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Ultrasonic-Assisted Extraction of Fucoxanthin from Marine Macroalga *Padina australis*: Optimization, Bioactivity, and Structural Characterization

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Abstract: Macroalgae serve as a potential feedstock for fucoxanthin extraction. Fucoxanthin, a bioactive pigment found in the chloroplasts of marine algae, exhibits significant pharmacological properties. As a member of the carotenoid family, fucoxanthin plays a crucial role in both the food and pharmaceutical industries. This research explores the effects of ultrasonics on the extraction of fucoxanthin from the marine macroalga *Padina australis*. In addition, various extraction techniques and the influence of solvents on the efficient separation of fucoxanthin from algae have been studied and compared. Using methanol, chloroform, and a combination of methanol and chloroform (1:1, v/v), conventional fucoxanthin extraction from *Padina australis* yielded 8.12 mg of fucoxanthin per gram of biomass. However, the ultrasonic-assisted extraction resulted in a significantly higher yield of 16.9 mg of fucoxanthin per gram of biomass, demonstrating that the use of ultrasonics enhances the extraction rate compared to conventional methods. Therefore, the efficient separation of fucoxanthin from *Padina australis* is highly dependent on ultrasonic-assisted extraction. The process conditions for the extraction were optimized to maximize the yield of fucoxanthin from seaweeds. The following parameters were selected for optimization studies: moisture content, particle size, mixing speed, extraction temperature, extraction duration, and solid-to-solvent ratio. The extracted fucoxanthin exhibited various biological activities, including antimicrobial and antioxidant properties, and its structure was elucidated through FTIR and NMR spectroscopy. Additionally, thin-layer chromatography of the crude algae extracts confirmed the presence of fucoxanthin in the marine algae. Given these findings, the optimized extraction process holds the potential for scaling up to large-scale fucoxanthin production. Fucoxanthin, as a potent pharmacological agent, offers promising applications in the treatment of various ailments.

Keywords: Marine algae; Fucoxanthin; Ultrasonics; Extraction; Optimization; TLC; DPPH; Antimicrobial antioxidant activities

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1. Introduction

The marine environment is a rich source of bioactive compounds, and marine algae, also known as macroalgae, hold great potential as a renewable resource for these valuable substances. Algae, which are photosynthetic organisms, form the foundational biomass of the intertidal zone [1]. Approximately 6,000 species of algae have been identified and classified into three major groups: green algae (chlorophytes), brown algae (phaeophytes), and red algae (rhodophytes). These classifications are based on the algae's nutrient content, pigments, and chemical composition [2,3]. Like terrestrial plants, algae contain numerous inorganic and organic compounds with the potential to benefit human health. Due to their ability to produce a wide range of secondary metabolites with diverse biological activities, algae are an excellent reservoir of bioactive compounds [4,5]. Bioactive compounds with antioxidant, antiviral, antifungal, and antibacterial properties can be found in brown, red, and green algae [6,7].

Although algae generally have lower lipid content compared to microalgae, lipids from brown algae are particularly noteworthy as they contain a variety of bioactive substances, including omega-3 polyunsaturated fats (PUFAs), fucoxanthin, fucosterols, and polyphenols [3,8,9]. Fucoxanthin, a key nutraceutical derived from brown algae, stands out due to its unique physiological effects stemming from a distinctive molecular mechanism [10]. Found in the chloroplasts of brown algae, fucoxanthin is a major carotenoid, accounting for over 10% of the estimated global carotenoid production, making it the most abundant carotenoid in these organisms [11]. Its complex formation with chlorophyll protein plays a crucial role in capturing and protecting light, ensuring efficient energy utilization, and regulating photosynthesis.

The unique structure of fucoxanthin, characterized by an allenic bond and a notable 5,6-monoepoxide linkage, is rare among natural carotenoids, with only about 40 out of 700 naturally occurring carotenoids possessing this feature. Fucoxanthin from brown algae, peridinin from microalgae, neoxanthin from higher plants, and fucoxanthin metabolites such as fucoxanthinol and amarouciaxanthin are the primary allenic carotenoids. The unique allenic bond of fucoxanthin enables it to function as an antioxidant even in hypoxic conditions, where other carotenoids may fail to counteract oxidative stress—an uncommon feature in natural food products. Fucoxanthin offers a wide range of health benefits, including anti-cancer, anti-inflammatory, antioxidant, and anti-obesity properties. It has demonstrated a potent inhibitory effect on prostate cancer, likely due to its antioxidant activity and its impact on apoptosis-related biomolecules. Furthermore, fucoxanthin has shown greater efficacy against human colon cancer cells than β-carotene and astaxanthin, highlighting its enhanced biological activity.

Given these promising effects, fucoxanthin has significant potential for commercial applications. In addition to its anti-obesity properties, it may reduce the risk of disorders such as type 2 diabetes by facilitating the removal of misfolded proteins. Fucoxanthin also has the potential to protect the liver and blood vessels, and it offers benefits for the brain, bones, skin, and eyes [12]. Although brown seaweed contains 3–10 g/kg of fucoxanthin, the chemical synthesis of the compound is challenging due to its high costs and low recovery rates.

Additionally, the occurrence of algal blooms can disrupt ecosystems by altering food chains and wildlife communities. Utilizing this biomass for fucoxanthin extraction not only provides a source of the compound but also helps mitigate environmental damage [13-15].

This preliminary study aimed to investigate and evaluate the fucoxanthin content of *Padina australis*. This research focused on optimizing the extraction process to maximize the yield of this bioactive compound from macroalga.

2. Materials and methods

2.1. Preparation of the sample

To remove contaminants such as sand, gravel, and mud, samples were first washed with fresh water and then

drained. All samples were transported to the laboratory for further examination after being wrapped in black plastic bags and stored in a cold box. The extraction of bioactive molecules from seaweeds followed previously reported protocols, which were also adopted for this optimization study. The methods used are outlined below.

2.1.1. Method 1

This methodology was based on a proposed extraction procedure for *Padina australis* algae specimens. The specimens were washed, dried, ground into powder, and subjected to extraction using methanol, chloroform, and a methanol-chloroform mixture. These extractions produced extracts representing the polar, non-polar, and low-polarity components of the algae. The extracts were then combined, concentrated under reduced pressure, and the fractions evaporated to dryness. The weight of the crude extract from each sample was recorded.

