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Effect of Fucoidan Extract from *Sargassum siliquosum* Using Combined Ultrasonic & Enzymatic Method on Its Functional Properties

Pengaruh Ekstrak Fucoidan dari *Sargassum siliquosum* Menggunakan Metode Gabungan Ultrasonik & Enzimatis terhadap Sifat Fungsionalnya

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ABSTRACT

Fucoidan is a sulfated polysaccharide derived from brown seaweed. It has attracted considerable attention due to its various biological properties. Gunung Kidul, Yogyakarta Province, has potential for seaweed producing. Fucoidan extraction there currently relies on conventional methods. However, this approach has its own shortcomings, namely long extraction time and low yields. To overcome these problems, this study evaluates the effectiveness of a combined ultrasound and enzyme extraction method in improving the yield and bioactive properties of fucoidan obtained from sargassum siliquosum. Cellulase enzyme concentrations (0.5 %,1 %, and 2% v/v) were investigated and compared with the conventional extraction method. The results showed that (UAE+EAE 2 %) method provided the highest yield (3.622 %), which is slightly higher than the conventional method (3.506 %), while the lower enzyme concentrations produced lower yields. FTIR analysis confirmed the presence of fucoidan functional groups, including sulfate ester (S=O) and glycosidic (C-O-C) bonds. The presence of fucoidan was confirmed by HPLC analysis, and the DPPH assay's evaluation of antioxidant activity showed that the bioactive characteristics of fucoidan were maintained. Overall, in comparison to conventional methods, the combined UAE+EAE approach shows great promise as a more effective and eco-friendly extraction technology. Further optimization and purification for extracted fucoidan are recommended to improve bioactivity for future applications.

Keywords: *Antioxidant activity; ultrasonic and enzyme-assisted extraction; Fucoidan; Bioactive polysaccharide; sargassum siliquosum.*

ABSTRAK

Fucoidan adalah polisakarida tersulfat yang berasal dari rumput laut cokelat. Senyawa ini telah menarik perhatian yang cukup besar karena berbagai sifat biologisnya. Gunung Kidul, Provinsi Yogyakarta, memiliki potensi sebagai penghasil rumput laut. Ekstraksi fucoidan di sana saat ini masih mengandalkan metode konvensional. Namun, pendekatan ini memiliki kekurangan tersendiri, yaitu waktu ekstraksi yang lama dan hasil yang rendah. Untuk mengatasi masalah ini, penelitian ini mengevaluasi efektivitas metode ekstraksi gabungan ultrasonik dan enzim dalam meningkatkan hasil dan sifat bioaktif fucoidan yang diperoleh dari Sargassum siliquosum. Konsentrasi enzim selulase (0,5%, 1%, dan 2% v/v) diteliti dan dibandingkan dengan metode ekstraksi konvensional. Hasil menunjukkan bahwa metode (UAE+EAE 2%) memberikan hasil tertinggi (3,622%), yang sedikit lebih tinggi daripada metode konvensional (3,506%), sedangkan konsentrasi enzim yang lebih rendah menghasilkan hasil yang lebih rendah. Analisis FTIR mengkonfirmasi keberadaan gugus fungsional fucoidan, termasuk ikatan ester sulfat (S=O) dan glikosidik (C-O-C). Keberadaan fucoidan dikonfirmasi oleh analisis HPLC, dan evaluasi aktivitas antioksidan dengan uji DPPH menunjukkan bahwa

karakteristik bioaktif fucoidan tetap terjaga. Secara keseluruhan, dibandingkan dengan metode konvensional, pendekatan gabungan UAE+EAE menunjukkan potensi besar sebagai teknologi ekstraksi yang lebih efektif dan ramah lingkungan. Optimalisasi dan pemurnian lebih lanjut untuk fucoidan yang diekstrak direkomendasikan untuk meningkatkan bioaktivitas untuk aplikasi di masa mendatang.

