

Validated TLC–Densitometric and UV–Vis Methods for Fucoxanthin Determination in *Sargassum ilicifolium*

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ABSTRACT

Fucoxanthin, the principal carotenoid pigment in *Sargassum ilicifolium*, possesses a distinct orange hue that makes it a promising natural compound for pharmaceutical, cosmetic, and food applications. This study aimed to develop and validate simple, accurate, and cost-effective analytical methods for fucoxanthin determination using UV–Vis spectrophotometry and TLC–densitometry. The measurements were conducted at wavelengths of 445 nm (UV–Vis) and 366 nm (TLC–densitometry). The methods demonstrated excellent linearity with correlation coefficients (r) of 0.9981 and 0.9887 within concentration ranges of 0.25–2.5 $\mu\text{g}/\text{mL}$ and 0.5–40 $\mu\text{g}/\text{mL}$, respectively. The limits of detection (LOD) and quantification (LOQ) were 0.17 $\mu\text{g}/\text{mL}$ and 0.52 $\mu\text{g}/\text{mL}$ for UV–Vis, and 2.5 $\mu\text{g}/\text{mL}$ and 7.5 $\mu\text{g}/\text{mL}$ for TLC–densitometry. Recovery values ranged from 95.07–98.06% (UV–Vis) and 100.50–102.00% (TLC–densitometry), with RSD values below 1%, fulfilling ICH and AOAC validation criteria. These validated methods provide reliable and accessible alternatives to advanced instrumentation, supporting fucoxanthin analysis in phytopharmaceutical research and quality control.



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Keywords:

Fucoxanthin; *Sargassum ilicifolium*; Method validation, UV–Vis Spectrophotometry; TLC–densitometry

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1. Introduction

Fucoxanthin is a bioactive compound in *Sargassum* sp. with numerous biological activities [1],[2],[3],[4]. It has been widely reported for its immunomodulatory, antibacterial, anti-inflammatory, anticancer, and antioxidant properties [5],[6],[7],[8]. In addition, fucoxanthin exhibits a distinct orange-brown color that makes it a promising natural pigment for use in medicines, cosmetics, food, and beverages. Synthetic

colorants commonly used in these industries may cause adverse health effects when consumed or applied beyond safe limits [9],[10]. Therefore, fucoxanthin has gained significant attention as a safer natural alternative.

Several analytical techniques have been developed to determine fucoxanthin levels in *Sargassum* species, particularly using UV-Vis spectrophotometry and TLC-densitometry, which are widely applied in pharmaceutical and phytochemical analyses due to their simplicity, cost-effectiveness, and rapid operation. Meanwhile, high-performance analytical instruments such as HPLC and LC-MS have also been used to analyze fucoxanthin [11],[12],[13]. Although these methods provide high sensitivity and specificity, they require expensive instrumentation, complex sample preparation, and high operational costs.

Mawaddah et al. (2017) developed a method for fucoxanthin analysis using maceration extraction with 96% ethanol followed by HPLC analysis [14]. Extraction by maceration has also been performed using acetone-methanol solvents (3:7, % v/v) followed by fractionation with *n*-hexane-ethyl acetate-methanol (6:3:1, % v/v), and the fucoxanthin content was determined using UV-Vis spectrophotometry at 448.2 nm in *Sargassum granuliferum*, *Sargassum polycystum*, and *Cystoseira trinodia* [15]. Lim et al. (2024) analyzed fucoxanthin using methanol-chloroform-water (4:2:2, % v/v) and determined the content spectrophotometrically at 420 and 672 nm [16]. Sodik et al. (2022) also extracted fucoxanthin with 96% ethanol followed by fractionation using *n*-hexane-acetone (6:4, % v/v) and analyzed it using HPLC [17].

An optimized method for identifying fucoxanthin in *Sargassum ilicifolium* using TLC-densitometry has been reported and showed good separation from other matrix components. In general, analytical methods have been applied to the identification and characterization of isolated fucoxanthin. However, a validated method for the determination of fucoxanthin from *Sargassum ilicifolium* as a raw material to ensure the quality of extracted compounds has not yet been fully developed in accordance with the validation requirements of the International Conference on Harmonization (ICH) [18].

