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The Potential of Fucoxanthin from *Sargassum polycystum*: Antioxidant, Antibacterial, and Photoprotective Properties

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ABSTRACT

Sargassum is a genus of seaweed that is known to contain fucoxanthin. The increasing incidence of health issues related to antimicrobial resistance (AMR), free radicals, and ultraviolet (UV) radiation has prompted the exploration of natural compounds as alternative sources of pharmaceutical agents. Fucoxanthin possesses the ability to protect cells from oxidative damage and offers a wide range of health benefits. This study aimed to evaluate the potential of fucoxanthin derived from *Sargassum polycystum* as an antioxidant, antifungal, antibacterial, and photoprotective (sunscreen) agent. The research methods included the extraction and isolation of fucoxanthin using Open Column Chromatography (OCC), phytochemical screening, thin-layer chromatography (TLC), antibacterial and antifungal assays, antioxidant activity assessment using the DPPH method, and determination of the Sun Protection Factor (SPF). The results showed that the extract of *Sargassum polycystum* contains secondary metabolites such as flavonoids, alkaloids, tannins, steroids, and saponins. The fucoxanthin fraction was successfully isolated, characterized by a distinct absorption peak at 447.5 nm and a retention factor (Rf) value of 0.93. The fucoxanthin fraction showed antibacterial activity against *Staphylococcus aureus*, as well as antifungal activity against *Trichoderma* sp. and *Candida albicans*. The antioxidant activity of the fucoxanthin fraction was categorized as strong, with an IC_{50} value of 67 ppm. Its photoprotective ability was classified as maximal, with a Sun Protection Factor value of 13.71. The fucoxanthin fraction derived from *Sargassum polycystum* exhibits promising potential as an antibacterial, antifungal, antioxidant, and photoprotective agent, and may serve as a valuable natural resource in pharmaceutical and cosmeceutical applications.

1. Introduction

Brown seaweeds (Phaeophyta) are a type of macroalgae that have garnered increasing attention due to their wide range of applications in food, industrial processes, cosmetics, and pharmaceuticals (Barbosa *et al.* 2019). One of the distinctive features of brown seaweeds is the presence of fucoxanthin, a natural orange pigment that imparts the characteristic brownish coloration to these algae (Mumu *et al.* 2022). Fucoxanthin is known to exhibit a broad spectrum of biological activities, including antidiabetic, anti-obesity, anticancer, anti-inflammatory, and antioxidant properties (Neumann *et al.* 2019; Karpiński *et al.* 2021; Mumu *et al.* 2022). Structurally, fucoxanthin contains an allenic bond and a 5,6-monoepoxide group, both of which are associated with its potent biological activities, particularly its antioxidant and antibacterial functions (Sulistiyani *et al.* 2021). These attributes make fucoxanthin a promising candidate for pharmaceutical development.

Sargassum spp., a group of brown seaweeds with high natural abundance, are commonly found in tropical regions. In Indonesia, *Sargassum* represents one of

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the most abundant seaweed genera. However, export activities have primarily focused on other genera such as *Kappaphycus* and *Gracilaria* (Puspita *et al.* 2020). Given its high fucoxanthin content and wide availability, *Sargassum* is considered a valuable and sustainable source of this bioactive compound. Although fucoxanthin can be synthesized artificially, naturally derived fucoxanthin is considered superior due to its eco-friendliness, ease of extraction, and cost-effectiveness (Lourenço-Lopes *et al.* 2020). Therefore, the exploration and utilization of *Sargassum* as a fucoxanthin source warrant further investigation and development.

Global health concerns and the rising incidence of disease have led to a growing interest in natural products as alternatives for therapeutic agents and pharmaceutical actives. Major modern health challenges, including antimicrobial resistance (AMR), diseases induced by oxidative stress, and skin cancers resulting from ultraviolet (UV) radiation exposure, represent significant medical concerns (Narayanan *et al.* 2010; Reuter *et al.* 2010; Prestinaci *et al.* 2015). Given its diverse and potent biological activities, fucoxanthin is proposed to be a great pharmaceutical agent, including as antibacterial, antifungal, antioxidant, and photoprotective agent (Peng *et al.* 2011). This study aims to explore the biological activities of fucoxanthin derived from *Sargassum polycystum*, focusing on its potential as an antibacterial, antioxidant, and photoprotective agent.