Kata kunci: Aktivitas antioksidan; ekstraksi ultrasonik dan berbantuan enzim; Fucoidan; Polisakarida bioaktif*; Sargassum siliquosum.*

1. INTRODUCTION

1.1 Background and Literature review

Brown algae are particularly rich in polysaccharide such as fucoidan, laminarin, and alginate. Fucoidan own Lots function biological like antimicrobial, inflammatory, antitumor, antioxidant, antiaging, and anticoagulant activities ((Bahrami et al., 2025). Due to these functional properties, fucoidan has attracted considerable interest for applications in the pharmaceutical, nutraceutical, and functional food industries. Indonesia, is a maritime country with extensive coastal areas, possesses abundant brown seaweed resources, particularly from the genus *Sargassum*, which represent a promising natural source for fucoidan production, however, its utilization as a fucoidan source remains limited (Bahrami et al., 2025; Tang et al., 2022).

Conventional extraction method of fucoidan depends on hot water at a certain pH value, usually use strong acids and bases or treatment hydrothermal. Although method extraction water based can selective to compound certain, there are require large amounts of water and chemicals, and requires long extraction time (and sometimes takes place at a high temperature). Additionally, conventional extraction conditions may cause degradation of sulfate groups, which are responsible for fucoidan's biological activity, thereby reducing its functional quality (Flórez-Fernández et al., 2018).

To the best of our knowledge, limited studies have investigated the synergistic extraction of fucoidan from *sargassum siliquosum* using combined (UAE&EAE) treatment, particularly focusing on functional and biological activity. Recent studies have examined the possible advantages of combining (UAE&EAE) to further improve the yield and efficiency of natural product extraction, even if each technique has advantages of its own. When ultrasonic waves and enzymatic treatment are combined, the mechanical disturbance brought on by cavitation and the biochemical breakdown aided by enzymes are both utilized. Fucoidan is better released into the extraction medium and the algal cell wall is broken down more effectively as a result of this combination

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Vol. 11, No. 1, Hal. 14-26 (July 2026)

strategy (Mapholi et al., 2025). For instance, (Wang et al., 2025). showed that combining enzymatic and ultrasonic techniques for peanut oil extraction greatly shortened processing times while simultaneously increasing extraction rates. Synergistic effects of UAE+EAE on brown algae remain unexplored. Although, there are a lot of previous studies about extraction fucoidan, but there is no study on combined method for *sargassum siliquosum* algae, as well as a combined evaluation of yield, FTIR, HPLC, and antioxidant activity.

1.2 Research Objective

This research aims to compare the effect of different extraction methods namely conventional method, and the combined ultrasound-assisted and enzymatic-assisted methods on the yield of extraction of fucoidan from brown seaweed *Sargassum sp*. To investigate the chemical structure of crude fucoidan extract and its antioxidant activities.

MATERIALS AND METHODS

2.1. Materials:

The dried brown seaweed *sargassum siliquosum* was obtained from Sepanjang Beach, Gunung Kidul, Special Region of Yogyakarta, Indonesia. All chemicals used in extraction such as ethanol, Cellulase enzyme, calcium chloride, NaOH, HCl and distilled water were purchased from a local chemical store in Yogyakarta

2.2. Conventional Method:

Initially, depigmented algal powder was mixed with water in a 1:30 (w/v) ratio for 30 min. The depigmented algal powder was heated for 60 min at 130 rpm at 65 °C using (B-One SWB-30 Shaker Water Bath). Then, mixture was filtered using filter paper to separate the residue from the supernatant. After extraction, 2 % $CaCl_2$ was added to precipitate calcium alginate and then kept in cold storage overnight. After cooling the solution, the leftover supernatant was combined with twice as much 96 % ethanol in ratio 1:2 and allowed to cool until the mixture precipitated in order to extract crude fucoidan. Then, the fucoidan was centrifugated at room temperature for 15 min at 4000 rpm. Finally, the fucoidan was dried before weighing. Finally, the dried fucoidan's weight was calculated after freeze dring at -55 ^{o}C using Freeze Dryer (VirTis Benchtop K). (Dwijayanto et al., 2025).