Therefore, this study aimed to develop and validate UV-Vis spectrophotometric and TLC-densitometric methods for the determination of fucoxanthin extracted from *Sargassum ilicifolium*. The application of these validated analytical methods is expected to provide valid, reliable, and accurate results that can be applied for fucoxanthin quantification and quality control in phytopharmaceutical research.

2. Methods

Materials and Reagents

Fucoxanthin standard ($\geq 99\%$, Sigma Aldrich), *n*-hexane, ethyl acetate, methanol, acetone, and ethanol 96% (all pro analysis grade, Merck) were used as solvents. TLC plates (silica gel 60 GF254) and silica gel GF25 (Merck) were employed for chromatographic separation. For sample preparation, standard laboratory glassware, a rotary evaporator (Heidolph), and glass columns were used. The instruments for fucoxanthin determination included a UV-Vis spectrophotometer (Agilent Cary 60) and a CAMAG TLC-densitometer.

Plant Material and Authentication

Sargassum ilicifolium was collected from Lampung, Indonesia. The sample was identified and authenticated under specimen number B-965/II.6.2/IR.01.02/4/2024 at the National Research and Innovation Agency (BRIN), Central Jakarta.

Extraction and Purification

Fresh *Sargassum ilicifolium* (harvested in 2024, Lampung) was washed, freeze-dried, ground, sieved (40-mesh), and weighed (200 g). The dried powder was extracted using 96% ethanol (1:10, % v/v) for 72 h and concentrated under reduced pressure at 40 °C using a rotary evaporator to obtain the crude extract. Purification of fucoxanthin was conducted by column chromatography using *n*-hexane-ethyl acetate-methanol (6:3:1, % v/v). Fractions (10 mL each) were collected and monitored by TLC using *n*-hexane-acetone (6:4, % v/v). Fractions exhibiting the same R_f as the fucoxanthin standard were combined and evaporated to dryness.

UV-Vis Spectrophotometric Conditions

The maximum absorption wavelength of fucoxanthin was determined by scanning standard and sample solutions from 200–800 nm. The absorbance of fucoxanthin fractions was measured at the determined maximum wavelength of 445 nm.

TLC-Densitometric Conditions

Chromatography was performed on silica gel 60 GF254 plates. Standard and sample solutions (8 µL) were applied as 2 mm bands spaced 8 mm apart and developed with *n*-hexane-acetone (6:4, % v/v). The chromatograms were scanned at 366 nm using a CAMAG TLC scanner to obtain the densitograms.

Method Validation (Selectivity, Linearity, Accuracy, Precision, LOD/LOQ)

The validation parameters were evaluated according to ICH Q2(R2) and AOAC guidelines [18],[19]. Selectivity was assessed by spiking standard fucoxanthin into the sample solution and examining potential interferences from other matrix components. In TLC-densitometry, selectivity was confirmed by ensuring sufficient separation ($R_s > 1.5$) between fucoxanthin and adjacent peaks. Linearity was determined by analyzing six concentrations of fucoxanthin within 0.25–2.5 µg/mL for UV-Vis and 2.5–40 µg/mL for TLC-densitometric methods. Calibration curves were plotted between concentration and response area. Accuracy was evaluated by recovery studies at three concentration levels (0.8, 1.0, and 1.2 µg/mL for UV-Vis; 5.3, 6.6, and 8 µg/mL for TLC-densitometry). Acceptable recovery was defined between 92–105%. Precision (repeatability) was determined by analyzing six replicate samples containing 100% fucoxanthin. Precision was expressed as the relative standard deviation (RSD) with a limit of $\leq 2\%$ [18],[19]. Limits of Detection (LOD) and Quantification (LOQ) were calculated using standard solutions in the concentration ranges of 0.625–2.5 µg/mL for the UV-Vis spectrophotometric method and 1–40 µg/mL for the TLC-densitometric method, following the procedures described by Kuczyńska et al., 2015 [20] and Wahyuningsih et al., 2024 [21]. The LOD and LOQ values were derived from the linear regression equations based on the slope and standard deviation of the response, in accordance with ICH Q2(R2) guidelines. All validation tests were conducted in triplicate, and data were statistically analyzed to confirm compliance with ICH Q2(R2) requirements.

