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Comprehensive Analysis of Fucoxanthin from Sargassum ilicifolium: Hydroxyl-Mediated Antibacterial Activity Against Vibrio parahaemolyticus

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Comprehensive Analysis of Fucoxanthin from *Sargassum ilicifolium*: Hydroxyl-Mediated Antibacterial Activity Against *Vibrio parahaemolyticus*

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Abstract

Fucoxanthin is a naturally occurring carotenoid from brown seaweeds that has been known to be an antibacterial agent. Apart from its health benefits, the potential use of fucoxanthin as an antibacterial agent in aquaculture remains poorly understood. The present study primarily focused on the quantitative and functional characterization of fucoxanthin derived from *Sargassum ilicifolium*, with a specific emphasis on identifying hydroxyl (-OH) functional groups and their contribution to its antibacterial efficacy against *Vibrio parahaemolyticus*, the principal pathogen affecting whiteleg shrimp (*Litopenaeus vannamei*). Ethanol, methanol, ethyl acetate, and water were used individually as solvents for purification and screening. LC-MS profiling was employed for qualitative component identification; HPLC was used to measure purity; FTIR spectroscopy was applied to determine functional groups. The maximum concentration of fucoxanthin (3.112 ± 0.0610 ppm) was detected in fresh Sargassum extracted with ethanol, whereas dried *Sargassum* (simplisia) extracted with ethyl acetate yielded 0.93 ppm. Both extracts exhibited a robust and sustained presence of hydroxyl groups and demonstrated the most significant antibacterial efficacy against *V. parahaemolyticus*. The molecular characteristics of fucoxanthin unequivocally indicate

its antibacterial efficacy, and there is compelling evidence that the hydroxyl group plays a role in the inhibitory mechanism against *V. parahaemolyticus*. The most effective lethal bactericidal concentrations were 100 g/disk. These latest results establish a scientific basis for using fucoxanthin as a natural, bioactive, marine-sourced agent to control pathogens in shrimp aquaculture.

Keywords: Fucoxanthin, *Sargassum ilicifolium*, Antibacterial activity, *Vibrio parahaemolyticus*, Hydroxyl groups, *Litopenaeus vannamei*

Introduction

Shrimp aquaculture ranks among the most lucrative segments of global food production, with output currently exceeding 8 million metric tons to meet ever-growing demand [1]. This sector provides livelihood to small-scale farmers in Asia, particularly in Indonesia and Vietnam [2]. However, significant risk factors for shrimp farming sustainability are mainly linked to bacterial pandemics, with the most infamous being *Vibrio parahaemolyticus*, which causes acute hepatopancreatic necrosis disease (AHPND), also called early mortality syndrome (EMS) [1, 3]. AHPND caused a staggering US$43 billion in economic damage following its first major outbreak in 2009, while production dropped by up to 60% in affected areas [1, 4]. Shrimp exposed to *V. parahaemolyticus* experienced nearly 100% mortality within 72 h [5]. In addition to production losses, this microorganism has zoonotic potential, meaning that human health can be compromised through consumption of seafood contaminated with it, thereby posing a dual risk to food security and public health [6, 7].

Traditional disease management in shrimp aquaculture largely relies on antibiotics and chemical disinfectants [8]. Although these agents can effectively suppress pathogens in the short term, their continuous use is unsustainable [8]. A large-scale use of antibiotics in pond systems not

only alters the host microbiota but also weakens innate immunity, leading to an increased spread of antibiotic resistance among the *Vibrio* [1]. An increase in the resistance of Vibrio strains isolated from aquaculture has recently been reported [9]. Consequently, governments are now restricting the use of prophylactic antibiotics, and there is a worldwide call for alternatives to existing therapeutics [10]. Researchers are focusing on developing effective and environmentally friendly antibacterial agents from natural marine products to support sustainable aquaculture [11].

Brown algae of the genus *Sargassum* are a rather extraordinary source of bioactive metabolites. These marine macroalgae produce several remarkable molecular players with well-documented biological effects, including unique carotenoids, polyphenols, and sulfated polysaccharides. Of these molecules, fucoxanthin isolated from brown algae has been the first choice for many researchers in medicine and pharmacology due to its diverse therapeutic properties, such as antioxidant, anti-inflammatory, anti-obesity, and antimicrobial effects across a wide spectrum. Oral administration of this marine xanthophyll carotenoid containing an allenic bond to a primate model reduced fasting blood glucose and insulin levels and body weight. Karpiński & Adamczak [12] found that isolated fucoxanthin exhibits growth-inhibitory activity against 13 species of aerobic bacteria, both Gram-positive and Gram-negative, with bacterial killing zone diameters ranging from 7.2 to 12.2 mm. Fucoxanthin disrupts the extracellular matrix in biofilms, limits bacterial attachment on surfaces, downregulates virulence factor production, and modifies cell-to-cell communication. In addition to the antibacterial effect of pure fucoxanthin, the crude extract of *Sargassum* species has demonstrated antibacterial activity in vitro against *V. parahaemolyticus* and *V. harveyi*. *Sargassum polycystum* is a source of various bioactive constituents with potential clinical applications. It is an intensively growing seaweed found in oceans worldwide, especially in tropical and subtropical regions. It offers excellent opportunities

for developing new functional products. Fucoxanthin is an important bioactive compound in *S. polycystum* [13, 14].

Most research shows that fucoxanthin is antibacterial, but no one has actually examined its -OH groups against *V. parahaemolyticus*. While articles discuss areas where growth ceases, they overlook the true connection between a molecule's structure and its biological effect. Carotenoids can disrupt bacterial membranes or induce cellular leakage, although there is no evidence that fucoxanthin directly binds to LPS layers or interferes with internal processes in this strain. Sargassum is a raw material abundant in Indonesia, and its potential as a tool for shrimp farming and phytoremediation is being studied [15]. However, the absence of field-based experimental data undermines the practical credibility of these findings. The current study aimed to isolate fucoxanthin from *S. ilicifolium*, elucidate its hydroxyl-mediated antibacterial activity against *V. parahaemolyticus*, and assess its potential as a sustainable alternative to antibiotics for the management of shrimp diseases [12, 16].