2. Materials and Methods

2.1. Extraction of *Sargassum polycystum*

Brown seaweed samples of *Sargassum polycystum* were collected from the coastal waters of Teluk Awur Beach, Indonesia. Fresh samples were thoroughly washed with clean water, chopped into approximately 1 cm pieces, and homogenized using a blender. The extraction process was carried out by maceration using ethanol as the solvent, with a sample-to-solvent ratio of 1:10 (w/v), for 24 hours in a dark room. After maceration, the extract was filtered and concentrated using a rotary evaporator at 35°C with a rotation speed of 100–120 rpm until a crude paste-like extract was obtained (Sulistiyani *et al.* 2021).

2.2. Phytochemical Screening

Phytochemical screening was conducted to identify the classes of secondary metabolites present in the crude *S. polycystum* extract. The screening included qualitative tests for flavonoids, tannins, alkaloids, saponins, and steroids. Each test was performed by reacting 0.5 mg of the extract with specific reagents according to standard qualitative procedures, as described by (Sabdoningrum *et al.* 2021).

2.3. Fucoxanthin Isolation

Fucoxanthin was isolated using the Open Column Chromatography (OCC) method as described by (Wibowo *et al.* 2022). K A silica gel-packed open chromatography column was initially eluted with *n*-hexane. The crude extract paste of *Sargassum polycystum* was then applied to the top layer of the column. Elution was continued using a solvent mixture of *n*-hexane and acetone at a ratio of 6:4 (v/v) to obtain the fucoxanthin fraction, which was identified by its characteristic orange color. The collected eluate was concentrated using a rotary evaporator, and the resulting fraction was stored at a temperature of 5–10°C.

2.4. Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was performed using a mixture of *n*-hexane:acetone (6:4, v/v) as the mobile phase, with silica gel plates serving as the stationary phase. Fucoxanthin fraction was dotted on the TLC plate then placed in a chromatography chamber until the solvent reach the upper boundary of the plate. Fucoxanthin content was identified based on the appearance of colored spots and their respective retention factor (Rf) values. The Rf value was calculated using the following formula:

$$Rf = \frac{\text{Distance traveled by substance}}{\text{Distance traveled by solvent front}}$$

Pigment identification of fucoxanthin was performed using a UV–Vis spectrophotometer. The pigment was dissolved in analytical-grade methanol (Pa), and its absorbance was measured across the wavelength range of 250–750 nm. Fucoxanthin was identified by its characteristic absorption peak within the range of 440–460 nm (Sulistiyani *et al.* 2021).

2.5. Antioxidant Activity Assay

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method as described by Fatmawati *et al.* (2022). The sample was dissolved in analytical grade methanol (Pa) and diluted to obtain a series of concentrations: 200 ppm, 150 ppm, 100 ppm, and 50 ppm. Each concentration of the sample was reacted with DPPH solution in a 1:1 (v/v) ratio and incubated in the dark for 30 minutes. Absorbance was measured using a UV–Vis spectrophotometer at a

wavelength of 517 nm. The percentage of inhibition was calculated using the following formula:

$$\%\text{Inhbition} = \frac{\text{Ac-As}}{\text{Ac}} \times 100\%$$

Where:
Ac : absorbance of control
As : absorbance of sample

The Inhibition Concentration (IC_{50}) value was determined graphically by plotting the percentage of inhibition against the extract concentrations. A linear regression equation of the form y = bx + a was obtained from the plotted data, and this regression line was used to calculate the 50% Inhibition Concentration (IC_{50}).

