial circle diameter (mm) of *S. flavicans* extract and tetracycline (300 µg/ml).

Bacterial species	Antibacterial circle diameter (mm) of *S. flavicans* extract and Tetracycline				
	37.5 (mg/ml)	75 (mg/ml)	150 (mg/ml)	300 (mg/ml)	Tetracyline
E. coli	$7.33^{b} \pm 0.58$	$7.67^{b} \pm 0.58$	$9.33^{a} \pm 0.58$	$10.33^{a} \pm 0.58$	$10.67^{a} \pm 0.58$
S. typhimunum	$3.33^{c} \pm 0.58$	$3.67^{c} \pm 0.58$	$5.33^{ab} \pm 0.58$	$6.67^{a} \pm 0.58$	$4.67^{bc} \pm 0.58$
L. innocua	$2,67^{b} \pm 0.58$	$3^{ab} \pm 1$	$3.33^{ab} \pm 0.58$	$4,67^{a} \pm 0.58$	$3.33^{ab} \pm 0.58$
B. cereus	$5.33^{c} \pm 0.58$	$7^{bc} \pm 1$	$7.33^{a} \pm 0.58$	$8.33^{a} \pm 0.58$	$4.33^{c} \pm 1.15$

Note: Same letters in the same row indicate 5% nonsignificant difference by Tukey's test.

the extract concentration used. In comparison to other strains, the inhibiting effect on *E. coli* with antibacterial ring diameters of 7.33 ± 0.58 to 10.33 ± 0.58 (mm) was higher at all tested concentrations (p-value ≤ 0.05). In contrast, the lowest results of resistance level were found on *L. innocua*. Algal polysaccharides inhibit bacteria by attaching to glycoprotein—receptors on the surface of the bacterial cell wall, DNA, and cytoplasmic membrane. This binding disrupts the bacterial cell, increasing membrane permeability, causing proteins to leak out, and damaging the bacterial DNA [45]. Since fucoidan has been shown to inhibit the growth of *E. coli*, it may have potential as a preventive measure against wound contamination. Marine algae produce polysaccharides including fucoidan that can inhibit bacteria. These are highly sought after for medical uses such as delivering drugs, covering wounds, and growing new tissues. They are safe for the body, break down naturally, and are not harmful, making them excellent candidates for medical applications. Importantly, these antibacterial polysaccharides can significantly improve healing by preventing infection. A study of Poveda-Castillo *et al.* [46] found fucoidan derived from *F. vesiculosus* brown algae with concentrations of 100, 200, and 1,000 µg/ml against *L. monocytogenes* bacteria after just 24 hours at 37°C. However, increasing the fucoidan concentration from 100 to 1,000 µg/ml did not significantly enhance its bactericidal effect. In addition, from this research of Poveda-Castillo *et al.* [46] fucoidan demonstrated the strongest bacteriostatic effect toward *Salmonella typhimurium* at concentrations between 5 and 50 µg/ml. The higher the fucoidan concentration, the greater its ability to inhibit bacterial growth. At concentrations ranging from 100 to 1,000 µg/ml, fucoidan was effective in completely stopping the growth of *S. typhimurium*.

Additionally, previous research by Lee *et al.* [47] found that fucoidan is highly effective against common Gram-positive and Gram-negative oral bacteria such as *Streptococcus mutans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*, among others. Fucoidan demonstrated bacteriostatic effects at concentrations between 150 and 500 µg/ml and bactericidal effects at concentrations between 250 and 1,000 µg/ml. Moreover, studies on the red alga *Gracilaria multipartite* found that its antimicrobial activity against *E. coli* was influenced by the time of year it was harvested [48]. Similar results were obtained when testing the antimicrobial properties of *Ulva rigida* using an antibiogram [49]. Marine algae contain compounds with diverse antimicrobial properties. These compounds can disrupt the permeability of cell membranes by interacting with their proteins and lipids. Additionally, they can also interfere with the activity of enzymes within cells [50,51]. Johnsi Christobel *et al.* [52] found that extracts from *Sargassum wightii* and *Padina tetrastromatica* seaweed showed promise in resistanting to *Staphylococcus aureus* and *Vibrio harveyi* bacteria. *Oscillatoria sancta* was shown to have potential antibacterial effects against certain bacteria, including *Proteus mirabilis, Proteus vulgaris,* and *Streptococcus pyogenes*, in a study conducted by Prakash *et al.* [53].