Materials and Methods

Seaweed Collection and Preparation

According to Senggagau [17], *S. ilicifolium* is among the top dominant *Sargassum* species found in Lampung waters. *S. ilicifolium* was obtained from the sea shore of Lampung, South Lampung Regency, Indonesia. The seaweed was first washed with clean water to remove surface contaminants. Then it was transported in an ice-filled cooler box to the lab. Two forms of biomass were made: (i) fresh *Sargassum* and (ii) dried *Sargassum* (simplisia), which was dried and cleaned according to a standard method before extracting. All samples were reduced in size by cutting before maceration.

Extraction of *Sargassum ilicifolium*

The extraction was performed following Jaswir's protocol [18], with some modifications. Briefly, 10 g of either fresh or dried *Sargassum* was separately macerated in 100 mL of water (WA), ethanol (ET) (Merck, Germany), ethyl acetate (EA) (Merck, Germany), or methanol (ME) (Merck, Germany) at a solid-to-solvent ratio of 1:10 (w/v) for 24 h at 18 °C in sealed amber glass bottles. The extracts were filtered through a 0.22-micron filter, and the solvents were removed by rotary evaporation under reduced pressure at 35°C and 60 rpm. Concentrated extracts were stored in dark glass bottles at 4 °C until further use.

Purification of Fucoxanthin from Concentrated *Sargassum* Extracts

Fucoxanthin was purified using open-column silica gel chromatography, adapted from Thom [19], with modifications. Silica gel 60 GF254 (Merck, Germany) was pre-equilibrated with n-hexane (Merck, Germany) for 24 h and packed in a chromatography column to a height of approximately 10 cm. Approximately 1 g of concentrated extract was loaded onto the column and eluted using a stepwise gradient: the first fraction employed 100% n-hexane to remove non-polar constituents, followed by a 6:4 (v/v) n-hexane:acetone (Merck, Germany) mixture to elute the fucoxanthin-enriched fraction, which was identified by its characteristic orange-red color. The fucoxanthin-containing fractions were pooled, evaporated to a paste under reduced pressure, dried under a nitrogen gas stream, and stored at 4 °C.

Molecular Characterization

Quantitative Analysis by HPLC

Fucoxanthin quantification was performed by HPLC using a modified method described by Truong [20]. The stable phase consisted of a Luna 5U C18 100 Å column (Phenomenex; 100 × 2.0 mm) maintained at 30 °C. Mobile phase A was made up of ultrapure water mixed with 0.1%

trifluoroacetic acid (TFA), and mobile phase B was made up of acetonitrile mixed with 0.1% TFA. It was sonicated for 30 minutes before each step. With a PDA detector set to 450 nm and a flow rate of 0.2 mL/min, a 0–58 min gradient program was used for the chromatographic separation. The amount of fucoxanthin in each fraction was determined by comparing it with a calibration curve generated from Sigma-Aldrich fucoxanthin solutions at 0.1, 0.5, 1.0, 1.5, and 2.0 ppm.

Qualitative Profiling by LC-MS

Fucoxanthin was identified qualitatively and molecularly at the Genomics Laboratory of the National Research and Innovation Agency (BRIN) in Cibinong, Bogor, Indonesia. Water (A) and analytical-grade acetonitrile (B) (Merck, Germany) were supplied at a total flow rate of 0.3 mL/min as the mobile phases. Mass spectra were processed using MassLynx v4.1 software, and compound identification was performed based on the molecular ion (m/z) values compared against molecular formulas in the Chemistry Structure Database (ChemSpider; www.chemspider.com). The molecular formula, molecular structure, molecular mass, and compound abundance were estimated from the chromatographic peak area.

FTIR Functional Group Analysis

FTIR analysis was performed following the procedure described by Renhoran [21] with minor modifications. Fucoxanthin fractions purified from each solvent system were used as samples. One milligram of dried sample was mixed with 200 mg of attenuated total reflectance (ATR) medium and pressed into pellets under vacuum for 20 min. Spectra were recorded using an FTIR spectrometer across the wavenumber range of 400–4000 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans. Spectral data were processed using Spectrum software version 6.3.4 to identify functional groups associated with antibacterial activity.

Antibacterial Activity Assessment

In Vitro Disc Diffusion Assay Against *V. parahaemolyticus*

Antibacterial activity was evaluated using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) [21]. *V. parahaemolyticus* at a concentration of 10^5 CFU/mL was uniformly spread across the MHA plates. Sterile paper discs were impregnated with fucoxanthin fractions at concentrations of 25, 50, and 100 µg/disc [22], as well as at 1, 2, and 3 ppm. Amoxicillin (25 µg/disc) was used as the positive control. Plates were incubated at 37 °C for 18–24 h, after which the diameter of the inhibition zone surrounding each disc was measured using a digital caliper. The inhibition zone diameter was calculated according to the method of Kipimbob [23]:

$$d = \frac{[(D_v - D_d) + (D_h - D_d)]}{2}$$

where d = inhibition zone diameter (mm); Dv = vertical diameter (mm); Dh = horizontal diameter (mm); Dd = disc diameter (mm).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values were determined using Bloomfield's linear regression method [24]. A linear regression curve was plotted between the natural logarithm of fucoxanthin concentration (ln Mo; x-axis) and the squared inhibition zone diameter (Z^2; y-axis). The x-intercept of this curve yielded the ln Mt value as follows: The MIC was calculated as 0.25 × Mt, and the MBC was defined as four times the MIC.

Statistical Analysis

Fucoxanthin concentration data and inhibition zone diameters were analyzed using one-way ANOVA in GraphPad Prism 10, and FTIR functional group data were analyzed descriptively in Origin Pro 2024 to assess antibacterial group profiles across solvent systems.