2.6. Antibacterial Assay

The antibacterial and antifungal activity of the fucoxanthin fraction was evaluated using the Kirby–Bauer paper disc diffusion method. Pathogenic bacteria *Staphylococcus aureus* and *Vibrio alginolyticus* were cultured in Nutrient Broth (NB), while pathogenic fungal *Trichoderma* sp. and *Candida albicans* were cultured in Potato Dextrose Broth (PDB) media and agitated for 24 hours. The culture was then diluted to achieve turbidity equivalent to 0.5 McFarland standard. The pathogenic bacteria were inoculated onto Mueller Hinton Agar (MHA) plates using the spread plate method, while the pathogenic fungi were inoculated on Potato Dextrose Agar (PDA) media. Fucoxanthin fraction was dissolved in 10% DMSO and impregnated into a paper disc. Amoxicillin (30 µg/disc) was used as a positive control for antibacterial assay, while antifungal used. Nystatin (30 µg/disc) was used as the positive control. Blank paper disc impregnated with 10% DMSO was used as a negative control. The Petri dishes were incubated at room temperature. Antibacterial and antifungal activity was indicated by the presence of a clear inhibition zone around the paper discs. Observations were made at 24, 48 and 72 hours.

2.7. Sun Protection Factor Assay

The SPF (Sun Protection Factor) assay was conducted in vitro using UV-Vis spectrophotometry. A total of 0.1 g of fucoxanthin fraction was dissolved in an appropriate solvent to obtain a stock solution with a concentration of 1,000 ppm. Serial dilutions were then performed to achieve concentrations of 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1,000 ppm. The absorbance of each concentration was measured across the wavelength range of 290–320 nm at 5 nm intervals. The SPF value was calculated using the equation proposed by Mansur (Aloanis *et al.* 2021).

$$\text{SPF spectrophotometric} = \text{CF} \times f(x) = \sum_{290}^{320} \text{EE}\,(\lambda) \times \text{I}\,(\lambda) \times \text{Abs}\,(\lambda)$$

Where:
EE : erythemal effect spectrum
I : solar intensity spectrum
Abs : absorbance of sunscreen product
CF : correction factor (=10)

3. Results

3.1. Phytochemical Screening

The phytochemical screening results indicated that the crude extract of *Sargassum polycystum* contains a variety of secondary metabolite compounds, as shown in Table 1. The crude extract tested positive for all compounds including flavonoids, tannins, alkaloids, steroids, and saponins.

The results of the reaction between the crude extract and the reagent are shown in Figure 1, as indicated by a color change in the solution.

3.2. Characterization and Purification of Bacteria

Fucoxanthin isolation using the Open Column Chromatography (OCC) method resulted in the separation of compounds, indicated by color differences. The orange color represented the fucoxanthin fraction that had been successfully separated from non-polar compounds and other impurities, while the green color in the upper layer of the column indicated the presence of chlorophyll c in the crude extract. The UV-Vis spectrophotometry analysis showed an absorption peak at a wavelength of 447.5 nm. This validates the successful isolation of fucoxanthin (Figure 2).

3.3. Thin Layer Chromatography (TLC)

TLC analysis revealed the presence of three significant spots with yellow, orange, and green colors. The orange spot exhibited an Rf value of 0.93, the yellow spot had an Rf of 0.73, while the green spot showed an Rf of 0.31 (Table 2). The yellow and orange spots are strongly presumed to be carotenoid compounds, with the orange spot in particular suspected to be fucoxanthin.