3.5. Cytotoxic Activities

Cancer is a significant global health problem that can develop in various organs. Chemotherapy is a common treatment for many cancers, but it often has side effects including headaches, muscle aches, hair loss, and stomach issues. Marine organisms produce compounds with unique structures that show promise in treating some diseases. Existing cancer treatments often have significant side effects such as vomiting, diarrhea, and fatigue. Therefore, it is crucial to find new anticancer drugs from natural sources such as microorganisms, plants, and seaweeds. These natural sources offer the potential for safer, more affordable, and less toxic treatments. Fucoidan, a naturally occurring substance, shows promise in treating certain illnesses without causing harm. Research by Kim *et al.* [54] suggests that fucoidan can combat cancer by triggering cell death (apoptosis) through various biological mechanisms . Natural polysaccharides, like fucoidans, can help fight cancer in several ways. They can control cell growth, trigger cell death, and hinder the formation of new blood vessels and the spread of cancer. They also boost the immune system. Fucoidans specifically work by influencing NF-κB signaling pathways, which helps stop cancer cells from multiplying [55].

As indicated in Figure 2, the survival rate of HepG2 cells was not lower than the negative control, indicating that the *S. flavicans* extract at the tested concentration was not toxic to cells. For the HeLa cell line, as shown in Figure 3 the cell survival rate after treatment with 400, 200, and 100 µg/ml of fucoidan extract was lower than the cell survival rate of the negative control. This demonstrated that the extracts exhibited a dose-dependent toxic effect on the cells, with higher concentrations leading to gradually greater cell death. These extracts might work because of the different phytochemical components they contain. Studies have shown that fucoidan, a substance extracted from the seaweed *Undaria pinnatifida*, can suppress the growth of various cancer cells, including those found in the lungs (A-549), breasts (MCF-7 and T-47D), skin (SK-MEL-29), and colon (WiDr) [56–58]. Seaweed compounds, such as fucoidan, translam, and ulvan, can reduce cell growth. Meanwhile, terpenes found in various seaweeds, such as caulerpenynes, mediterraneol, and usneoidones, can inhibit cells from dividing. A specific sesquiterpene from *Caulerpa taxifolia* has been shown to be effective against cancer cells [59]. The findings suggest a broad-spectrum toxic effect on cells. Nevertheless, considering the variability in the bioactive components of the extract such as algal species, collection site, timing, and extraction solvent on the chemical makeup of the extract, additional studies are necessary to delve deeper into its chemical composition and biological activities.

3.6. Antifungal Activity

DMSO 10% was used as a solvent to dilute the extract, therefore, the antibacterial activity was also examined. The results showed that DMSO 10% did not affect the growth of the fungus *C. albicans*. The results of the estimation of the antifungal activity of the *S. flavicans*