Results and Discussion

Solvent Optimization for Fucoxanthin Extraction

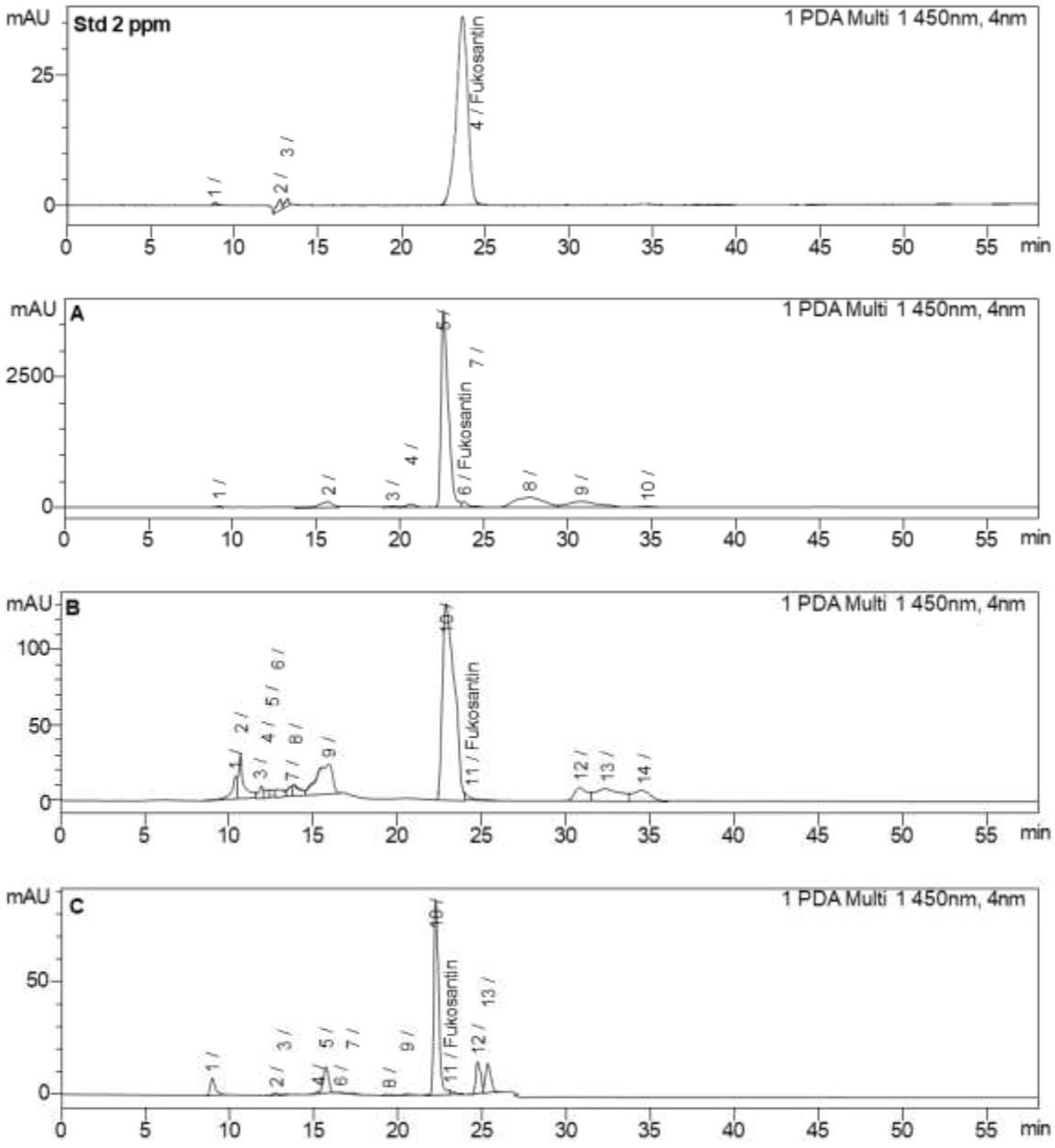
HPLC examination indicated that the fucoxanthin level was markedly greater in extracts from fresh Sargassum biomass compared to dried simplisia preparations (Table 1). Sample A, derived from fresh *S. ilicifolium* extracted with ethanol, exhibited the greatest quantity of fucoxanthin at 3.112 ± 0.0610 ppm. The simplisia form attained a maximum concentration of 0.939 ± 0.0392 ppm with ethyl acetate as the extraction solvent.

Table 1 Fucoxanthin concentration in fresh and dried *S. ilicifolium* extract in different solvents

Sample	*S. ilicifolium* form	Solvents Extract	Fucoxanthin concentration (ppm) (mean ± SD) n = 3
A	Fresh	Ethanol	3.112 ± 0.0610
B	Fresh	Methanol	0.168 ± 0.0060
C	Fresh	Ethyl acetate	0.041 ± 0.0020
D	Fresh	Water	0.009 ± 0.0004
E	Dried	Ethanol	0.324 ± 0.0130
F	Dried	Methanol	0.601 ± 0.0233
G	Dried	Ethyl acetate	0.939 ± 0.0392
H	Dried	Water	0.022 ± 0.0015

The superior fucoxanthin yield from fresh biomass using ethanol can be attributed to a synergy of physicochemical factors. Fresh biomass retains the complete tissue architecture, minimizing oxidative degradation and preserving fucoxanthin in its original form [25]. Ethanol is a semipolar solvent that penetrates the algal cell membrane and dissolves polar and semi-lipophilic

pigments such as fucoxanthin [26, 27]. In addition, the fresh biomass is not subjected to any thermal processing, thereby avoiding thermally induced isomerization and degradation of the conjugated polyene backbone, which are often consequences of high-temperature drying [28, 29]. When extraction parameters such as solvent volume, temperature, contact time, and mechanical disruption are well controlled, fresh biomass consistently yields higher fucoxanthin recovery than dried material [30].