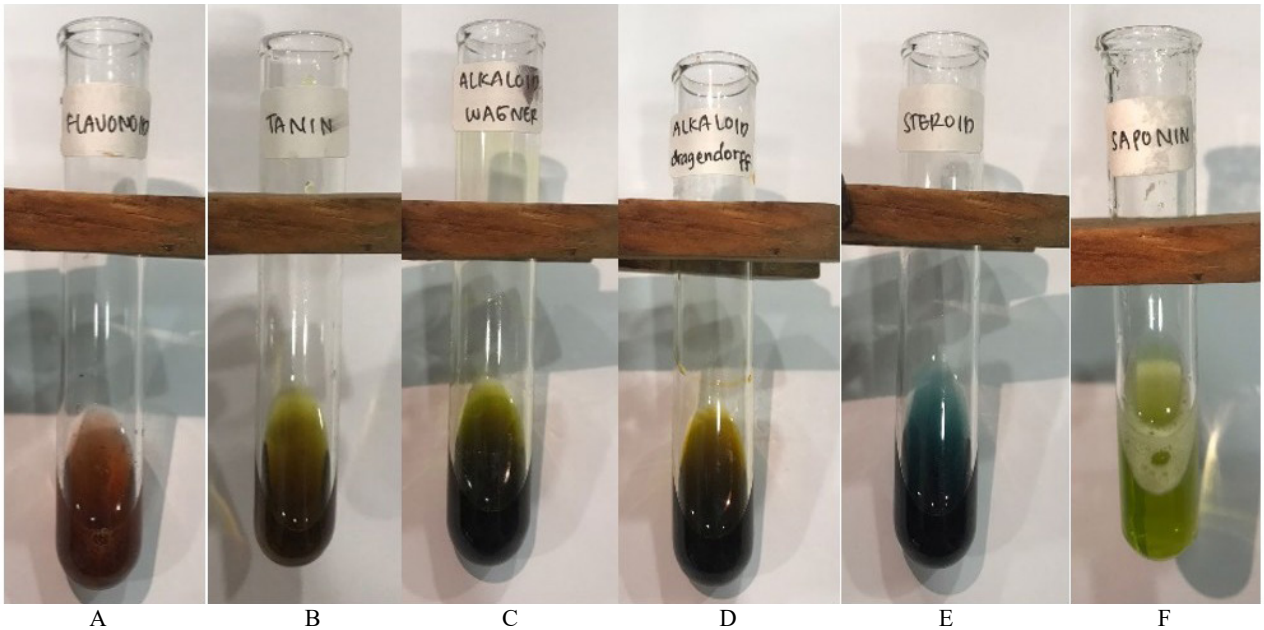
Table 1. Phytochemical screening result of *Sargassum polycystum* crude extract

Secondary metabolite	Indicator	Result
Flavonoid	Yellow/orange/red	+
Tanin	Green/blue	+
Alkaloid	-Wagner: orange suspension	+
	-Dragendorff: brownish-orange suspension	+
Steroid	Green	+
Saponin	Foam formed	+

+ (positive result), - (negative result)

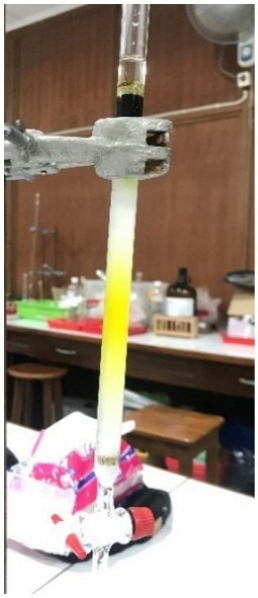
3.4. Antibacterial and Antifungal Assay

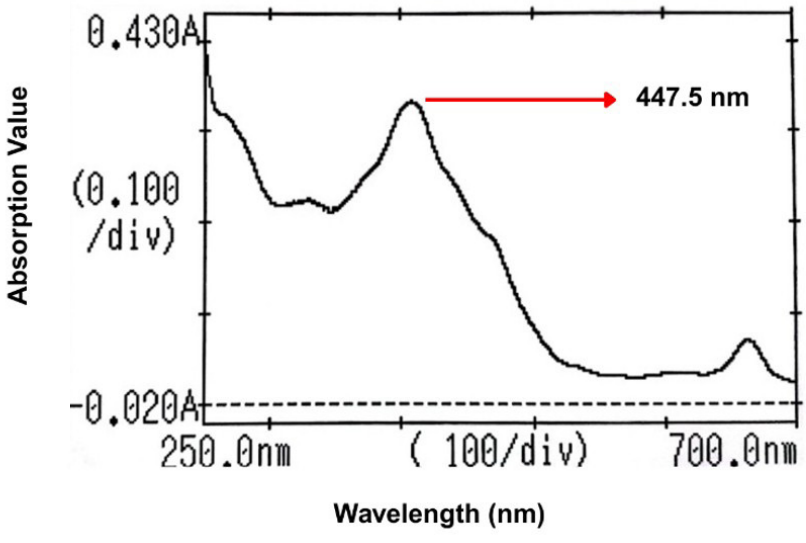
Antibacterial and antifungal activity in this assay is indicated by the presence of a clear zone surrounding the paper disc. The stronger the antibacterial activity, the larger the inhibition zone formed. Table 3 shows that the fucoxanthin fraction from *Sargassum polycystum* exhibits antibacterial activity against *Staphylococcus aureus*, but not against *Vibrio alginolyticus*. The fucoxanthin fraction exhibited antifungal activity against both *Trichoderma* sp. and *Candida albicans*.