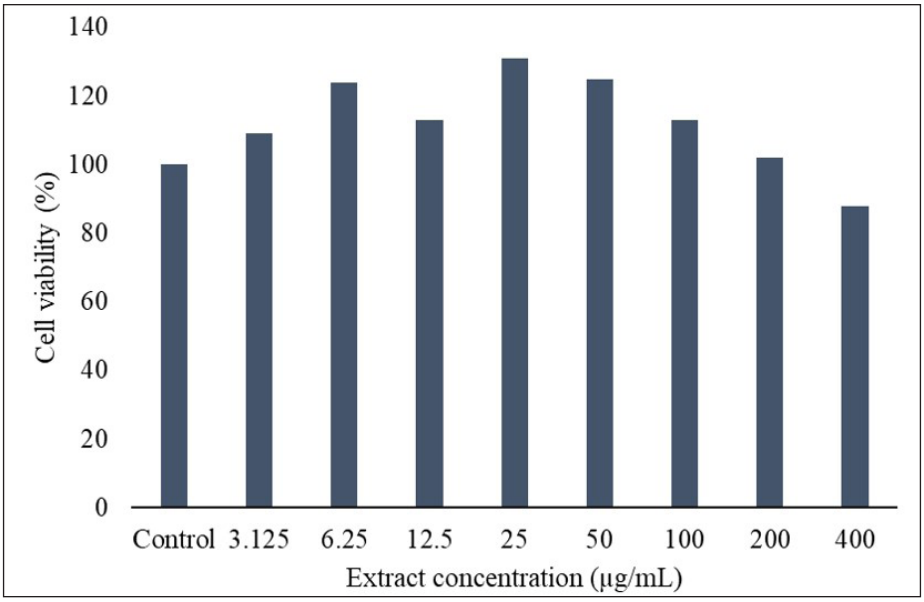

Figure 2. Cytotoxicity on HepG2 cell line.

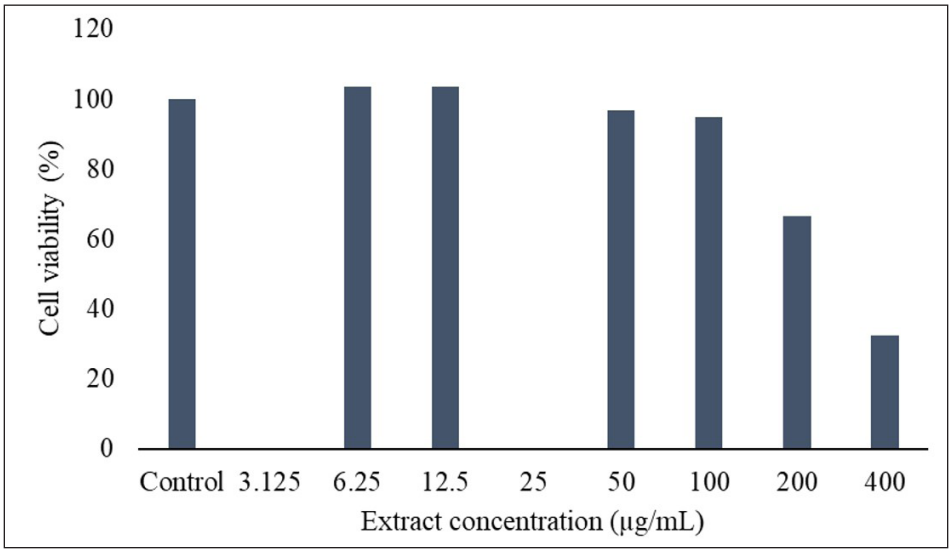

Figure 3. Cytotoxicity on Hela cell line.

Table 7. Antifungal circle diameter (mm) of *S. flavicans* extract and Nystatin (1 mg/ml).

Microorganism	Antifungal circle diameter (mm) of *S. flavicans* extract and Nystatin				
	37.5 (mg/ml)	75 (mg/ml)	150 (mg/ml)	300 (mg/ml)	Nystatin
C. albicans	$8.67^{b} \pm 0.58$	$9.67^{b} \pm 0.58$	$11.33^{a} \pm 0.58$	$11.67^{a} \pm 0.58$	$12.67^{a} \pm 0.58$

Note: Same letters in the same row indicate 5% nonsignificant difference by Tukey's test.

extract at different concentrations ranging from 37.5 to 300 mg/ml and Nystatin (1 mg/ml) on the fungus strain were determined based on the ability to inhibit the growth of the fungus shown through the diameter of the antifungal zone created on the petri dish (mm). Inhibition zones ≥20 mm were declared as strong, 15–19 mm as moderate, and ≤14mm as weak activities [23].