3. Results and Discussion

Extraction and Purification

The extraction of fucoxanthin from *Sargassum ilicifolium* was carried out using 96% ethanol (1:10, % v/v) for 72 h, followed by concentration under reduced pressure at 40 °C to obtain the crude extract. The resulting extract was purified by column chromatography using *n*-hexane-ethyl acetate-methanol (6:3:1, % v/v). The

fractionation yield obtained from 200 g of dried *Sargassum ilicifolium* powder was 0.71%. Fractionation was conducted to minimize matrix interference and improve separation efficiency. The identification of fucoxanthin in the purified fraction was confirmed by TLC.



A = fraction B standard fucoxanthine

Figure 1. TLC identification of fucoxanthin extracted from *Sargassum ilicifolium* using *n*-hexane-ethyl acetate-methanol (6:3:1, % v/v). Spot A: fucoxanthin standard; Spot B: purified fraction with similar R_f values (0.53 and 0.52).

As shown in **Figure 1**, the R_f values of the fucoxanthin standard and the *n*-hexane-ethyl acetate-methanol (6:3:1, % v/v) fraction were 0.53 and 0.52, respectively, confirming the presence of fucoxanthin in the isolated fraction. The similarity of R_f values indicates successful purification with minimal interference from other matrix components. These findings are consistent with previous reports showing effective isolation of fucoxanthin using solvent systems of comparable polarity [6],[15],[16].

Method Validation Selectivity

The selectivity of the UV-Vis spectrophotometric and TLC-densitometric methods was evaluated by comparing the spectra and chromatograms of fucoxanthin standards with those of the extracted fractions (**Figure 2**). The UV-Vis spectrum of fucoxanthin exhibited a maximum absorbance at 450 nm, indicating no interference from matrix components. Similarly, the TLC-densitometric chromatograms of the fucoxanthin standard and the spiked fraction showed distinct and well-separated peaks with a resolution (R_s) value greater than 1.5. These results confirm that both analytical methods are selective for fucoxanthin determination and fulfill the ICH and AOAC criteria for specificity [18],[19].

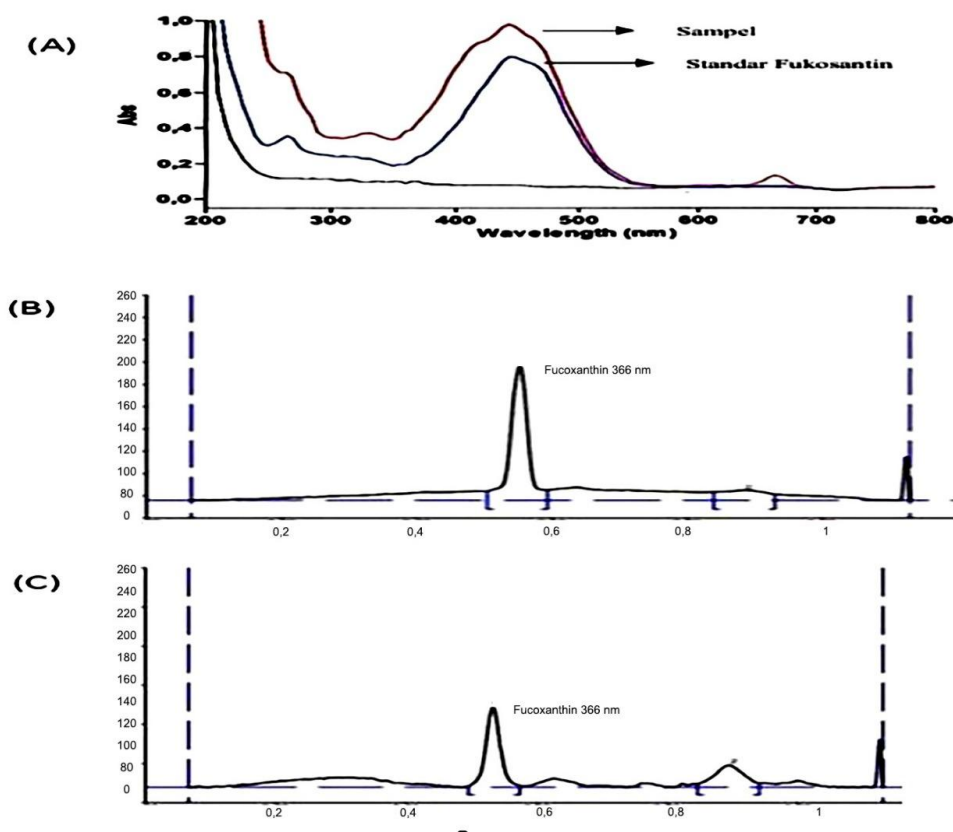


Figure 2. Spectra and chromatograms of fucoxanthin standard and sample. (A) UV-Vis spectra of fucoxanthin standard and fraction at 450 nm; (B) TLC-densitogram of fucoxanthin standard at 366 nm; (C) TLC-densitogram of fraction spiked with fucoxanthin standard showing identical peaks.