2.1.2. Method 2

In this approach, a modified version of a previous study was used to extract and purify fucoxanthin from *Padina australis* algae. The algae were collected, cleaned, shade-dried, ground into powder, and then used for further experiments. A mixture of acetone and methanol was added to the dried and powdered algae, homogenized, and filtered. This process was repeated three times. The resulting extracts were combined and evaporated, and the residue was dissolved in methanol.

2.1.3. Method 3

In this extraction procedure for marine algae *Padina australis*, the samples were washed, freeze-dried, and blended. The samples were then ground into powder and treated with 80% methanol for 24 hours at room temperature. The methanolic extracts were filtered and dried under a vacuum. Proper washing and handling of the samples were critical steps in this extraction process.

2.1.4. Ultrasonics-based method

Cell disruption is a crucial step in recovering intracellular components from biomass, improving the bioavailability and absorption of pigments within cells. In a study focusing on the marine macroalga *Padina australis*, ultrasonication of dried and powdered samples was employed to enhance fucoxanthin extraction efficiency. The application of ultrasound was found to induce acoustic cavitation in the solvent, facilitating deeper penetration of the solvent into the tissue. Additionally, ultrasound increased the interface area between the solid and liquid phases, promoting rapid diffusion of solutes from the solid phase into the solvent. Three different extraction techniques utilizing ultrasonication were employed for fucoxanthin extraction. In this method, 1 g of dried powdered macroalgae was placed in a glass flask with a solid-to-solvent ratio of 1:10. The mixture was stirred with a magnetic stirrer and subjected to ultrasound-assisted extraction in a sonication water bath. The ultrasound parameters were set to a fixed frequency of 40 kHz and a power of 250 W. The extract was then filtered and analyzed to determine the fucoxanthin content [16].

2.2. Optimization of extraction parameters

Several factors can affect fucoxanthin extraction; however, six parameters were chosen and optimized to increase the extraction yield. The parameters selected for optimization were the solid-to-solvent ratio, temperature, time, mixing speed, moisture content, and particle size.

Table 1. Ranges of extraction parameters for fucoxanthin optimization

Extraction parameters	Range of the parameters
Moisture content (%)	2.08–9.82
Particle size (mm)	0.246–0.053
Mixing speed (rpm)	200–800
Extraction temperature (˚C)	35–65
Extraction time (min)	10–70
Solid-to-solvent ratio	1:2–1:14

2.2.1. Moisture content

Padina australis was thoroughly washed with fresh water, transported to the laboratory in iced conditions, and shade-dried (38 ± 2°C) for about 20 hours. The moisture content of the samples varied between 2.08% and 9.82%. The moisture content was determined using the following equation:

$$Moisture\ content\ (\%) = \frac{M_i - M_f}{M_i} \times 100$$

where M_i is the initial weight of the sample (g), and M_f is the final weight after drying (g). The dried samples, with specific moisture contents, were extracted using ultrasonication and a methanol-chloroform (1:2) solvent system. The sample was stirred for 30 minutes at 400 rpm, and ultrasound-assisted extraction was performed in a sonication bath. The extraction temperature was maintained at 40°C. The extracts were then evaporated under reduced pressure using a rotary evaporator and analyzed.

2.2.2. Particle size

The dried sample with the optimal moisture content, determined from the above procedure, was ground into powders with particle sizes ranging from 0.246 mm to 0.053 mm. By holding the other parameters constant, the same procedure was used to extract fucoxanthin from these samples.

2.2.3. Mixing speed

The dried sample with optimal moisture content and particle size was used for fucoxanthin extraction. The same protocol was followed, but mixing was performed at varying speeds (200–800 rpm). After extraction, the fucoxanthin content was analyzed.

2.2.4. Extraction temperature

Following the previous procedure, the dried sample with an optimal moisture content of 8.17% and particle size of 0.063 mm was used for fucoxanthin extraction. Ultrasonication was performed using a 1:1 methanol-chloroform solvent mixture and a mixing speed of 700 rpm. The extraction temperature was varied from 35°C to 65°C while maintaining the extraction time at 30 minutes with a solid-to-solvent ratio of 1:10.

2.2.5. Extraction time

Extraction time is a key parameter for fucoxanthin extraction from *Padina australis*. It helps determine the optimal duration for the extraction process. The dried sample with optimal moisture content, particle size, mixing intensity, and temperature was used for fucoxanthin extraction. Extraction times varied from 10 to 70 minutes, after which the extracts were analyzed.

2.2.6. Solid-to-solvent ratio

The solid-to-solvent ratio for fucoxanthin extraction was varied between 1:12 and 1:14 while maintaining the other parameters at their optimal levels: moisture content at 8.17%, particle size at 0.063 mm, mixing speed at 700 rpm, extraction temperature at 60°C, and extraction time at 60 minutes.

2.3. Analysis of fucoxanthin extract

Fucoxanthin extracted from *Padina australis* was analyzed using Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectroscopy, and high-performance liquid chromatography (HPLC) techniques. A modified version of the Wright *et al*. [17] HPLC method was employed, with solvent gradients consisting of methanol (A), acetonitrile (B), and pure ethyl acetate (C). A linear solvent gradient program was used (**Table 2**). The solvents were degassed using a sonicator and filtered through a 0.25 µm filter before use. The injection volume was 20 µL, and the flow rate was 1 mL/min. All samples were dissolved in a 1:1:2 methanol solution. Separation was carried out at room temperature using a C18 column, and fucoxanthin was detected using a UV-VIS detector at 450 nm.

Table 2. Gradient program for the separation of brown seaweed pigments

Time (min)	Flow rate (mL/min)	A (%)	B (%)	C (%)
0–10	1	80	10	10
10–25	1	80	16	4

Abbreviation: A, methanol; B, acetonitrile; C, pure ethyl acetate.

2.4. Bioactivity studies on fucoxanthin applications

2.4.1. Antimicrobial activity of fucoxanthin

A well-diffusion assay was conducted. After autoclaving, 1% of 24-hour cultures of indicator organisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhi*) were added to nutrient agar medium, cooled to 40°C, and aseptically poured into sterile Petri dishes (100 × 15 mm). Each plate was left to solidify for three hours, after which wells were bored into the agar. Different concentrations (250 µg, 500 µg, 750 µg) of crude fucoxanthin extract were added to each well. The plates were incubated at 37°C for 24 hours, and the diameters of inhibition zones around the wells were measured horizontally and vertically and averaged.