Nystatin, similar to other antifungal drugs like natamycin and amphotericin B, targets ergosterol, a component of fungal cell membranes. When enough nystatin molecules attach to ergosterol, they create pores in the membrane. These pores allow potassium ions to escape from the fungal cell, leading to cell death [60]. Similarly, it is believed that marine macroalgae extract has antimicrobial properties. This is thought to occur by targeting the cell membranes of harmful microorganisms, disrupting their structure and function. This disruption leads to imbalances in the cell's internal environment, ultimately causing cell death [60].

Results showed that the algal extract at concentrations of 150 and 300 mg/ml and standard Nystatin (1 mg/ml) reached a similar inhibitory ability to *C. albicans* (p-value ≤0.05). Additionally, Table 7 indicates the level of fungal resistance depended on the concentration of the extract used. At the extract concentration of 300 mg/ml, the inhibitory zone reached the diameter of the antifungal ring of 11.67 ± 0.58 (mm). The clear area around the agar well might be caused by antifungal compounds in the extract spreading out and preventing the growth of microorganisms. Previous research suggested that algal extracts have substances that can inhibit fungal growth, create internal damage, and disrupt the normal function of fungal cells [61].

Polysaccharides such as fucoidan and laminarin can successfully curb the growth of bacteria such as *S. aureus* and *E. coli*. They can also reduce the formation of *Helicobacter pylori* biofilms in the stomach lining. Sulphated polysaccharides, such as modified fucoidans, exhibit antibacterial properties by interacting with bacterial cell membranes. This interaction disrupts the membrane, ultimately leading to cell death [62]. Polysaccharides extracted from the seaweed *Sargassum swartzii* have been shown to inhibit the growth of both Gram-positive and Gram-negative bacteria [63]. Additionally, polysaccharides extracted from the red seaweed *Pterocladia capillacea* and the brown seaweed *Dictyopteris membranacea* using hot and cold water methods have been found to inhibit the growth of several bacterial species, including *B. cereus, S. aureus, Pseudomonas fluorescens,* and *E. coli* [64]. Collectively, the effectiveness of antifungal agents from algae varies depending on the efficiency of the extraction method, the type of algae used, and the ability of the fungi to resist these agents. Moreover, studies indicated that fucoidan might not directly kill the target organism. Instead, it could hinder their growth by limiting their access to essential nutrients [45]. Research on the antifungal effects and how algal polysaccharides like fucoidan work against fungi is still relatively limited [45].

4. CONCLUSION

The results of this present work showed a preliminary evaluation of the bioactive properties of the *S. flavicans* extract. The *S. flavicans* brown seaweed collected from Hon Son island, Kien Giang, Vietnam, has been proven to be a source of fucoidan with noticeable biopharmaceutical properties. The antibacterial and antifungal activity of the extracted fucoidan shows high levels as the concentration increases; this study showed the highest at 300 mg/ml. Antioxidant activity using the DPPH method of *S. flavicans* fucoidan with the IC_{50} value 206.21 ± 0.69 µg/ml. Fucoidan extracted from *S. flavicans* was proven to have a good inhibit α-amylase enzyme with the IC50 value of 10.87 ± 0.25 µg/ml. Besides, it also showed good cytotoxicity against the Hela cell line at 400 µg/ml. According to research, algae extract efficacy was influenced by several factors. The conditions under which the extracts were obtained and the solvents used during the process appear to be important contributors to their bioactivities potential. This research contributes to addressing environmental challenges caused by excessive algae growth in coastal waters. From an economic standpoint, it is expected to boost economic growth by developing a novel production method that utilizes algae as a raw material for pharmaceutical products.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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