Linearity

Linearity was assessed by analyzing six concentrations of fucoxanthin standards within the ranges of 0.25–2.5 µg/mL for the UV-Vis spectrophotometric method and 2.5–40 µg/mL for the TLC-densitometric method. The calibration curves were obtained by plotting concentration against absorbance (UV-Vis) or peak area (TLC-densitometry) as shown in **Figure 3**.

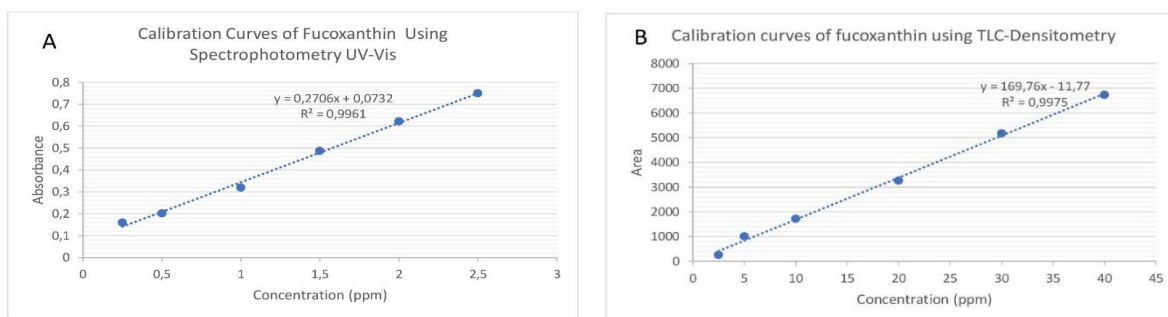


Figure 3. Calibration curves of fucoxanthin (A) UV-Vis spectrophotometric (B) TLC-Densitometric

Accuracy and Precision

The accuracy of the UV-Vis spectrophotometric and TLC-densitometric methods was assessed through recovery studies by spiking known quantities of fucoxanthin standards into the sample matrix at three concentration levels (80%, 100%, and 120%). The recovery results for each method are presented in **Table 2**. The UV-Vis spectrophotometric method showed recovery values ranging from 94.67% to 102.68%, while the TLC-densitometric method yielded recovery values between 102.10% and 104.57%. The mean recoveries were within the acceptable range of 92–105% as recommended by the AOAC and USP guidelines, indicating that both analytical methods exhibited high accuracy [19], [22].

Table 2. Accuracy of *fucoxanthin* determination in the *n*-hexane-ethyl acetate-methanol (6:3:1, % v/v) fraction using UV-Vis spectrophotometric and TLC-densitometric methods

	Standard Addition	Replication	Amount Added ($\mu\text{g/mL}$)	Amount Found ($\mu\text{g/mL}$)	Recovery (%)	Mean \pm RSD (%)
UV-Vis Spectrophotometric	80%	1	0.80	0.76	95.00	95.83 \pm 1.23
		2	0.80	0.78	97.50	
		3	0.80	0.76	95.00	
	100%	1	1.00	0.96	96.00	95.67 \pm 0.49
		2	1.00	0.96	96.00	
		3	1.00	0.95	95.00	
	120%	1	1.20	1.18	98.33	98.06 \pm 1.06
		2	1.20	1.16	96.67	
		3	1.20	1.19	99.17	
TLC-Densitometric	80%	1	5.30	5.35	100.94	100.50 \pm 0.62
		2	5.30	5.28	99.62	
		3	5.30	5.35	100.94	
	100%	1	6.60	6.63	100.45	100.81 \pm 0.25
		2	6.60	6.66	100.91	
		3	6.60	6.67	101.06	
	120%	1	8.00	8.17	102.13	102.00 \pm 0.10
		2	8.00	8.15	101.88	
		3	8.00	8.16	102.00	

The precision of the developed methods was evaluated based on repeatability (intra-day precision) using six replicate measurements of fucoxanthin at a concentration equivalent to 100% of the standard. The results are summarized in **Table 3**. The relative standard deviation (RSD) values obtained were 0.04% for the UV-Vis spectrophotometric method and 0.88% for the TLC-densitometric method. Both values were below the 2% limit specified by the AOAC [19], confirming that the developed methods demonstrate excellent repeatability and precision.