2.5. Qualitative analysis of antioxidant activity

2.5.1. Thin layer chromatography (TLC)

Plant extracts were loaded onto pre-coated silica plates and developed using a methanol-chloroform solvent system (0.75:9.25 ratio). Spots were visualized under iodine vapor, far light, and UV light. The Rf value was calculated as the ratio of the solute's travel distance to the solvent's travel distance.

2.5.2. TLC bioautography for antioxidant screening

Plant extracts were applied to thin-layer chromatography sheets, developed, and allowed to dry. The plates were sprayed with a 0.2% DPPH solution in methanol or ethanol, left to sit at room temperature for 30 minutes, and observed under white light. Antioxidant activity was indicated by a color change from purple (DPPH) to yellow.

2.6. Antioxidant assay for DPPH radical scavenging activity

The effect of fucoxanthin extract from *Padina australis* on DPPH radicals was assessed. Fucoxanthin extracts (10, 20, 50 µg) were added to 4 mL of distilled water and combined with 1 ml of methanolic DPPH solution.

After one minute of vortexing, the mixture was left to stand at room temperature in the dark for 30 minutes. Absorbance was measured at 517 nm. The scavenging effect was calculated using the formula:

$$Scavenging\ effect\ (\%) = [1 - \frac{A_{sample} - A_{blank}}{A_{control}}] \times 100$$

where $A_{control}$ is the absorbance of the control (DPPH solution without sample), A_{sample} is the absorbance of the test sample (DPPH solution plus test sample), and A_{blank} is the absorbance of the sample alone (without DPPH solution) [18].

3. Results

Various extraction methodologies and the influence of solvents on the effective isolation of fucoxanthin from algae have been reported and compared. Methanol, chloroform, and a methanol-chloroform combination (1:1 v/v) followed by chloroform resulted in a fucoxanthin yield of 8.12 mg per gram of biomass using Method 1 for fucoxanthin extraction from *Padina australis*. The application of an ultrasonic-assisted approach led to a fucoxanthin yield of 16.9 mg per gram of biomass, demonstrating that ultrasonic assistance enhances extraction efficiency compared to the non-ultrasonic method. Hence, the effective separation of fucoxanthin from the marine macroalga *Padina australis* is contingent upon ultrasonic-assisted extraction [16]. When compared to other techniques, ultrasound-assisted extraction of fucoxanthin from *Padina australis* produced a greater yield. The optimal technique demonstrates the potential for process development and scaling up for fucoxanthin extraction from seaweeds. This bioactive compound can be utilized to treat cancer by inducing apoptosis in various cancer cell types, exhibiting anti-tumor properties, inhibiting cancer cell invasion, and blocking enzyme activity in cancer cells.

3.1. Optimization of parameters influencing extraction

3.1.1. Effect of moisture content

Moisture content is a crucial factor in fucoxanthin extraction. **Figure 1** illustrates how moisture levels affect the extraction of fucoxanthin, with a range of 2.08% to 9.82% moisture content tested. By holding other parameters constant—such as particle size (0.147 mm), stirrer speed (400 rpm), extraction temperature (40°C), extraction time (30 minutes), and solid-to-solvent ratio (1:10)—the impact of moisture content on fucoxanthin extraction from *Padina australis* was investigated. Based on **Figure 1**, it can be deduced that fucoxanthin extraction increases from 2.08% to 8.17% moisture content, then gradually decreases as moisture content continues to rise.

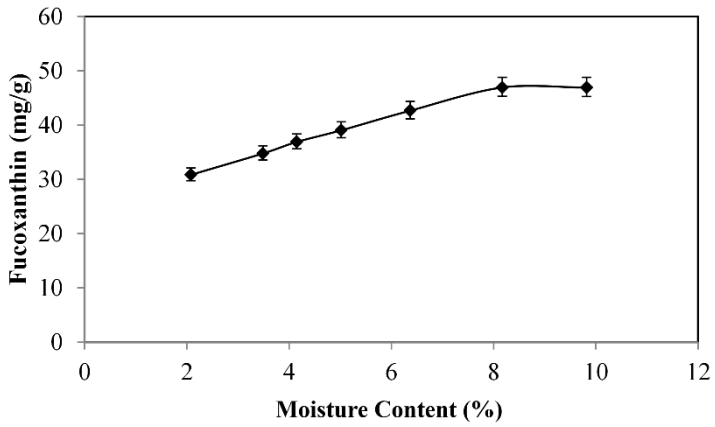

Figure 1. Effect of moisture content on fucoxanthin extraction

3.1.2. Effect of particle size

Particle size significantly affects fucoxanthin extraction from *Padina australis*. Smaller biomass particles provide a greater interfacial area between the solid and liquid phases, leading to higher fucoxanthin yield. Maximum extraction was achieved with a particle size of 0.063 mm and optimal moisture content of 8.17% at 40°C, 400 rpm, and a solid-to-solvent ratio of 1:10 for 30 minutes. **Figure 2** shows that the fucoxanthin yield gradually increased as particle size decreased from 0.246 mm to 0.063 mm. However, further reduction in particle size did not significantly improve fucoxanthin extraction.

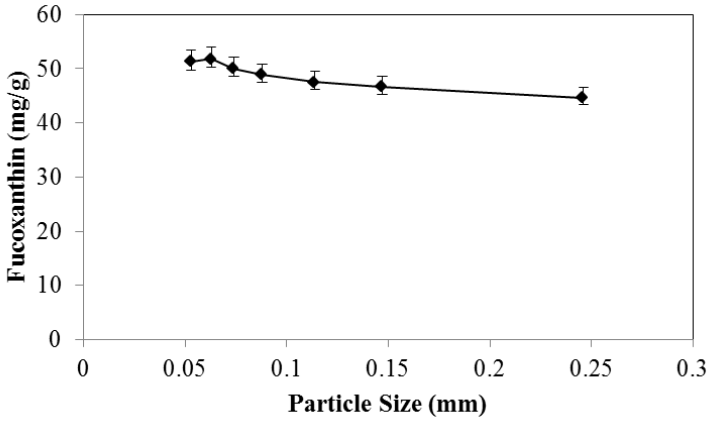

Figure 2. Effect of particle size on fucoxanthin extraction

3.1.3. Effect of mixing speed

Mixing intensity is a key factor in fucoxanthin extraction. **Figure 3** shows how stirrer speed affects extraction, with speeds ranging from 200 to 800 rpm. It was discovered that increasing the stirrer speed from 200 to 700 rpm enhanced fucoxanthin extraction, with the highest yield achieved at 700 rpm. However, speeds above 700 rpm did not significantly increase extraction efficiency.