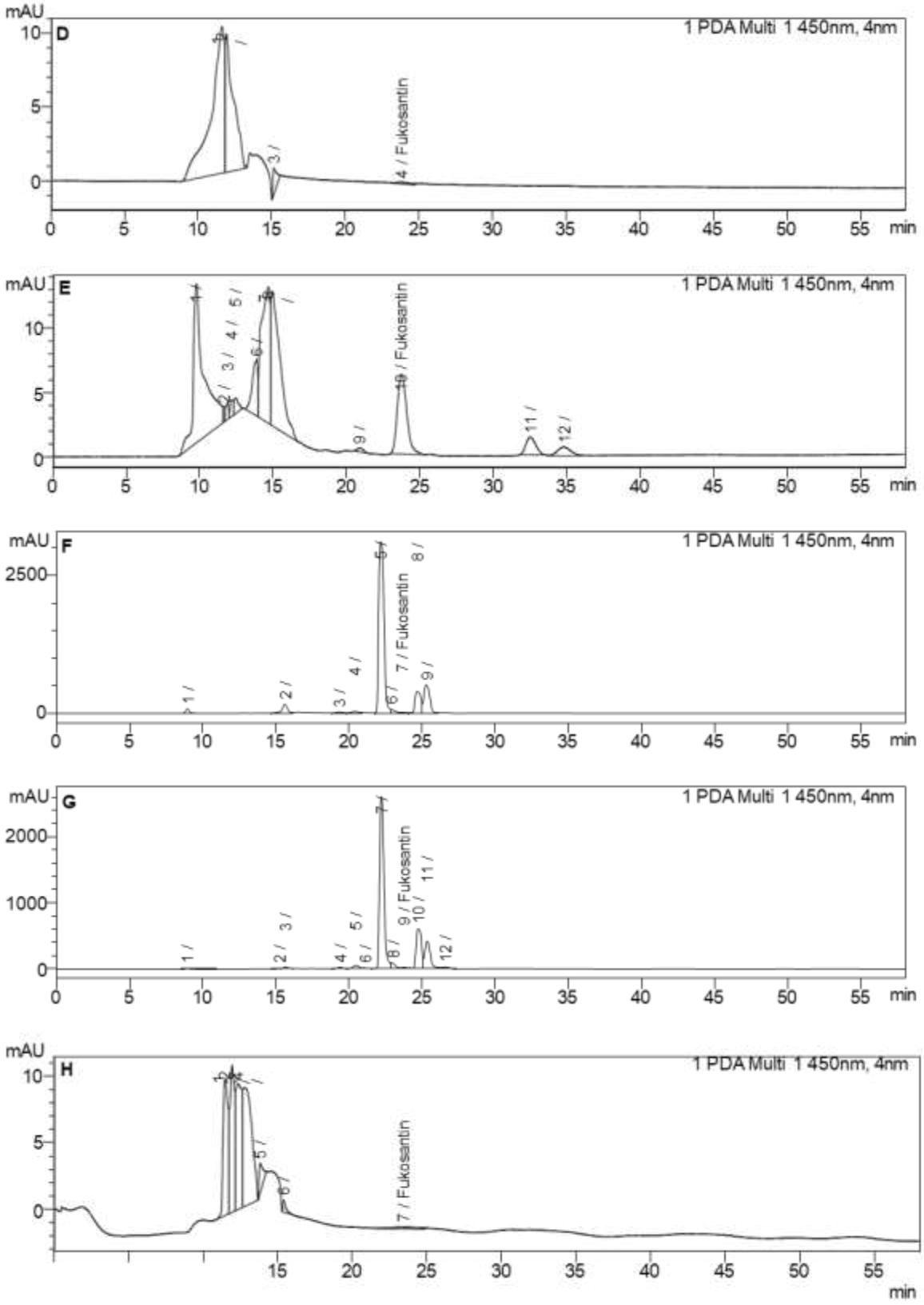

mAU
D
1 PDA Multi 1 450nm, 4nm
4 / Fukosantin
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10 / Fukosantin
11 /
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7 / Fukosantin
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G
1 PDA Multi 1 450nm, 4nm
9 / Fukosantin
min
mAU
H
1 PDA Multi 1 450nm, 4nm
7 / Fukosantin
min

Fig. 1 Chromatogram of fucoxanthin from a 2 ppm standard, fresh (A−D) and dried (E−H) *S. ilicifolium* in different solvents. (A and E: ethanol; B and F: methanol; C and G: ethyl acetate; D and H: water).

The presence of Fucoxanthin compounds in all types of solvents, both in fresh and dried (simplisia) *S. illicifolium* is presented in Fig 1. The effectiveness of ethyl acetate for extracting fucoxanthin from dried simplisia can be elucidated using complementary methods. Desiccation disrupts cellular membranes, increasing tissue permeability and allowing solvent infiltration into the lipid matrix where fucoxanthin is stored [28]. Simultaneously, moisture removal enhances the concentration of fucoxanthin on a dry-weight basis, hence increasing apparent extraction yields [31]. Ethyl acetate, a moderately polar solvent, selectively interacts with lipophilic carotenoids such as fucoxanthin and exhibits greater extraction efficiency than highly polar solvents like methanol [32]. Ethyl acetate serves as an efficient extractant for fucoxanthin from desiccated biomass.

Table 2 HPLC result for retention time, area, and height of fucoxanthin from a 2 ppm standard, fresh (A−D) and dried (E−H) *S. ilicifolium* in different solvents. (A and E: ethanol; B and F: methanol; C and G: ethyl acetate; D and H: water).

Sample	Retention Time (min)	Area	Height
2 ppm standard	23.608	1712681	36224
A	23.820	2655092	114851
B	23.973	143178	5073
C	23.088	35307	1707

D	23.838	7867	126
E	23.682	276329	6213
F	23.593	512919	17069
G	23.721	801255	24877
H	23.436	19119	151

Table 2 showed that the presence of the fucoxanthin compound occurs, on average, at 23 minutes across all solvent types, in both fresh and dried (simplisia) *S. illicifolium*. The elevated fucoxanthin concentration in fresh *S. ilicifolium* compared to simplisia aligns with the heat-sensitive characteristics of fucoxanthin, which is prone to oxidative destruction during high-temperature drying [29]. Extended exposure to light and oxygen during the storage of simplisia significantly enhances the oxidation of fucoxanthin [30]. The unaltered cellular architecture of fresh biomass provides a protective milieu, whereas cellular disruption during drying renders fucoxanthin susceptible to enzymatic and oxidative degradation [25]. When simplisia preparation is necessary, freeze-drying or low-temperature drying techniques are advised to reduce fucoxanthin degradation [28].