Table 3. Precision of *fucoxanthin* determination in the *n*-hexane–ethyl acetate–methanol (6:3:1, % v/v) fraction using UV–Vis spectrophotometric and TLC–densitometric methods

Replication	Fucoxanthin Concentration (µg/mL)	Mean (µg/mL)	SD	RSD (%)	Fucoxanthin Concentration (µg/mL)	Mean (µg/mL)	SD	RSD (%)
UV-Vis Spectrophotometric					TLC-Densitometric			
1	1.7966				15.5365			
2	1.7977				15.5742			
3	1.7981	1.7987	0.0007	0.04	15.6719	15.6291	0.0535	0.34
4	1.7985				15.6596			
5	1.7985				15.6719			
6	1.7987				15.6602			

These results collectively confirm that the UV–Vis and TLC–densitometric methods are accurate, precise, and reliable for fucoxanthin quantification in *Sargassum ilicifolium* extracts, fulfilling international validation requirements for analytical performance.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the UV–Vis spectrophotometric and TLC–densitometric methods was evaluated based on the limit of detection (LOD) and limit of quantification (LOQ). These parameters were calculated using the standard deviation of the response and the slope of the calibration curve in accordance with the ICH Q2(R2) and AOAC guidelines, as described by Wahyuningsih et al., 2024 [21].

The UV–Vis spectrophotometric method produced LOD and LOQ values of 0.17 µg/mL and 0.52 µg/mL, respectively. For the TLC–densitometric method, the LOD and LOQ were 2.50 µg/mL and 7.50 µg/mL. The corresponding regression equations were $y = 0.2714x + 0.0717$ ($R^2 = 0.9972$) for the UV–Vis method and $y = 169.76x - 11.77$ ($R^2 = 0.9975$) for the TLC–densitometric method.

Table 4. Limit of detection (LOD) and limit of quantification (LOQ) of *fucoxanthin* using UV–Vis spectrophotometric and TLC–densitometric methods

Method	Regression Equation	R ²	SD	Slope (m)	LOD (µg/mL)	LOQ (µg/mL)
UV-Vis spectrophotometric	$y = 0.2714x + 0.0717$	0.9972	0.015	0.2714	0.17	0.52
TLC-densitometric	$y = 169.76x - 11.77$	0.9975	5.00	169.76	2.50	7.50

As shown in **Table 4**, the UV–Vis method demonstrated higher sensitivity due to its lower detection limits, while the TLC–densitometric approach exhibited better selectivity as a result of chromatographic separation. Both methods displayed sufficient sensitivity for fucoxanthin determination in *Sargassum ilicifolium* extracts and are suitable for routine analytical applications in phytopharmaceutical research.

Quantification of Fucoxanthin

The validated UV–Vis spectrophotometric and TLC–densitometric methods were subsequently applied to determine the fucoxanthin content in the purified *Sargassum ilicifolium* extract. The quantification results are presented in **Table 5**. The

fucoxanthin content obtained using the UV-Vis spectrophotometric method was 0.22% (w/w), while the TLC-densitometric method yielded 0.56% (w/w).

Table 5. Fucoxanthin content in *Sargassum ilicifolium* extract determined using UV-Vis spectrophotometric and TLC-densitometric methods

Method	Wavelength / Detection (nm)	Concentration Range (µg/mL)	Regression Equation	R ²	Fucoxanthin Content (% w/w)
UV-Vis spectrophotometric	445	0.25-2.5	$y = 0.2706x + 0.0732$	0.9961	0.22 ± 0.01
TLC-densitometric	366	2.5-40	$y = 169.76x - 11.77$	0.9975	0.56 ± 0.02

The difference in fucoxanthin concentration between the two methods may be attributed to differences in analytical selectivity and matrix interference. The UV-Vis spectrophotometric method, although more sensitive, may experience absorbance interference from coexisting pigments such as chlorophylls and β-carotene that have overlapping absorption bands. In contrast, the TLC-densitometric method provides chromatographic separation that minimizes matrix interference, leading to higher and more specific quantification results.