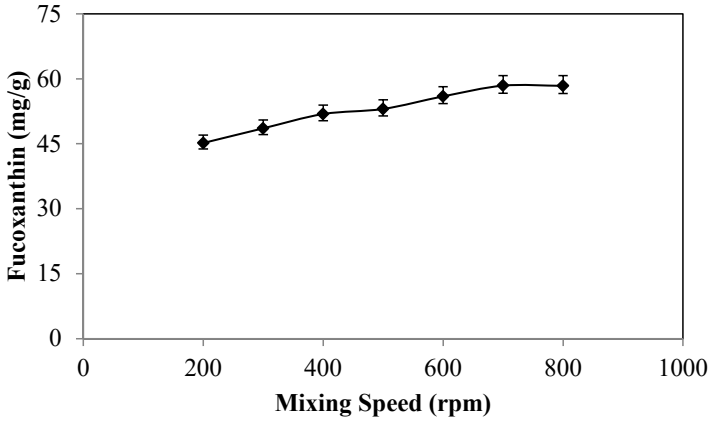

Figure 3. Effect of mixing speed on fucoxanthin extraction

3.1.4. Effect of extraction temperature

Temperature is one of the most crucial factors in fucoxanthin extraction. **Figure 4** illustrates the effect of temperature on extraction from *Padina australis*, tested within a range of 35°C to 65°C. By keeping other parameters constant at optimal levels (moisture content: 8.17%, particle size: 0.063 mm, stirrer speed: 700 rpm) and using a solid-to-solvent ratio of 1:10 for 30 minutes, the impact of temperature was investigated. It was

found that fucoxanthin extraction increased as the temperature rose, due to the improved dissolution capacity of the solvent system. According to **Figure 4**, extraction efficiency increased as temperature rose from 35°C to 60°C, then gradually decreased with further temperature increases.

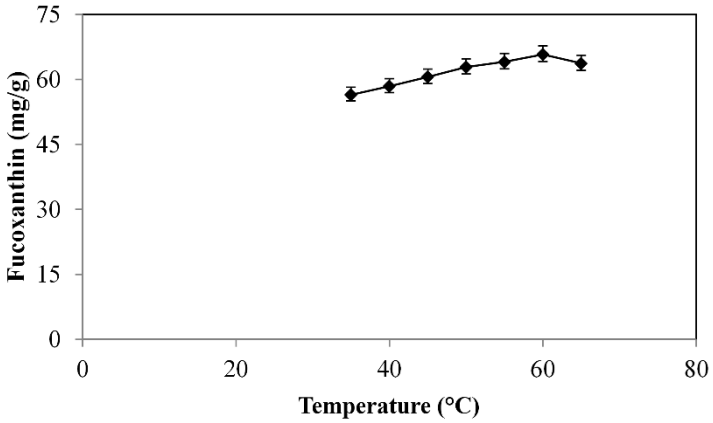

Figure 4. Effect of extraction temperature on fucoxanthin extraction

3.1.5. Effect of extraction time

The effect of extraction time was examined using intervals ranging from 10 to 70 minutes (**Figure 5**). The findings showed that fucoxanthin extraction increased over time, reaching its peak at 60 minutes under optimal conditions (8.17% moisture content, 0.063 mm particle size, 700 rpm stirrer speed, and 60°C temperature). Extending the extraction time beyond 60 minutes to 70 minutes did not significantly improve the yield. Thus, the optimal extraction time was determined to be 60 minutes.

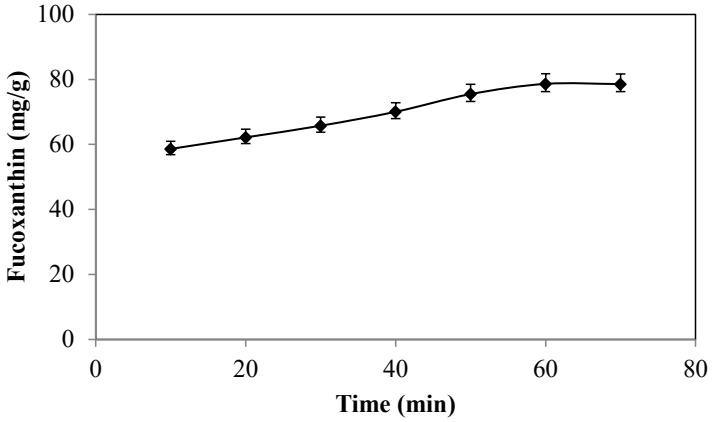

Figure 5. Effect of extraction time on fucoxanthin extraction

3.1.6. Effect of solid-to-solvent ratio

The solid-to-solvent ratio is another crucial element in the extraction process. A larger solvent volume can improve extraction yield by more efficiently dissolving the target components, but excessive solvent use leads to waste. Conversely, a lower solvent volume results in lower yields. **Figure 6** illustrates the effect of the solid-to-solvent ratio on fucoxanthin extraction. By keeping all other parameters at optimal levels, the impact of ratios ranging from 1:2 to 1:14 was investigated. The fucoxanthin yield increased as the solid-to-solvent ratio rose from 1:2 to 1:14, but further increases beyond a ratio of 1:12 did not significantly improve extraction. Thus, the optimal solid-to-solvent ratio of 1:12 was selected for process scale-up.