A B C D E F

Figure 1. Results of phytochemical screening, (A) flavonoid test, (B) tannin test, (C) wagner's alkaloid test, (D) dragendorff's alkaloid test, (E) steroid test, (F) saponin test





A B

Figure 2. Fucoxanthin isolation and identification using (A) Open Column Chromatography (OCC), and (B) UV-Vis spectrophotometry

3.5. Antioxidant Activity

The DPPH method measures antioxidant activity through a color change, as antioxidants donate hydrogen atoms to stabilize DPPH free radicals, turning them from purple to yellow (Figure 3). The fucoxanthin fraction derived from *Sargassum* sp. in this study exhibited strong antioxidant activity, as shown in Table 4. RSA (Radical Scavenging Activity) of fucoxanthin fraction increased alongside increasing concentrations, ranging from 37.37% at 50 ppm to 88.95% at 200 ppm (Figure 4). The IC_{50} value represents the concentration of a compound required to inhibit 50% of free radical activity, serving as an indicator of its antioxidant effectiveness. In this study, the IC_{50} value of fucoxanthin was 67 ppm, indicating that half of the maximum antioxidant activity was achieved at this concentration, while the IC_{50} value of ascorbic acid was 11 ppm (Table 5).

Table 2. Thin layer chromatography results of the fucoxanthin fraction from *Sargassum polycystum*, Rf value, and spots in silica plate

TLC result		Retention factor (Rf)	Visible light
Visible light	UV 366		
		0.93	Orange
		0.73	Yellow
		0.31	Green

Table 3. Antibacterial and antifungal activity of fucoxanthin fraction against *S. aureus* and *V. alginolyticus*

Pathogenic bacteria	Time of observation		
	24 hour	48 hour	72 hour
S. aureus	+	+	+
V. alginolyticus	-	-	-
Trichoderma sp.	+	+	+
Candida albicans	+	+	-
Control (+)	+	+	+
Control (-)	-	-	-

+ (antibacterial activity observed), - (no antibacterial activity observed). Amoxicillin (30 µg/disc) was used as a positive control, 10% DMSO was applied to blank paper discs as a negative control