When compared with previous studies, the fucoxanthin content obtained in this study is consistent with the range reported for *Sargassum* species. Noviendri et al. (2017) reported a fucoxanthin content of 0.47 mg/g in *Sargassum sp.*, while Sulistiyani et al. (2021) found levels between 0.90–3.01 mg/g depending on species and solvent polarity [16],[24]. The observed variations among studies can be influenced by factors such as geographical location, harvesting season, light exposure, and solvent systems used during extraction.

Overall, both analytical approaches demonstrated reliable quantification of fucoxanthin, confirming that *Sargassum ilicifolium* from Lampung, Indonesia, represents a potential natural source of fucoxanthin for further phytopharmaceutical applications.

Summary of Validation and Quantitative Findings

The present study successfully developed and validated two simple, reliable, and cost-effective analytical methods—UV-Vis spectrophotometric and TLC-densitometric—for the quantitative determination of fucoxanthin in *Sargassum ilicifolium*. Both methods demonstrated excellent analytical performance in terms of selectivity, linearity, accuracy, precision, and sensitivity, fulfilling the validation requirements outlined in ICH Q2(R2) and AOAC guidelines.

The UV-Vis spectrophotometric method exhibited lower limits of detection and quantification (0.17 µg/mL and 0.52 µg/mL, respectively), indicating higher sensitivity. However, this method was more susceptible to interference from other pigments such as chlorophylls and carotenoids due to overlapping absorption spectra. In contrast, the TLC-densitometric method achieved better chromatographic separation and selectivity, enabling more accurate quantification of fucoxanthin despite slightly higher LOD and LOQ values (2.50 µg/mL and 7.50 µg/mL, respectively).

The recovery values of both methods were within 94.67–104.57%, and RSD values were below 2%, confirming high accuracy and repeatability. The quantification results showed that the fucoxanthin content in the purified *Sargassum ilicifolium* extract ranged between 0.22% (UV-Vis) and 0.56% (TLC-densitometric), consistent with previously reported levels in other *Sargassum* species [16],[24]. Variations in fucoxanthin

content among studies are likely influenced by species differences, geographical factors, environmental conditions, and solvent polarity used during extraction.

Overall, these findings highlight that both validated methods are suitable for fucoxanthin determination and can be implemented for routine quality control and standardization of marine-derived carotenoids in phytopharmaceutical research [25].

This study was limited by the use of a single sampling site and harvest period for *Sargassum ilicifolium*, which may not fully represent the variability of fucoxanthin content across different geographical locations and seasons. Additionally, cross-validation using advanced chromatographic techniques such as HPLC or LC-MS was not performed, which could further confirm the accuracy and specificity of the developed methods. The degradation behavior and stability of fucoxanthin during extraction and analysis were also not evaluated in detail, although these factors may influence quantitative results. Future studies should therefore include multi-site sampling, seasonal variation assessment, and stability testing, as well as comparative validation using more sensitive instrumental methods to strengthen the analytical robustness and applicability of these techniques.

4. Conclusion

The UV-Vis spectrophotometric and TLC-densitometric methods developed in this study were successfully validated for the quantitative determination of fucoxanthin in *Sargassum ilicifolium*. Both methods met the analytical performance criteria required by ICH Q2(R2) and AOAC guidelines, demonstrating excellent selectivity, linearity, accuracy, precision, and sensitivity. The UV-Vis method exhibited higher sensitivity with lower detection limits, while the TLC-densitometric method provided superior selectivity through effective chromatographic separation. The validated methods yielded fucoxanthin contents of 0.22% and 0.56% (w/w), respectively, and both are reliable for routine quantification. These validated analytical approaches offer practical, accurate, and low-cost alternatives for fucoxanthin analysis in brown algae, supporting phytopharmaceutical standardization, marine natural product research, and quality control of carotenoid-based formulations. These findings are expected to encourage the application of validated low-cost analytical methods in marine bioactive compound research and industrial-scale quality control.