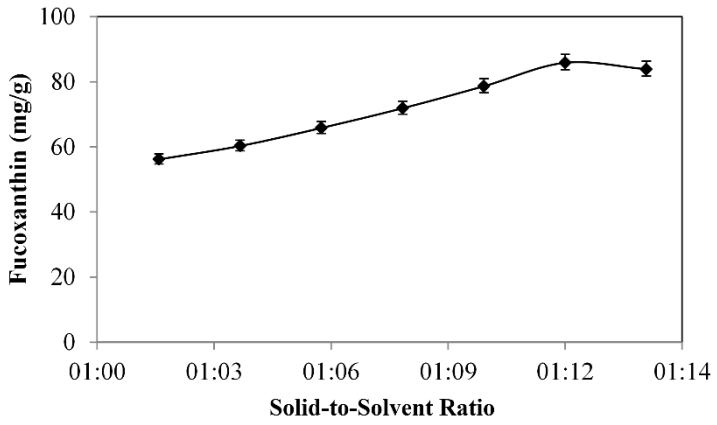

Figure 6. Effect of solid-to-solvent ratio on fucoxanthin extraction

3.2. Characterization of fucoxanthin

3.2.1. Fourier transform infrared (FTIR) analysis

FTIR analysis was conducted on the fucoxanthin extract from Padina australis to identify the associated functional groups (**Figure 7**). The FTIR spectrum of the sample was compared with that of standard gallic acid. The FTIR spectrum of the fucoxanthin extract showed the same number of peaks, ranging between 3904.5 cm^{-1} and 1108.2 cm^{-1}.

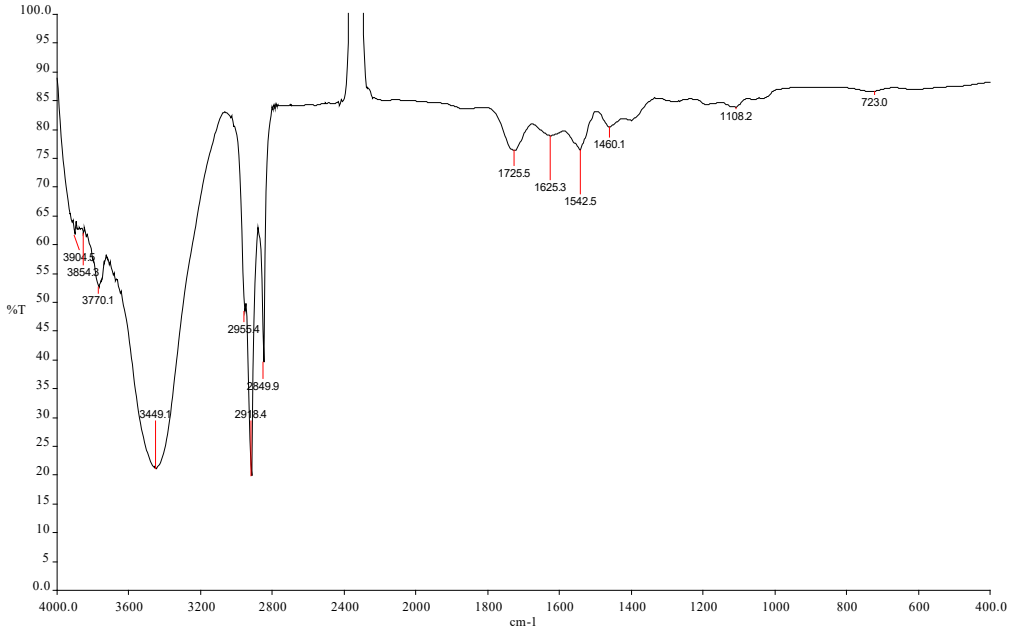

Figure 7. FTIR analysis of fucoxanthin

Table 3. FTIR spectral analysis of fucoxanthin extract

Frequency range (cm^{-1})	Bond	Compound type
3600–3200	O-H	Hydrogen bonded – Alcohols, Phenols (stretch)
2950–2850	C-H	Alkanes (stretch)
1725–1640	C-H	Phenyl ring substitution
1470–1350	C-H	Alkanes (bending)
1110–1000	C-O	Alcohols, Ethers, Esters (stretch)

3.2.2. Nuclear magnetic resonance spectroscopy

Fucoxanthin was characterized using NMR spectroscopy (**Figure 8**). Based on the NMR spectral analysis, the fucoxanthin extract was identified as all-trans fucoxanthin because its NMR spectral data matched those of an authentic all-trans fucoxanthin compound, as reported by Englert *et al*. [19].