The polarity of the solvent is a key factor influencing the efficacy of fucoxanthin extraction. Fucoxanthin is a semipolar xanthophyll soluble in ethanol, methanol, and ethyl acetate, although virtually insoluble in water [12, 33]. A prior investigation indicated that ethanol yielded the highest fucoxanthin concentration (0.59 mg/g dry weight) among the solvents assessed, consistent with the current results [18, 34]. The polar characteristics of ethanol facilitate the effective dissolution of pigment–protein complexes in algal chloroplasts, therefore liberating bound carotenoids [26].

While additional parameters such as temperature, time, and solvent-to-biomass ratio modulate extraction efficiency, solvent polarity remains the primary driver of fucoxanthin selectivity [33].

LC-MS Qualitative Molecular Profiling

Fucoxanthin Standards and Sample Identification

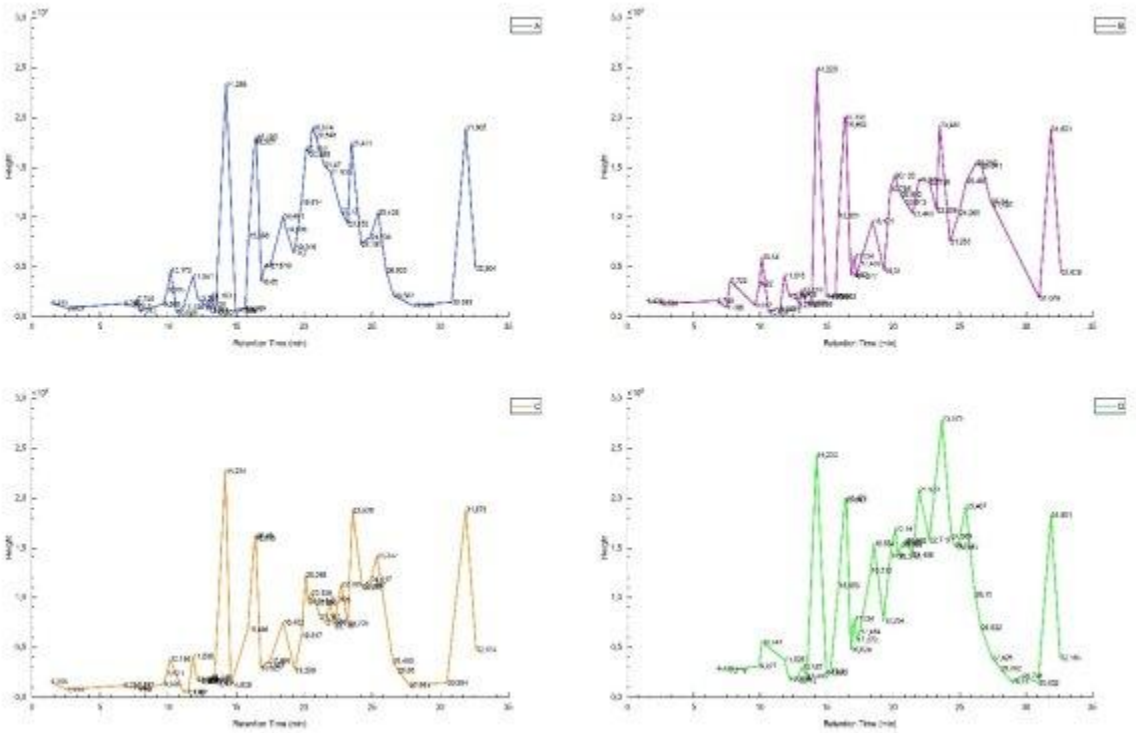
In the LC-MS analysis, we confirmed the protonated molecular ion $[M+H]^+$ characteristic of fucoxanthin at m/z 659.8600 in the fucoxanthin standards of 1 and 2 ppm, which is very close to the theoretical molecular mass of 659.9 Da (Table 3). The 1 ppm standard showed a single, well-resolved chromatographic peak with a very high signal intensity at 19.706 min, 100% signal purity and fucoxanthin was confirmed as the main component. The minor ions at m/z 581.6, 623.5, 641.6, and 681.6 represent characteristic carotenoid adducts and fragments [35].

Table 3 LC-MS results for retention time, mass-weight (MW) targeted, % area, and percentage height of fucoxanthin compound from fresh and dried *S. ilicifolium* in different solvents

Samples	Retention time (min)	MW target (m/z)	% Area	% Height
2 ppm Std	19.761	659.86	94.802	91.41
	24.584	659.86	5.198	8.59
1 ppm Std	19.706	659.86	100	100
A	20.097	659.86	14.926	19.833
	24.563	659.86	85.074	80.167
B	20.104	659.86	21.758	25.666
	24.597	659.86	78.242	74.334
C	20.068	659.86	95.719	90.508
	21.562	659.86	4.281	9.492
D	20.092	659.86	100	100

E	20.074	659.86	52.462	51.253
	24.541	659.86	47.538	48.747
F	19.967	659.86	74.347	67.898
	21.064	659.86	25.653	32.102
G	29.513	659.86	100	100
H	20.106	659.86	54.506	57.91
	28.534	659.86	45.494	42.09

(a)



(b)