Table 4. Antioxidant activity of fucoxanthin

Concentration (ppm)	%RSA	IC_{50}
200	88.95	67
100	60.70	
50	44.38	
25	37.37	

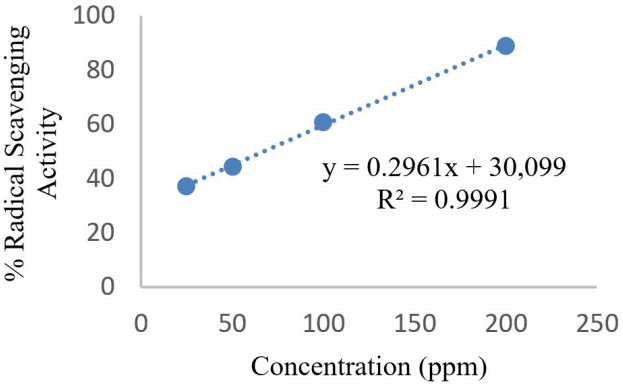
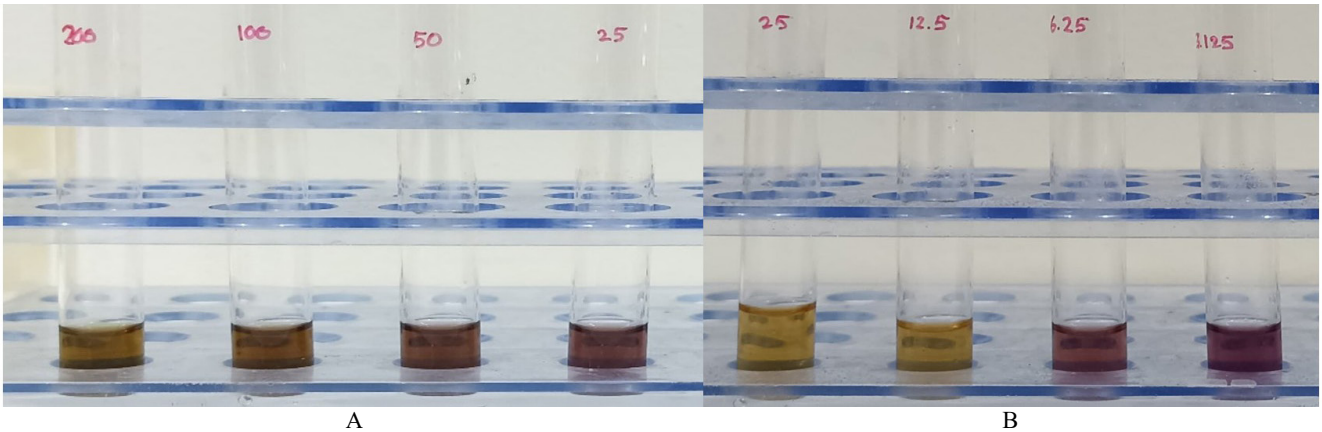


Figure 4. Graphic of antioxidant activity of fucoxanthin



A B

Figure 3. DPPH radical scavenging activity of (A) the fucoxanthin fraction and (B) ascorbic acid as a positive control

3.6. Sun Protection Factor (SPF) Assay

The fucoxanthin fraction exhibited an SPF value of 13.71, suggesting that it provides maximum protection against UVB radiation. The calculation and absorbance are shown in Table 6.

4. Discussion

The crude extract of *Sargassum polycystum* tested positive for all compounds including flavonoids, tannins, alkaloids, steroids, and saponins. Similar findings were reported by Meinita *et al.* (2024), where *Sargassum polycystum* from the waters of Java tested positive for alkaloids, saponins, flavonoids, and steroids. These results indicate the biological potential present in *Sargassum polycystum* seaweed. The simultaneous detection of these metabolite classes implies that *S. polycystum* synthesizes multiple bioactive compounds as adaptive responses to environmental stresses such as salinity, UV exposure, and microbial competition in the marine habitat. Flavonoids are secondary metabolite compounds known for their anticancer, anti-inflammatory, antioxidant, and antibacterial properties (Ekalu & Habila 2020). Alkaloids are utilized as antimicrobial agents due to their ability to inhibit enzyme activity involved in DNA and protein synthesis, ultimately leading to bacterial cell death (Meinita *et al.* 2024). The presence of saponins in the test was indicated by the formation of white foam, resulting from a hydrolysis reaction. Saponins exhibit antibacterial, anti-inflammatory properties, and are also known to modulate specific immune responses (Santiago *et al.* 2021). Tannins are polyphenolic compounds capable of binding to proteins and other molecules. Their ability to scavenge free radicals contributes to their high antioxidant activity. Steroids function as structural components and signaling molecules. They are widely used as anti-inflammatory agents and have the capacity to influence various metabolic processes in the body (Them *et al.* 2019).