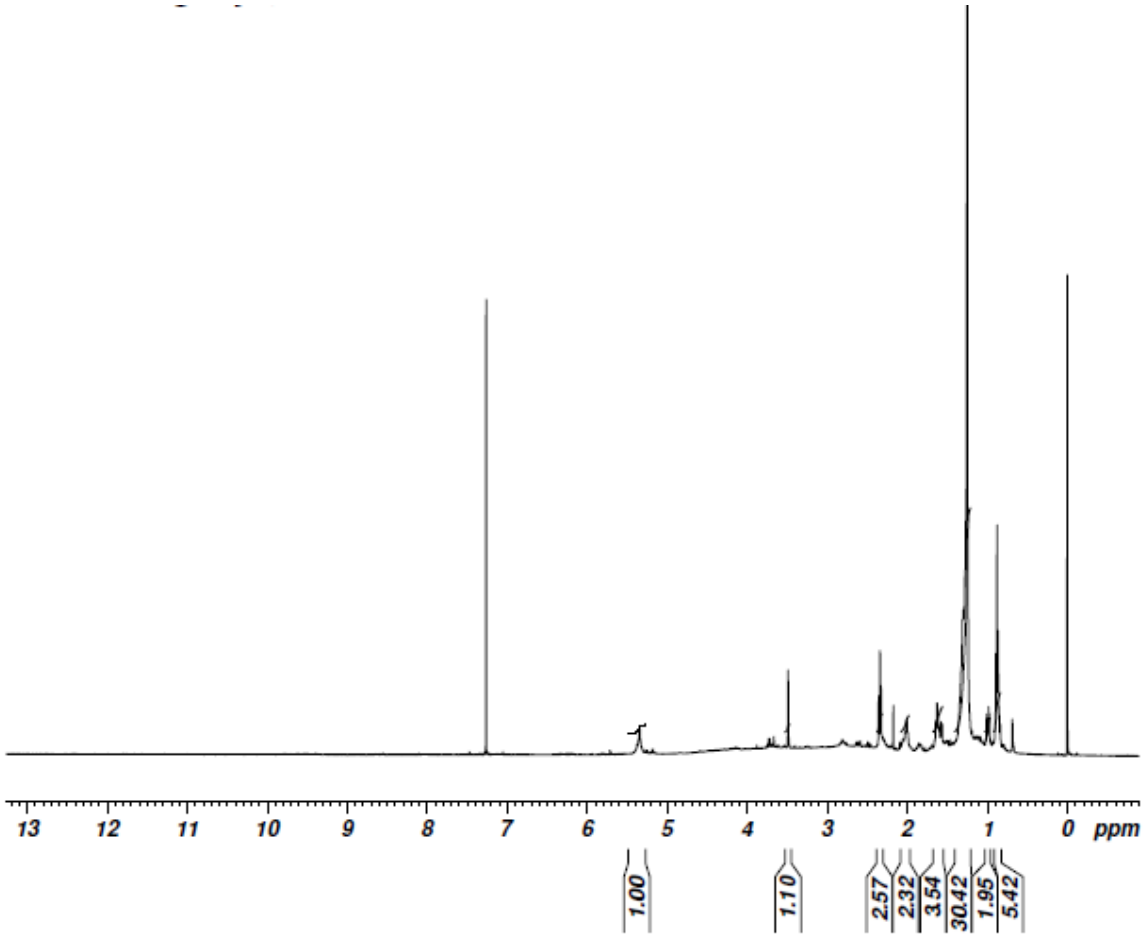

Figure 8. NMR spectral analysis of fucoxanthin

Table 4. NMR results of fucoxanthin extract

No.	Functional group	Peaks (ppm)
1	-(CH_3-C) – terminal methyl group	0.866 – 1.009
2	-$(CH_2)_n$ – backbone CH_2	1.254 – 1.312
3	-(CH_2CH_2COOH) – β methylene proton	1.566 – 1.645
4	-(CH_2COOH) – α methylene group to acid	2.004 – 2.016
5	-(CH=CH) – olefinic proton	5.341 – 5.351

3.2.3. HPLC analysis of fucoxanthin extract

The fucoxanthin extract from *Padina australis* was analyzed using HPLC (**Figure 9**). The HPLC chromatograms of the crude pigment extract were detected at 450 nm. Two peaks were observed at retention times of 10.08 and 17.22 minutes, which were consistent with the standard fucoxanthin reported in the literature.

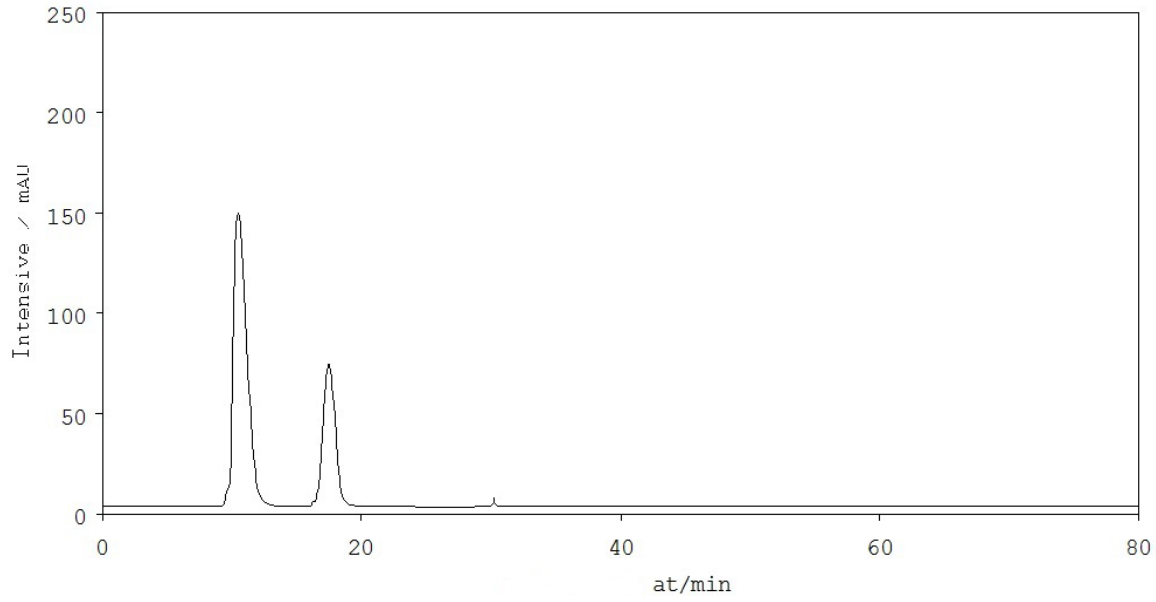

Figure 9. HPLC analysis of fucoxanthin extract

3.3. Studies on the application of fucoxanthin

3.3.1. Determination of antimicrobial activity using well diffusion assay

The extract obtained from *Padina australis* was investigated for its antimicrobial activity. Concentrations of 250 µg, 500 µg, and 750 µg of the algae extract inhibited the growth of microorganisms. The fucoxanthin extracts from *Padina australis* were particularly effective against Gram-positive bacteria, especially *B. subtilis* and *S. aureus*, as well as Gram-negative bacteria, especially *E. coli* and *S. typhi*. The results indicate that the algal extracts are generally more effective against Gram-positive than Gram-negative bacteria (**Figure 10**), likely due to the more complex structure of the Gram-negative bacterial cell wall.