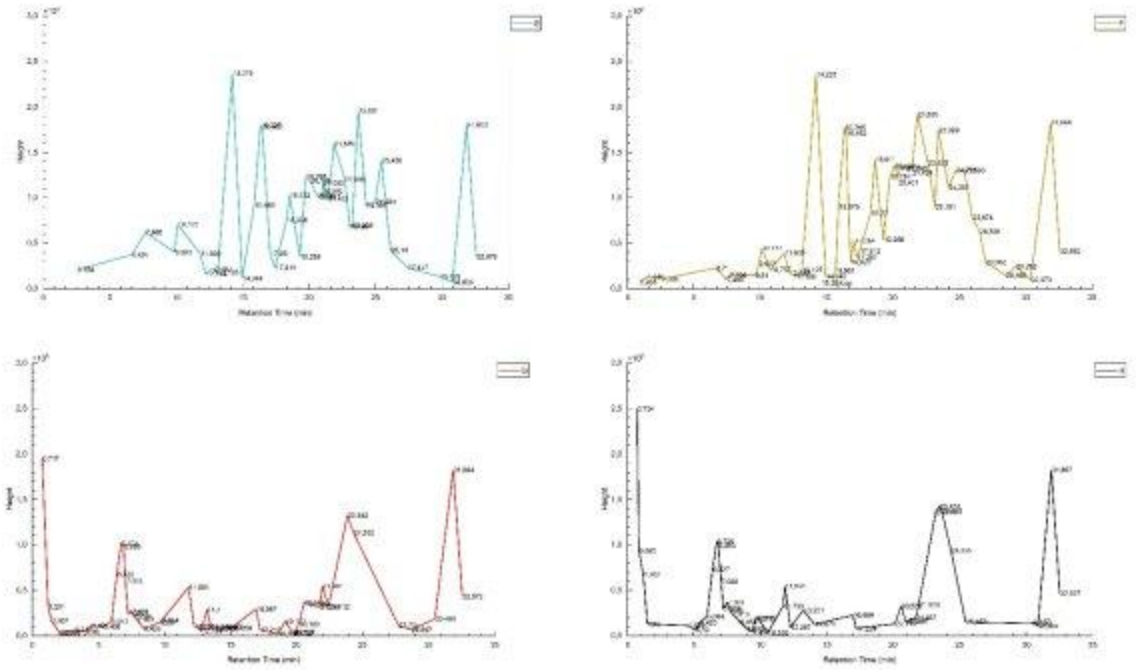


Fig. 2 TIC of (a) fresh and (b) dried *S. ilicifolium* in different solvents (A and E: ethanol, B and F: methanol, C and G: ethyl acetate, D and H: water).

The 2 ppm standard exhibited two chromatographic peaks at retention times of 19.761 min (area: 5,790,814; 94.8%) and 24.584 min (area: 317,520; 5.2%), both at m/z 659.8. The appearance of a secondary peak at higher concentrations may reflect the presence of fucoxanthin isomers, including all-trans and 9′-cis forms, which are commonly generated under conditions of elevated concentration or analytical stress [36, 37]. A comparative study of 1 ppm and 2 ppm standards reveals that the 1 ppm sample exhibited a cleaner, more straightforward chromatographic profile, whereas the 2 ppm sample showed signal distribution toward a secondary peak, potentially attributable to partial isomerization. This observation highlights the necessity of regulating sample concentration and analytical conditions to preserve signal integrity [37].

Fig 2 showed the TIC from the LC-MS fractionation of fresh and dried *S. illicifolium* samples with various solvents. Several bioactive compounds, in addition to fucoxanthin, were yielded by

all solvents. This can occur because LC-MS selects compounds based on the molecular weight of the target and its derivatives.

LC-MS Analysis of *Sargassum*-Derived Fucoxanthin Fractions

There was no doubt that fucoxanthin was present in all eight fractions (samples AH) at m/z = 659.8600. The retention times ranged from approximately 19.7 to 29.5 minutes, with differences attributable to changes in sample matrix composition, possible co-elution of isomers, or minor differences in chromatographic column conditions. Samples A and B showed the highest total signal areas (around 36.76×10^6 and 24.20×10^6, respectively), which supports that their fucoxanthin content, as determined by HPLC, was also high. The main peak in sample A at 24.563 minutes (area: 31 274 699) is a good indication of the efficient extraction and improved stability of fucoxanthin in the fresh biomass matrix obtained from ethanol.

The multiple chromatographic peaks observed in several samples suggest the co-occurrence of geometric isomers (all-trans and 9′-cis fucoxanthin) and/or oxidation products, as is well documented in carotenoid chemistry [36, 37]. Samples G and H showed lower signal intensities but still confirmed the presence of fucoxanthin, reflecting the lower efficiency of the simplisia-water and simplisia-ethyl acetate combinations. Supplementary ions at m/z values of approximately 637.4, 681.7, and 727.5 in multiple samples correspond to characteristic carotenoid fragmentation ions, further supporting compound identity.

FTIR Functional Group Analysis

FTIR spectral analysis of the samples confirmed the presence of fucoxanthin-characteristic functional groups, including: carbonyl (C=O) stretching at 1700–1740 cm^{-1}; free and hydrogen-bonded hydroxyl (–OH) groups at 3200–3600 cm^{-1}; epoxide vibrations; and conjugated C=C stretching, consistent with the known structural features of fucoxanthin [38–41].

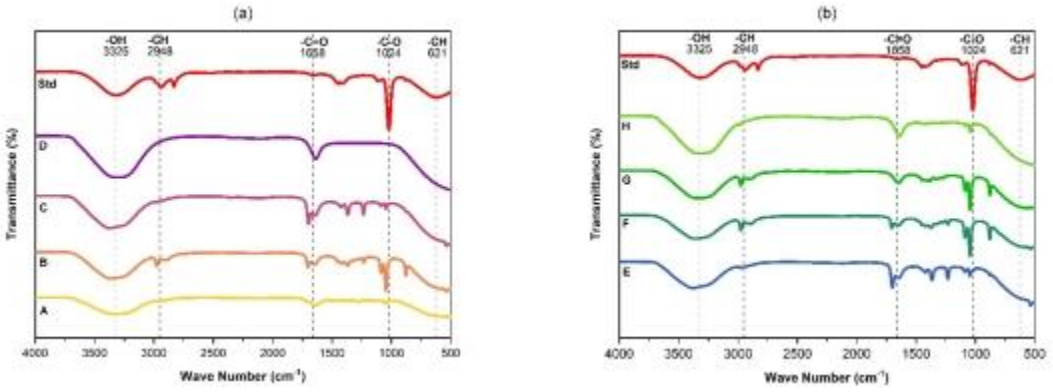


Fig. 3 The functional group characteristics of fucoxanthin from fresh (a) and dried (b) *S. ilicifolium* in different solvents (A and E: ethanol; B and F: methanol; C and G: ethyl acetate; D and H: water; Std: standard fucoxanthin: 1 ppm) samples by FTIR analysis.