2.3. Combined (UAE&EAE) Method:

This extraction method started with depigmented algal powder was mixing with water in a 1:30 (w/v) ratio for 30 min. Next, sonication was preformed using an ultrasonic homogenizer device with

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Vol. 11, No. 1, Hal. 14-26 (July 2026)

frequency of 25 kHz and power of 250 W at a temperature of 50 °C for 10 min, to disrupt cell walls and release active compound. After filtration, cellulase enzyme was added in concentration of (1% v/v) was added with constant stirring to accelerate polysaccharides hydrolysis, and the mixture incubated at 4 °C for 24 hours. pH value during enzymatic hydrolysis between 4.5-5.5 The supernatant mixed with 96% ethanol and left at 4 °C for 24 hours to precipitate fucoidan. The mixture was filtered to separate the residue. The filtrate obtained was centrifuged at 4000 rpm for 15 min at room temperature for separate suspended particles. The fucoidan dried using Freeze dryer to obtain fucoidan in form powder. Finally, the dried fucoidan's weight was calculated after freeze dried at -55 ºC using Freeze Dryer (VirTis Benchtop K). This extraction was repeated three times(n=3) for each different concentrations of cellulase enzyme which they are 0.5 %,1 %, and 2% (Dwijayanto et al., 2025).

2.4. Fucoidan yield:

The fucoidan yield % was determined using the following formula (Dwijayanto et al., 2025).

$$\boldsymbol{Fucoidanyield\%ww}\ \frac{\text{Weight of dried fucoidan (g)}}{\text{Weight of depipgmented algal powder (g)}}\ 100 \quad \textbf{(1)}$$

2.5. Fucoidan characterization:

The extracted fucoidan samples were carried out using Fourier Transform Infrared Spectroscopy (FTIR) to identify the extracted fucoidan's functional groups, and High-Performance Liquid (HPLC) to evaluate fucoidan’s presence.

2.6. Analysis of antioxidant activity:

Antioxidant activity was assessed using the 2,2-Diphenyl-1- picrylhydrazyl (DPPH) free radical inhibition assay. Several concentrations of fucoidan (0, 60, 120, 180, 240, and 300 µg/mL) were prepared for the sample solution. The absorbance was measured at a wavelength of 517 nm. The following formula was used to determine the inhibition percentage.

$$\%\text{inhibition} = \frac{(\text{Ablank} - \text{Asample})}{\text{Ablank}} \times 100\% \quad \text{……(2)}$$

and then the linear plot of %inhibition versus concentration was analyzed using the following equation.

$$Y = a + bX \quad \text{……..(3)}$$

Where x is the concentration of the measured substance and y is the % inhibition. Meanwhile, the IC_{50} value was determined as the x value of this equation when y was equal to 50%.

All experiments have done in duplicate (n = 2), and results are expressed as mean ± standard deviation (SD).

p. ISSN: 2354-9610, e. ISSN:2614-5081
Vol. 11, No. 1, Hal. 14-26 (July 2026)

Statistical analysis was performed using an independent sample t-test to compare the yield and antioxidant activity between extraction methods. Differences have considered statistically significant at a confidence level of 95% ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Fucoidan yield:

The cell wall of brown algae seaweed is primarily composed of sulfated polysaccharides containing fucose and polyanionic polysaccharides called alginates. Approximately 25–30% of the dry fucoidan, a sulfated polysaccharide, makes up the bulk of brown seaweeds (Salmeán et al., 2017)

Table 1 shows the yields of fucoidan extracted using conventional, and combined ultrasound and enzymatic-assisted extraction techniques. The results from Table 1 demonstrated that the yield of fucoidan may be increased by employing (UAE+EAE).