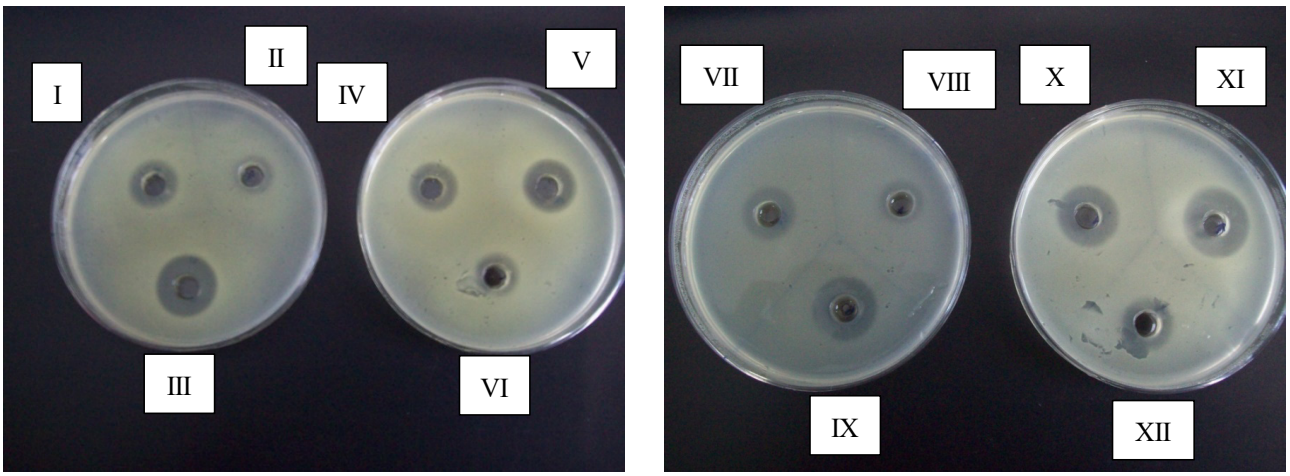

Figure 10. Well diffusion assay

Table 5. Zone of inhibition against different microorganisms

No.	Microorganism	Extract concentration	Inhibition zone (mm)
I	*Bacillus subtilis*	250 µg	12
II	*Bacillus subtilis*	500 µg	17
III	*Bacillus subtilis*	750 µg	18
IV	*Staphylococcus aureus*	250 µg	12
V	*Staphylococcus aureus*	500 µg	16
VI	*Staphylococcus aureus*	750 µg	20
VII	*Escherichia.coli*	250 µg	12
VIII	*Escherichia.coli*	500 µg	16
IX	*Escherichia.coli*	750 µg	18
X	*Salmonella typhi*	250 µg	15
XI	*Salmonella typhi*	500 µg	20
XII	*Salmonella typhi*	750 µg	22

3.4. TLC bioautography for antioxidant property screening

In this study, compounds in the selected crude extract were first separated using analytical TLC. When the chromatographic profile of the crude extract was viewed under light, it revealed three vibrant bands on the TLC plate (**Figure 11 left**). Fucoxanthin was identified as the primary colorful pigment in *Padina australis*, suggesting that this pigment may be responsible for the brown and olive-green bands observed in the TLC. The bands were visible on the analytical TLC at Rf = 0.91, 0.85, and 0.74. TLC bioautography was also used to assess the potential biological properties of the separated compounds. To determine antioxidant capacity, the developed plate was sprayed with DPPH˙ reagent. The results showed that only band 2 (Rf = 0.85), which turned yellow against a purple background, exhibited antioxidant capacity (**Figure 11 right**). The intensity of the yellow color indicates the quantity and type of radical scavengers present in the sample.

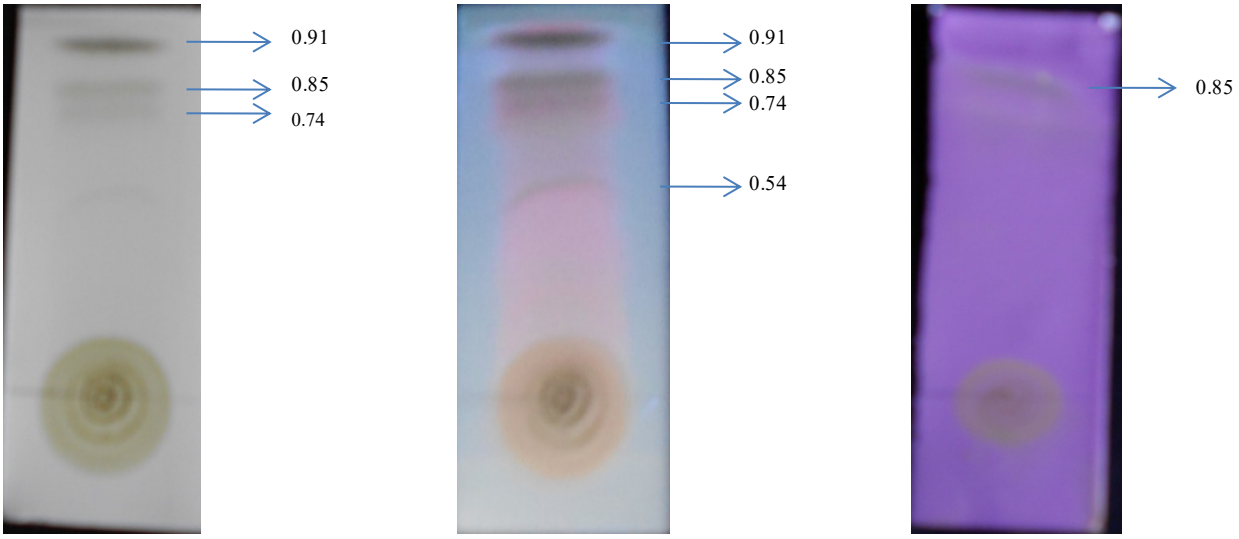

Figure 11. TLC bioautography under white light **(left)**, under UV **(middle)**, and sprayed with 0.2% DPPH **(right)**

3.5. DPPH radical scavenging activity

DPPH is a useful reagent for examining a compound's free radical scavenging ability. In the DPPH test, the extracts were able to convert the stable radical DPPH from purple color to yellow color when neutralized. The method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant, resulting in the formation of the non-radical DPPH-H. The crude extract of *Padina australis* demonstrated DPPH radical scavenging activity in a concentration-dependent manner.

Table 6. Percentage of antioxidant activity

Extract concentration (µg)	Antioxidant activity (%)
100	9.08
200	14.36
300	17.68

4. Discussion

A method for preparing fucoxanthin from leftover Kombu (*Laminaria japonica*) waste parts was reported by Kazuki *et al*. in 2008. The analysis of Kombu waste components aimed to produce high-quality fucoxanthin. Temperature enhanced fucoxanthin recovery, and further washing with tap water reduced the extract's salt content. After two extractions with three volumes of absolute ethanol, Kombu showed the highest fucoxanthin recovery and the lowest salt content. The extract was subjected to silica gel column chromatography to remove chlorophylls, ultimately yielding 1,490 g of fucoxanthin with an 82% recovery rate from 10 t of Kombu waste. After six months of storage at 4°C, the obtained fucoxanthin remained stable, with only a 2% reduction [20]. Therefore, leftover Kombu culture material serves as a valuable bioresource for fucoxanthin production.