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Conflicts of Interest:

The authors declare no conflict of interest regarding the publication

References

- [1] R. A. Aryatikta, S. Winarni, S. N. W. Pramono, J. Soedarto, K. Undip Tembalang, and L. Resmi, "Literature review on the potential of *Sargassum* sp. as a nutraceutical," *Food Scientia Journal of Food Science and Technology*, vol. 2, no. 2, pp. 139–159, 2022. [Online]. Available: <https://doi.org/10.33830/fsj.v2i1.3083.2022>
- [2] E. D. L. R. Arguelles and A. B. Sapin, "Chemical composition and bioactive properties of *Sargassum aquifolium* (Turner) C. Agardh and its potential for pharmaceutical application," *Philippine Journal of Science*, vol. 151, no. S1, pp. 9–24, 2022. [Online]. Available: <https://doi.org/10.56899/151.S1.02>
- [3] V. Stiger-Pouvreau et al., "A concise review of the highly diverse genus *Sargassum* C. Agardh with wide industrial potential," *Journal of Applied Phycology*, Aug. 2023. [Online]. Available: <https://doi.org/10.1007/s10811-023-02959-4>
- [4] M. Puspita et al., "Indonesian *Sargassum* species bioprospecting: potential applications of bioactive compounds and challenges for sustainable development," *Advances in Botanical Research*, vol. 95, pp. 113–161, Jan. 2020. [Online]. Available: <https://doi.org/10.1016/bs.abr.2019.12.002>
- [5] M. K. A. Sobuj et al., "Qualitative and quantitative phytochemical analysis of brown seaweed *Sargassum polycystum* collected from Bangladesh with its antioxidant activity determination," *Food Chemistry Advances*, vol. 4, p. 100565, Jun. 2024. [Online]. Available: <https://doi.org/10.1016/j.focha.2023.100565>
- [6] V. Sodik, S. Tamat, T. Suwarno, and D. Noviendri, "Extraction and purification of fucoxanthin from brown seaweed *Sargassum* sp. as an antioxidant," *Health Research Journal of Poltekkes Bandung*, vol. 14, no. 1, pp. 123–133, May 2022. [Online]. Available: <https://doi.org/10.34011/juriskesbdg.v14i1.2057>
- [7] W. Koch et al., "Fucoxanthin: From chemical properties and sources to novel anticancer mechanistic insights and synergistic therapeutic opportunities," *Current Research in Biotechnology*, vol. 6, p. 100203, Jan. 2024. [Online]. Available: <https://doi.org/10.1016/j.crbiot.2024.100203>
- [8] E. Wahyuningsih, R. Nurhayati, and A. K. Putri, "Effect of hydrochloric acid concentration on phenolic compounds and antioxidant activity in methanol extract of *Sargassum oligocystum*," *Scientific Journal of Science*, vol. 24, no. 1, pp. 80–86, Apr. 2024. [Online]. Available: <https://doi.org/10.35799/jjs.v24i1.53431>
- [9] L. Kadir, R. J. Saleh, and Y. Mokodompis, "Prevalence and environmental risk factors of dermatitis in Gorontalo Class IIA Correctional Facility, Indonesia," *Journal of Syifa Sciences and Clinical Research*, vol. 7, no. 2, pp. 215–225, Aug. 2025. [Online]. Available: <https://doi.org/10.37311/jsscr.v7i2.33454>
- [10] E. Yustati, A. S. Suryadinata, and S. Al-Ma'arif, "Risk factors for dermatitis in children who come to primary health care centers," *Cendekia Medika: Journal of STIKES Al-Ma'arif Baturaja*, vol. 7, no. 1, 2022. [Online]. Available: <https://doi.org/10.52235/cendekiamedika.v7i1.102>
- [11] B. Zhong, N. A. Robinson, R. D. Warner, C. J. Barrow, F. R. Dunshea, and H. A. R. Suleria, "LC-ESI-QTOF-MS/MS characterization of seaweed phenolics and their antioxidant potential," *Marine Drugs*, vol. 18, no. 6, Jun. 2020. [Online]. Available: <https://doi.org/10.3390/md18060331>
- [12] P. Crupi et al., "Determination of fucoxanthin isomers in microalgae (*Isochrysis* sp.) by high-performance liquid chromatography coupled with diode-array detector and multistage mass spectrometry," *Rapid Communications in Mass*