Samples extracted using polar solvents (A B E, F) exhibited pronounced absorption bands of polar functional groups, primarily OH and C=O, whereas extracts from semi-polar solvents (C and G) were distinguished by predominant ester carbonyl and aliphatic CH bands, indicating selective extraction of lipophilic fractions (Fig. 3). Water-based extracts (D and H) exhibited significantly diminished intensities of all functional group bands, reflecting the restricted solubility of carotenoid pigments in water. Fucoxanthin standards exhibited the most distinct and intense spectrum patterns, characterized by notable carbonyl overtones and highly reactive free hydroxyl groups at >3900 cm, indicating a significant level of chemical purity and potential for biological reactivity.

The ethyl acetate extract of dried *S. ilicifolium* had a pronounced carbonyl peak at 1704 cm^{-1} and an active hydroxyl group at 3359 cm^{-1}, in addition to classic C–O and C–N stretching bands linked to microbial membrane contacts and enzyme inhibition [39, 41, 42]. Sample A (fresh biomass and ethanol) displayed pronounced indications of polar functional groups. The structural characteristics align with the known antibacterial action of fucoxanthin, which involves disruption

of membrane permeability, inhibition of enzymes, and the generation of oxidative stress in bacterial cells [39].

Antibacterial Activity Against *Vibrio parahaemolyticus*

Fucoxanthin fractions demonstrated the ability to kill *V. parahaemolyticus* bacteria in a dose-dependent manner across six concentrations (25, 50, 100 g/disc). Clearly distinct inhibition zones were observed at all these doses; however, differences in the nature of inhibition depended on the incubation concentrations and times.

At 24 hours of incubation, all the doses gave measurable inhibitory zones. However, 96 hours (4-24 hours) of incubation was the time at which the test with a dose of 100 g/disc of fucoxanthin revealed that it was bactericidal, as indicated by the failure of bacterial regrowth in the inhibitory zones after an extended incubation period. But the 25 g/disc and 50 g/disc doses appeared to have only a bacteriostatic effect at or near the MIC level, allowing for bacterial regrowth after extended incubation. Doses greater than 100 µg/disc appear to reach a saturation point in effectiveness, likely due to diffusion saturation in the agar matrix [43].

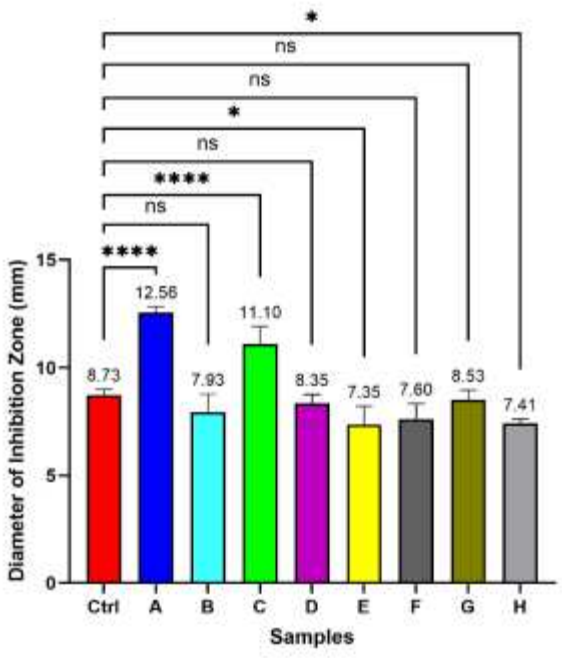


Fig. 4 Diameter of inhibition zone (mm) from different solvent extracts (A and E: ethanol; B and F: methanol; C and G: ethyl acetate; D and H: water) of fresh (A to D), dried *S. ilicifolium* (E to H), and control (amoxicillin 30 µg) against *V. parahaemolyticus*.

Sample A (fresh *S. ilicifolium*, ethanol) exhibited the highest inhibition zone diameter against *V. parahaemolyticus*, followed by sample C (fresh *S. ilicifolium*, ethyl acetate). Only samples A (12.56 ± 0.26 mm) and C (11.10 ± 0.82 mm) demonstrated bactericidal properties; the remaining samples exhibited bacteriostatic effects, with bacterial regrowth observed after 4 days of incubation (Fig. 4).

The superior antibacterial activity of sample A is attributed to the capacity of ethanol to co-extract fucoxanthin along with phenolic compounds, flavonoids, and steroids that synergistically disrupt the integrity of Gram-negative cell membranes [21, 44]. Fucoxanthin acts through multiple antibacterial mechanisms: disruption of membrane permeability, leading to ion leakage; inhibition of essential enzymes and DNA replication; and induction of oxidative stress [12, 39]. The

lipopolysaccharide-containing outer membrane of *Vibrio* spp. is particularly susceptible to semi-polar and lipophilic bioactive compounds [45, 46].

The differences in the functional group composition and solvent polarity explain the bactericidal versus bacteriostatic distinction observed among samples. Lipophilic substances, including flavonoids, carotenoids, and steroids, generally exert bactericidal effects by compromising membrane integrity [47], whereas chemicals that inhibit protein synthesis or DNA replication without causing direct membrane damage exert bacteriostatic effects [45, 48]. Ethanol extracts, in contrast to water extracts, can yield a wider variety of semipolar molecules, including those with membrane-lytic action [49, 50]. Environmental variables, including pH, temperature, and matrix composition, also influence antibacterial activity [51, 52]. The Gram-negative nature of *V. parahaemolyticus* and the structure of its outer membrane dictate its varying susceptibility to fucoxanthin in contrast to Gram-positive pathogens [33, 53].