Table 5. Antioxidant activity of ascorbic acid

Concentration (ppm)	%RSA	IC_{50}
25	74.25	11
12.5	52.78	
6.25	40.38	
3.125	35.35	

Table 6. SPF value of fucoxanthin fraction

Wavelength	EE x I	Abs	EE x I x Abs
290	0.0150	1.310	0.0197
295	0.0817	1.275	0.1042
300	0.2874	1.297	0.3728
305	0.3278	1.353	0.4435
310	0.18864	1.444	0.2692
315	0.0839	1.569	0.1316
320	0.0180	1.721	0.0310
Total			1.3719
Correction factor			10
SPF value			13.7187

Fucoxanthin was successfully isolated in this study using the open column chromatography (OCC) method, yielding an orange-colored solution. Subsequent UV–Vis spectrophotometric analysis revealed a characteristic absorption peak at 447.5 nm, consistent with the typical absorbance range reported for fucoxanthin (440–460 nm) in previous studies (Sulistiyani *et al.* 2021; Bárcenas-Pérez *et al.* 2021). Thin-layer chromatography (TLC) analysis further confirmed the presence of fucoxanthin, showing three distinct spots with yellow, orange, and green coloration. The orange spot exhibited an Rf value of 0.93 and was predicted to be fucoxanthin. This result is in agreement with the findings of Sari *et al.* (2022), who observed fucoxanthin as an orange spot with an Rf value of 0.96. In contrast, Sulistiyani *et al.* (2021) reported fucoxanthin with orange coloration and Rf values ranging from 0.44 to 0.46. The variation in Rf values among studies is likely attributable to differences in eluent type and ratio used during TLC separation (Roviqowati *et al.* 2019). Additionally, the presence of a green spot with an Rf value of 0.31 indicates residual chlorophyll (Munawaroh *et al.* 2019), suggesting that the fucoxanthin fraction obtained in this study still contains impurities and requires further purification. Future studies employing fucoxanthin standards are recommended to confirm the identity and purity of the isolated compound.

Fucoxanthin fraction of *S. polycystum* in this study showed antibacterial activity against *Staphylococcus aureus*, but no antibacterial activity was observed against *Vibiro alginolyticus*. The difference in antibacterial activity against the two tested pathogens is presumed to be due to the different bacterial types (Breijyeh *et al.* 2020). *Staphylococcus aureus* is a Gram-positive bacterium characterized by a thick peptidoglycan layer in its cell wall, but it possesses a relatively simple wall structure. In contrast, *Vibrio alginolyticus* is a Gram-negative bacterium, which has a more complex cell wall structure than Gram-positive bacteria, consisting of lipoproteins, polysaccharides,

and peptidoglycan (Epand *et al.* 2016). This structural complexity renders Gram-negative bacterial cell walls less permeable to external compounds. Fucoxanthin has been previously reported to exhibit antibacterial activity against several pathogenic bacteria, including *Staphylococcus epidermidis, Streptococcus agalactiae, Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cereus, Klebsiella pneumoniae*, and others (Karpiński & Adamczak 2019; Karpinski *et al.* 2021; Mumu *et al.* 2022). Fucoxanthin antibacterial activity was presumed to be caused by its ability to increase membrane permeability and inhibit nucleic acid synthesis in bacterial cells (Karpiński *et al.* 2021).

Fucoxanthin fraction in this study exhibited antifungal activity against both *Trichoderma* sp. and *Candida albicans*. The antifungal activity of the fucoxanthin fraction against *Trichoderma* sp. was consistently observed across all incubation periods (24–72 hours), suggesting a stable inhibitory effect. In contrast, inhibition of *C. albicans* was observed only at 24 and 48 hours, with reduced activity at 72 hours. This decline may indicate time-dependent degradation or reduced stability of active compounds in the fraction, or possible adaptive responses from *C. albicans*. The consistent inhibition of *Trichoderma* sp. suggests that fucoxanthin and associated bioactive compounds may possess broad-spectrum antifungal properties with varying degrees of persistence depending on fungal physiology. These findings align with previous reports describing the antifungal potential of fucoxanthin through mechanisms such as disruption of fungal cell membrane integrity, oxidative stress induction, and inhibition of spore germination (Al Aboody & Mickymaray 2020).