UAE+EAE 2% showed a higher mean compared to conventional extraction, however, the difference is not significant ($p > 0.05$)

Ultrasonic enzymatic extraction (UAE + EAE) shows promising results in restoring bioactive compounds, from sample materials. In this study, three concentrations of cellulose enzymes (1%, 2%, and 0.5%) were evaluated, giving an average variation of 2.685% ±0.364, 3.622% ±0.175 and 2.825% ±0.015, respectively. These results highlight that the concentration of the enzyme can be effective in which moderate enzyme levels optimize mass transfer in the extraction process. As well as the reduced yields at 0.5% and 1% enzyme concentrations indicates that, these concentrations were not ideal for full cell wall matrix breakdown. That is related to some process conditions during the extraction process such as, pH value or temperature, and so on.

It was noted that the concentration of 2% reaches the highest average yield (3.622%), slightly higher than the conventional method (3.505%). This implies that the algal cell wall can be broken down and fucoidan released more effectively by the combined use of ultrasound and an adequate enzyme concentration than by conventional extraction method.

Table 1. Fucoidan extraction yields obtained using the conventional, and (UAE + EAE)

Method	Yield
UAE+EAE 0.5%	2.825 % ±0.015
UAE+EAE 1 %	2.685 % ±0.364
UAE+EAE 2%	3.622% ±0.175
Conventional	3.506 % ±0.348

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Vol. 11, No. 1, Hal. 14-26 (July 2026)

The results of this study show that it is higher compared to a previous study they used ultrasound method and microwave and they obtain (2.772% and 2.493%), respectively (Dwijayanto et al., 2025). Fucoidan release may be limited by inadequate hydrolysis of cellulose and hemicellulose at lower enzyme concentrations (0.5–1%). Similar patterns of incomplete cell wall disintegration due to insufficient enzyme dose were documented by [Tang 2022 / Rhein-Knudsen 2023].

Table 2 summarizes the crude fucoidan content from *Sargassum* genera with some extra information. It indicates that the kind of algae and the extraction technique had an impact on the fucoidan yield.

Table 2. Summary of crude fucoidan content reported for several algal species from *Sargassum* genera

Species	**Location**	**Crude Content (%DW)**	**Ref.**
Sargassum binderi	Malaysia	6.2	(Lim et al., 2014)
Sargassum fusiforme	China	3.94–11.24	(Deng et al., 2020)
Sargassum hemiphyllum	China	2.72 ± 0.18	(R. Li et al., 2023)
Sargassum hemiphyllum	China	4.69 ± 1.05	(Wang et al., 2021)
Sargassum henslowianum	China	6.25	(Lin et al., 2022)
Sargassum polycystum	China	3.41 ± 0.77	(Wang et al., 2021)
Sargassum siliquosum	China	5.08 ± 1.17	(Lin et al., 2022)
Sargassum vachellianum	China	5.5 ± 0.25	(Jesumani et al., 2020)
Sargassum sp	Egypt	5.99–36.79	(Wang et al., 2021)
Sargassum wightii	India	10.59-14.61	(Hanjabam et al., 2019)
Sargassum cinereum	Egypt	21.79±4.99	(El-Sheekh et al., 2024)
Sargassum polycystum	Bangladesh	4.1 – 6.1	(Roy et al., 2024)
Sargassum horneri	China	2.91±0.17	(W. Li et al., 2023)
Sargassum Zhangii	China	2.85±0.35	(R. Li et al., 2023)
Sargassum polycystum	Malaysia	1.16 ± 0.07	(Mohd Fauziee et al., 2021)
Sargassum wightii	India	0.7–1	(Ramu et al., 2020)
Sargassum cinereum	India	9.4 ± 1.9	(Somasundaram et al., 2016)

3.2. Functional group analysis (FTIR):

The O–H stretching vibrations present in polysaccharides are indicated by a broad absorption band in the FTIR spectra of all the fucoidan samples, which ranged from 3359 to 3283 cm^{-1}. The 1029–1027 cm^{-1} region also showed distinct peaks that corresponded to C–O and C–O–C stretching vibrations associated with pyranose ring structures in glucose and galactose units. Furthermore, symmetric and asymmetric stretching vibrations of carboxylate groups are responsible for absorption bands located between 1595 and 1618 cm^{-1}, which suggest the presence of uranic acids or trace amounts of proteinaceous contaminants in the samples. The presence of carboxyl functional groups in the fucoidan structure is further