In 2011, Noviendri *et al*. isolated and purified fucoxanthin from two species of brown seaweed in Malaysia: *Sargassum binderi* and *Sargassum duplicatum*. HPLC analysis confirmed that fucoxanthin had a purity level of >99%. The total lipid and fucoxanthin contents, along with the fatty acid composition of the seaweeds, indicated that both samples contained significant amounts of these components. *S. duplicatum* had a fucoxanthin content of 21.3 ± 0.10 mg/g dry-weight and a total lipid content of 1.01 ± 0.10 mg/g, while *S. binderi* had lower values of 16.6 ± 4.10 mg/g for total lipids and 0.73 ± 0.39 mg/g for fucoxanthin. Both seaweed varieties also contained significant levels of unsaturated fatty acids. Notably, *S. duplicatum* showed higher concentrations of docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, linoleic acid, and alpha-linolenic acid (0.76, 2.55, 13.64, 5.81, and 5.35%, respectively) than *S. binderi* (0.70, 1.82, 9.13, 6.37, and 4.39%, respectively). Palmitic acid (C16:0) was found to be the dominant saturated fatty acid in both samples [21].

Shang *et al*. found that fucoxanthin extraction efficiency was significantly influenced by temperature and ethanol concentration. The maximum predicted fucoxanthin extraction was 0.42 mg/g at 110°C and 90% ethanol [22]. Takeshi *et al*. explored the possibility of producing a high-purity fucoxanthin-rich product using *Cladosiphon okamuranus* as the raw material. Drying and pulverization techniques were optimized to reduce fucoxanthin degradation. The results revealed that 50 µm freeze-dried powder exhibited the best qualities. Additionally, the algal extract demonstrated strong DPPH radical scavenging activity [23].

A study was conducted to examine the antioxidant properties of three specific types of Indian brown seaweeds: *Sargassum marginatum*, *Padina tetrastomatica*, and *Turbinaria conoides*. The total phenolic content and reducing power of crude methanolic extracts were analyzed [18]. The total antioxidant activity, DPPH radical

scavenging ability, and deoxyribose assay were evaluated for total methanolic extract and five distinct fractions: petroleum ether, ethyl acetate, dichloromethane, butanol, and aqueous. Among these, the ethyl acetate fraction of *S. marginatum* showed significantly higher total antioxidant activity (39.62 mg ascorbic acid equivalent per gram of extract or 0.31 mg ascorbic acid equivalent per gram of seaweed on a dry weight basis). This fraction also exhibited superior DPPH scavenging activity (23.16%), while the petroleum ether fraction of *T. conoides* showed lower scavenging activity. The deoxyribose activity of *T. conoides* was 47.81%, lower than the other samples. The aqueous fraction of *T. conoides* exhibited the highest phenolic content (49.16 mg gallic acid equivalent (GAE) per gram of extract or 0.86 mg GAE per gram of seaweed on a dry weight basis). The *in vitro* antioxidant activity of the methanolic extracts increased with concentration, suggesting a dose-dependent antioxidant property.

In another study by Hii *et al*. [24], the stability of fucoxanthin extracted from *S. binderi* was assessed under various storage conditions. The stability was tested across different pH levels, with and without the addition of the antioxidant ascorbic acid, under both light and dark conditions over four weeks. Fucoxanthin exhibited the highest stability in a dark environment. Stability was also greater in an alkaline pH environment compared to neutral or acidic conditions. The addition of 1.0% w/v ascorbic acid further delayed fucoxanthin degradation, particularly under dark conditions, with the pigments showing a high retention rate. In conclusion, fucoxanthin was sensitive to light exposure and acidic pH conditions, but ascorbic acid was effective in stabilizing the pigments.

Algal blooms pose significant threats to ecosystems, disrupting food chains and altering faunal communities. Taylor *et al*. noted that increased algal biomass displaces natural seagrass and higher plant communities [25]. Similarly, macroalgal blooms impact the biogeochemical cycles of carbon, nitrogen, phosphorus, and sulfur [26]. The macroalgal biomass requires attention for cleanup, making it a useful resource for fucoxanthin extraction. According to Zailanie *et al*., *P. australis* and *T. conoides* are rich sources of fucoxanthin, making them promising candidates for cultivation as edible brown seaweed [27]. Roh *et al*. extracted fucoxanthin from *Undaria pinnatifida* using supercritical carbon dioxide (SCO_2) and ethanol as a co-solvent. The ethanol flow rate was 3.0% (v/v) compared to the SCO_2 flow rate [28].

The utilization of marine macroalgae for fucoxanthin extraction offers dual benefits. It serves as a source for fucoxanthin while mitigating environmental impacts. Therefore, the primary aim of this study was to examine and evaluate the levels of fucoxanthin in *P. australis*, *L. japonica*, and *U. pinnatifida*, while exploring their characteristics and potential pharmacological effects.

5. Conclusion

To conclude, the utilization of ultrasound-assisted extraction resulted in a higher yield of fucoxanthin from the marine macroalga Padina australis, surpassing the results of other studied extraction techniques. Padina australis demonstrated a higher fucoxanthin content compared to Laminaria japonica and Undaria pinnatifida. The highest yield of fucoxanthin was obtained using a methanol and chloroform mixture in a 1:2 ratio. Under optimal conditions, the maximum fucoxanthin content reached 85.85 mg/g. These conditions included a moisture content of 8.17%, a particle size of 0.063 mm, a mixing intensity of 700 rpm, an extraction temperature of 60℃, an extraction time of 60 minutes, and a solid-to-solvent ratio of 1:12. The extracted fucoxanthin was analyzed through agar well diffusion and DPPH assays, revealing its antimicrobial and antioxidant properties. This confirms the pharmacological potential of fucoxanthin and its suitability for medicinal applications.

Disclosure statement

The authors declare no conflict of interest.

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