- Spectrometry*, vol. 27, no. 9, pp. 1027–1035, May 2013. [Online]. Available: <https://doi.org/10.1002/rcm.6531>
- [13] C. Lourenço-Lopes et al., "An HPLC–DAD method for identifying and estimating the content of fucoxanthin, β -carotene, and chlorophyll a in brown algal extracts," *Food Chemistry Advances*, vol. 1, Oct. 2022. [Online]. Available: <https://doi.org/10.1016/j.focha.2022.100095>
- [14] R R. Mawaddah, D. Noviendri, I. Setyaningsih, and Uju, "Extraction and purification of fucoxanthin from *Sargassum* sp.," *Journal of Fishery Product Processing Indonesia*, vol. 20, no. 2, pp. 370–379, 2017. [Online]. Available: <https://doi.org/10.17844/jphpi.v20i2.18105>
- [15] M. Renhoran, D. Noviendri, I. Setyaningsih, and U. Uju, "Extraction and purification of fucoxanthin from *Sargassum* sp. as an anti-acne agent," *Journal of Fishery Product Processing Indonesia*, vol. 20, no. 2, pp. 370–379, Aug. 2017. [Online]. Available: <https://doi.org/10.17844/jphpi.v20i2.18105>
- [16] V. Sodik, S. Tamat, T. Suwarno, and D. Noviendri, "Extraction and purification of fucoxanthin from brown seaweed *Sargassum* sp. as an antioxidant," *Health Research Journal of Poltekkes Bandung*, vol. 14, no. 1, pp. 123–133, 2022. [Online]. Available: <https://doi.org/10.34011/juriskesbdg.v14i1.2057>
- [17] V. Sodik, S. Tamat, T. Suwarno, and D. Noviendri, "Extraction and purification of fucoxanthin from brown seaweed *Sargassum* sp. as an antioxidant," *Health Research Journal of Poltekkes Bandung*, vol. 14, no. 1, pp. 123–133, 2022. [Online]. Available: <https://doi.org/10.34011/juriskesbdg.v14i1.2057>
- [18] U.S. Pharmacopeia, "International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use: ICH harmonised tripartite guideline stability testing Q1B," *U.S. Pharmacopeia (USP)*, 2024. [Online]. Available: <https://www.usp.org>
- [19] AOAC International, "Guidelines for validation of botanical identification methods," *Journal of AOAC International*, vol. 95, no. 1, pp. 268–272, 2012. [Online]. Available: <https://pmc.ncbi.nlm.nih.gov/articles/PMC3600167/>
- [20] L. M. S. Kuczyńska, A. Szymczak, and J. W. Wasilewska, "TLC-densitometric method for the preliminary evaluation of fucoxanthin-based products," *Acta Chromatographica*, vol. 27, no. 4, pp. 665–678, 2015. [Online]. Available: <https://doi.org/10.1556/AChrom.27.2015.4.7>
- [21] E. Wahyuningsih, V. L. P. Amannillah, G. Febriananda, Z. N. Aliyyah, and F. A. Z. Wijatmoko, "Optimization of the method and determination of detection limit for fucoxanthin analysis in *Sargassum ilicifolium* (Turner) C. Agardh using TLC–densitometry," *Camellia: Clinical, Pharmaceutical, Analytical and Pharmacy Community Journal*, vol. 3, no. 1, pp. 171–179, 2024. [Online]. Available: <https://doi.org/10.30651/cam.v3i1.22837>
- [22] AOAC International, "Appendix F: Guidelines for standard method performance requirements," *AOAC Official Methods of Analysis*, 2016, pp. 1–18. [Online]. Available: https://www.aoac.org/wp-content/uploads/2019/08/app_f.pdf
- [23] U.S. Pharmacopeia, "Validation of compendial procedures," *USP–NF*, 2024. [Online]. Available: https://doi.org/10.31003/USPNF_M99945_04_01
- [24] D. Noviendri, H. M. Salleh, and K. Miyashita, "Fucoxanthin extraction and fatty acid analysis of *Sargassum binderi* and *S. duplicatum*," *Marine Science Research*, 2011. [Online]. Available: <https://www.researchgate.net/publication/287564183>

- [25] L. Cai, "Thin layer chromatography," *Current Protocols in Essential Laboratory Techniques*, vol. 6, no. 3, pp. 6.3.1-6.3.18, 2014. [Online]. Available: <https://doi.org/10.1002/9780470089941.et0603s08>