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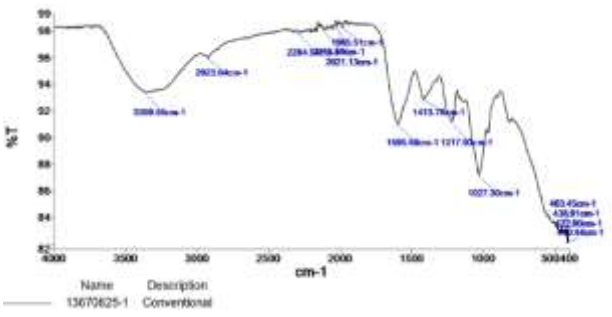
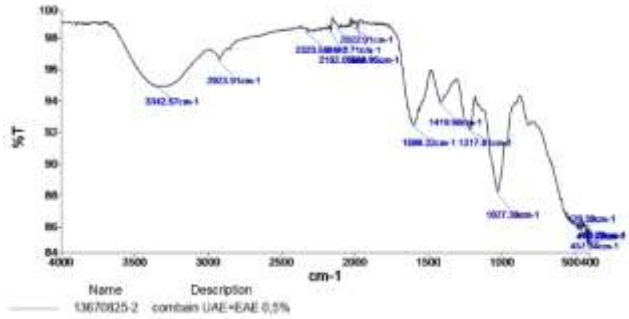
Vol. 11, No. 1, Hal. 14-26 (July 2026)

supported by the spectral signature at about 1412 cm^{-1} (Flórez-Fernández et al., 2018)

The absorption band about 1220–1270 cm^{-1}, which is frequently ascribed to the S=O asymmetric stretching vibration of sulfate ester groups, is a crucial sign of sulfation in fucoidan. A constant absorption around ~1217 cm^{-1} across all samples indicates the existence of sulfate esters, even though the automatic peak-picking output did not show a distinct peak at 1250 cm^{-1}. This is further corroborated by the UAE+EAE 1% and 2% samples showing a peak at ~964 cm^{-1}, which is frequently linked to C–O–S stretching or vibrations related to sulfate. Furthermore, the peak lists could not resolve the expected C–O–S bending signal, which is usually seen around 820–845 cm^{-1} and indicates sulfate substitution patterns on sugar residues. This could be because of weak intensity or overlapping signals. However, the existence of these sulfate-associated bands suggests that the fucoidan extracted by ultrasound-assisted enzyme methods (UAE+EAE) maintains more sulfate groups than the conventional extraction method, especially when the enzyme concentration is higher (Flórez-Fernández et al., 2018; Zayed & Ulber, 2020). The FTIR analysis for all the samples is shown in Figure 1.

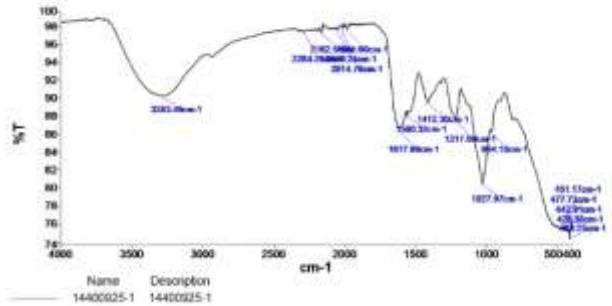


(a)



(b)

(c)



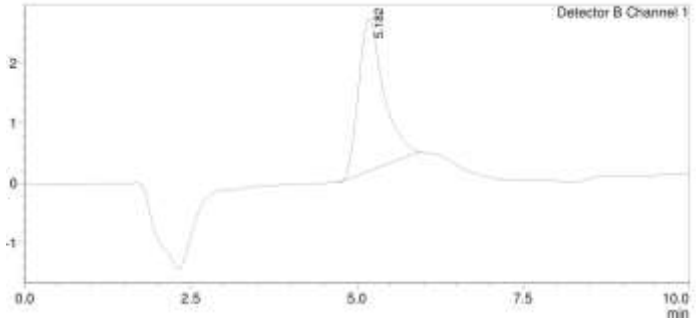
(d)

p. ISSN: 2354-9610, e. ISSN:2614-5081
Vol. 11, No. 1, Hal. 14-26 (July 2026)