Fucoxanthin is a carotenoid commonly found in various types of algae and seaweed, known for its numerous health benefits. In this study, the % RSA (Radical Scavenging Activity) of fucoxanthin increased with increasing concentrations, ranging from 37.37% at 50 ppm to 88.95% at 200 ppm (Figure 4). A similar trend was observed for ascorbic acid, which served as the positive control (Figure 5). Yamauchi *et al.* (2024) similarly reported that antioxidant activity increases proportionally with extract concentration. Molyneux classified antioxidant activity into four categories: very strong (IC_{50} <50 ppm), strong (IC_{50} 50–100 ppm), moderate (IC_{50} 100–150 ppm), and weak (IC_{50} >150 ppm). Based on this classification, the fucoxanthin fraction in this study exhibited strong antioxidant activity, with an IC_{50} value of 67 ppm. This

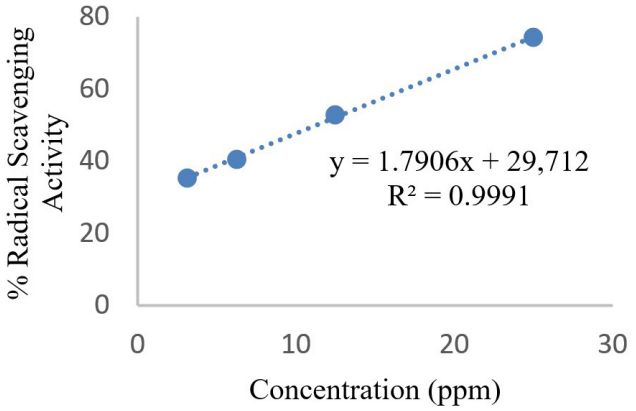


Figure 5. Graphic of antioxidant activity of ascorbic acid

result is comparable to that reported for *Sargassum duplicatum* (IC_{50} = 78.52 ppm), which also exhibited strong antioxidant capacity, and higher than that of *Phaeodactylum tricornutum* (IC_{50} = 201 ppm), classified as weak (Neumann *et al.* 2019; Savira *et al.* 2021). The antioxidant activity of fucoxanthin can be attributed to its unique chemical structure, which allows it to donate electrons and neutralize free radicals. This mechanism helps prevent oxidative damage to cells and molecules, thereby contributing to its health benefits (Fernandes & Mamatha 2023).

Sun Protection Factor (SPF) indicates the effectiveness of a sunscreen in protecting the skin from UVB radiation, which is responsible for sunburn, skin damage, and can contribute to skin cancer. According to the U.S. Food and Drug Administration (FDA), sunscreen efficacy is categorized as minimal (SPF 2–4), moderate (SPF 4–6), extra (SPF 6–8), maximal (SPF 8–15), and ultra protection (SPF >15). Sari *et al.* (2019) reported that the highest SPF value in sunscreen cream containing *Sargassum* sp. was achieved with the addition of 2% fucoxanthin fraction, yielding an SPF of 3.362. This value is lower than the Sun Protection Factor (SPF) of the fucoxanthin fraction determined in the present study, which reached 13.71 and is classified within the maximal protection category (Table 6). Other studies have found that sunscreen formulations incorporating solid lipid nanoparticles combined with fucoxanthin exhibit higher SPF ratings (Rajasekar *et al.* 2024). These findings suggest that the formulation and methods used can significantly influence the resulting SPF value.

In conclusion, phytochemical screening revealed that the *S. polycystum* crude extract contains secondary metabolites including flavonoids, alkaloids, tannins, steroids, and saponins. The fucoxanthin fraction

was successfully isolated from the brown seaweed *Sargassum polycystum*. This fucoxanthin fraction demonstrated antibacterial activity against the pathogen *Staphylococcus aureus*, as well as antifungal activity against *Trichoderma* sp. and *Candida albicans*. Its antioxidant activity was classified as strong, with an IC_{50} value of 67 ppm, while its Sun Protection Factor (SPF) was categorized as maximal, with a value of 13.71. These findings highlight the value of fucoxanthin as a natural candidate for pharmaceutical and cosmetic applications. Future studies should focus on further purification processes, bioavailability testing, formulation stability, and *in vivo* validation to support its development into effective and safe bio-based products.

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