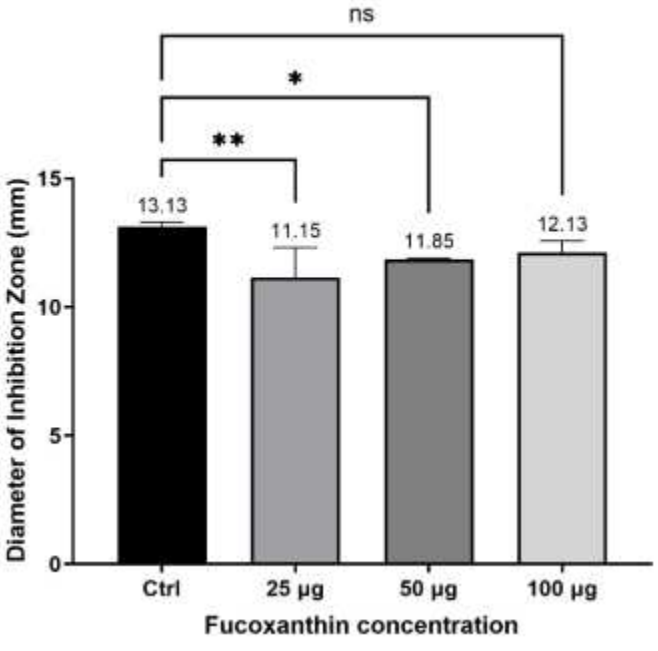


Fig. 5 Diameter of inhibition zone (mm) from different fucoxanthin concentrations and control (amoxicillin 25 µg) against *V. parahaemolyticus*.

Fucoxanthin showed antibacterial activity against *V. parahaemolyticus* in disc diffusion tests at 25, 50, and 100 g/disk, using amoxicillin (25 g/disk) as a reference. It produced inhibition zones ranging from 11. 15 to 12.13 mm (Fig. 5), less than amoxicillin's 13.13 mm zone, so differences were real at lower doses, $p < 0.01$ at 25 g, $p < 0.05$ at 50 µg, plus, and this uses Bloomfield's regression method (1991), the minimum inhibitory concentration was found at 10. 25 ppm and the minimum bactericidal concentration at 41 ppm. These values indicate strong antibacterial action. Ouchari [54] defines inhibition zones as weak (0–5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (over 20 mm). The area of inhibition includes the entire paper disk.

The current investigation revealed that fucoxanthin displayed significant antibacterial activity against *V. parahaemolyticus*; however, its effectiveness was inferior to that of the positive control (amoxicillin, 25 µg/disk). Although the relationship is not perfectly linear, these findings indicate that fucoxanthin exhibits dose-dependent antibacterial activity.

The diminished inhibitory zone at lower concentrations may be attributed to the restricted bioavailability of fucoxanthin, a carotenoid known for its hydrophobic nature and susceptibility to degradation under light and oxygen exposure [39]. At elevated doses, the activity neared that of amoxicillin, suggesting possible synergistic interactions with bacterial membrane constituents. In bacteria, fucoxanthin compromises lipid bilayers, hinders biofilm formation, and regulates oxidative stress pathways [55]. These pathways may elucidate its inhibitory activity against *V. parahaemolyticus*, a significant problem in aquaculture due to its involvement in vibriosis outbreaks.

Though the antibacterial property of fucoxanthin is weaker than that of the other medicines. This limitation underscores the importance of designing delivery systems, such as nanoencapsulation, to enhance stability and bioavailability. When fucoxanthin is encapsulated in

chitosan nanoparticles, not only are its antibacterial and antioxidant activities enhanced, but it also becomes more effective at maintaining health in aquaculture [56]. Besides, substances of natural origin, such as fucoxanthin, might serve as primary agents for the production of medicines, thereby reducing reliance on synthetic antibiotics and helping mitigate the threat of antimicrobial resistance.

Incorporation of fucoxanthin into functional feeds or biomaterials for shrimp aquaculture would provide natural protection against *Vibrio* infections. By being both an antioxidant and an antibacterial agent, it significantly enhances its potential to promote aquatic animal health. Research efforts should be directed toward improving the formulation technique, evaluating in vivo effects, and exploring synergistic interactions with other bioactive agents.

Current data support the antibacterial activity of fucoxanthin against different pathogens, including *Staphylococcus aureus* and *Propionibacterium acnes* [21, 30, 33, 44, 57, 58]. To our knowledge, this is among the first few comprehensive studies investigating the effects of fucoxanthin from *S. ilicifolium* on *V. parahaemolyticus*, providing fresh support for its use in the marine aquaculture sector.

Conclusions

This research shows that fucoxanthin taken from *S. ilicifolium* has strong antibacterial activity against *V. parahaemolyticus*. Effectiveness is strongly related to liquid polarity, biomass condition, and concentration. The highest concentration of fucoxanthin was found in fresh *Sargassum* biomass recovered with ethanol (3.112 ± 0.0610 ppm), while the highest concentration was found in dried simplisia with ethyl acetate (0.939 ± 0.0392 ppm). The HPLC study showed that quantification was highly accurate ($R^2 > 0.99999$), and LC-MS profiling consistently detected fucoxanthin at $[M+H]^+$ = m/z 659.8600 across all samples, with different isomeric forms observed

in different fractions. FTIR spectroscopy revealed the presence of functional groups important for antibacterial activity, including hydroxyl, carbonyl, epoxide, and conjugated C=C groups. Samples A (fresh biomass, ethanol) and G (simplisia, ethyl acetate) had the greatest antibacterial effects, with bactericidal activity confirmed after 96 hours of incubation at a concentration of 100 µg/disk. These results establish a scientific foundation for developing *Sargassum*-derived fucoxanthin as a natural antibacterial agent in shrimp aquaculture, particularly for managing vibriosis in *L. vannamei* production systems.

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Author Contribution

Betutu Senggagau contributed to conceptualization, methodology, investigation, formal analysis, data curation, writing the original draft, and visualization. **Sukenda** contributed to supervision, conceptualization, methodology, validation, and review. **Widanarni** contributed to supervision, validation, formal analysis, and review. **Alimuddin** contributed to supervision, validation, resources, and review. **Brata Pantjara** contributed to investigation, validation, and writing and editing. **Manja Meyky Bond** contributed to data curation, formal analysis, visualization, writing review and editing. All authors have read and approved the final version of the manuscript.

Data Availability

This published paper contains all of the data created or analyzed during this investigation.

Declarations

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Ethics Approval Neither humans nor animals were used in this study.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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