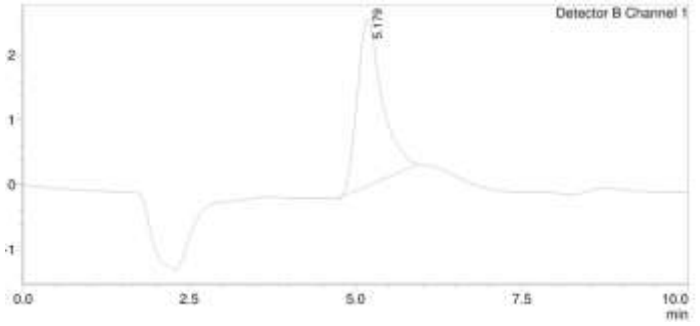
Figure.1. FTIR spectra of fucoidan extracted from *sargassum siliquosum* using extraction method: (a) conventional, (b) UAE+EAE 0.5% method, (c) UAE+EAE 1% method, (d) UAE+EAE 2

3.3. Functional group analysis (HPLC):

All samples show a major peak at around 5.18 min, indicating that fucoidan was successfully extracted under all conditions. Given that crude fucoidan frequently consists of heterogeneous sulfated polysaccharide fractions, the presence of an additional peak (6.97 min) in the UAE+EAE 2% sample suggests either a co-extracted impurity, a secondary fucoidan fraction with a different molecular weight, or a structural variant (Rhein-Knudsen et al., 2023). Similar observations have been reported by other studies, where crude fucoidan exhibited multiple chromatographic peaks due to differences in sulfation patterns and molecular weight distribution (Zayed & Ulber, 2020). The additional peak observed at 6.97 min (about 8.7% area) in the UAE+EAE 2% sample may therefore reflect either co-extracted impurities such as proteins, phenolics, or oligosaccharides, or indicate the presence of fucoidan fractions with different molecular weights or degradation products formed during processing. Quantitative sulfate content and molecular weight analysis were not performed and should be included in future studies. The HPLC analysis for all the samples is shown in Figure 2



(a)



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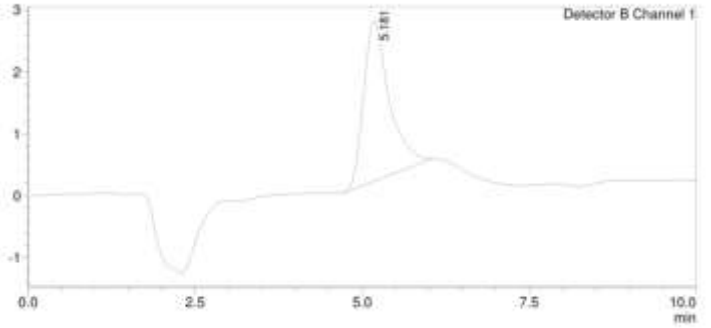

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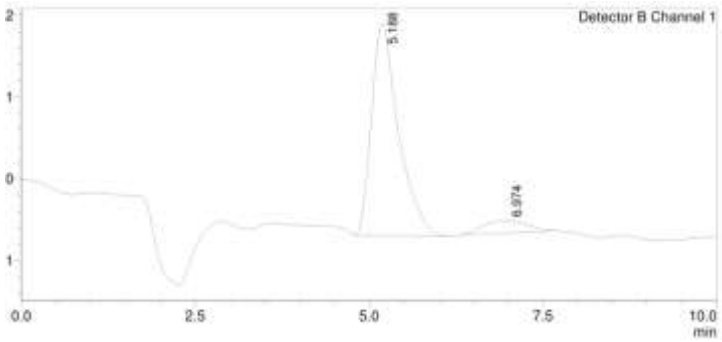

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p. ISSN: 2354-9610, e. ISSN:2614-5081
Vol. 11, No. 1, Hal. 14-26 (July 2026)

Figure.2. HPLC spectra of fucoidan extracted from *sargassum siliquosum* using extraction method: (a) conventional, (b) UAE+EAE 0.5% method, (c) UAE+EAE 1% method, (d) UAE+EAE 2 % method. (x axis is time in minutes, y axis is intensities)

3.4. Antioxidant activity:

Using standard DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assays, the IC_{50} values for the fucoidan extracts were obtained by first calculating the percent inhibition at each tested concentration using the relationship:

$$\%\text{Inhibition} = \frac{\text{Acontrol} - \text{Asampel}}{\text{A control}} \; x \; 100\% \quad (3)$$

Then fitting a linear regression to the concentration vs. percent-inhibition data and solving the regression equation at 50% inhibition to obtain the concentration corresponding to IC_{50}. This method explains why the conventional extract could not be assigned an IC50: the maximum inhibition detected was only 45.66% even at the highest tested concentration (19.254 mg·mL⁻¹), meaning that the 50% threshold was never attained and no reliable extrapolated IC_{50} could be reported. On the other hand, the combined method of ultrasound and enzyme assisted extraction (UAE+EAE) samples produced concentration dependent scavenging curves with robust linear fits, yielding IC_{50} values of 12.739 ± 0.891 mg·mL⁻¹ (0.5% enzyme), 12.089 ± 0.845 mg·mL⁻¹ (1% enzyme) and 8.903 ± 0.623 mg·mL⁻¹ (2% enzyme) As shown in table 3.

The IC50 value category is strong if the IC50 value is less than <10 mg/mL, moderate if the IC50 value is between 10 and 20 mg/mL, and weak if the IC50 value is less than 20 mg/ml (idrianny, 2015).

Table 3. Average IC50 values of fucoidan obtained using the conventional, and (UAE + EAE)

Extraction Method	IC_{50} (mg/mL)	Antioxidant Strength
UAE+EAE 2%	8.903 ± 0.623	Strong
UAE+EAE 1%	12.089± 0.845	Moderate
UAE+EAE 0.5%	12.739± 0.891	Moderate
Conventional	No IC50 (max = 45.66%)	Very weak / ineffective

However, due to the limited number of replication(n=2), statistical significance should be interpreted cautiously. The independent sample t-test indicated that the reduction in IC_{50} at 2% enzyme concentration was statistically significant compared to 0.5% ($p<0.05$) while the difference between 1% and 0.5% was not statistically significant.

Based on comparison with the previous study performance. And the IC_{50} for conventional extraction could not be determined in this study. So, the

UAE+EAE 2% extraction provided the most effective fucoidan for DPPH radical scavenging, yielding a slightly strong IC_{50} value of 8.903 ± 0.623 mg/mL.

It must note that statistical interpretation is limited by the small number of replicates (n =2). To validate these results, more research with bigger sample size is advised.

CONCLUSION

This study successfully demonstrated that the combined method of ultrasound-assisted and enzymatic extraction (UAE+EAE) effectively enhances the recovery of crude fucoidan from *sargassum siliquosum*. The use of 2% (v/v) cellulase resulted in the highest yield and the strongest antioxidant activity among the tested samples, indicating a synergistic effect between ultrasonic cavitation and enzymatic hydrolysis. The presence of sulfated polysaccharides and phenolic co-extractives is responsible for the fucoidan's moderate radical scavenging action. The environmentally friendly extraction conditions and the utilization of crude extracts demonstrate the promise of this technology for sustainable and scalable production, even though the yield was lower than that reported for purified fucoidan from other Sargassum species, the environ-mentally friendly extraction conditions and the use of crude extracts highlight the potential of this method for sustainable and scalable production. The results highlight that the combine method of UAE+EAE does not only improve the yield but also enhances the functional groups and bioactive properties of fucoidan, indicating great potential for scalable and sustainable high-value future applications in the pharmaceutical, and functional food industries.

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