

# Anti-obesity Activity of Hexane Fraction and Fucoxanthin Isolate from *Sargassum polycystum* as an Inhibitor of Pancreatic Lipase Enzyme *in Vitro*

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## Abstract

**Background.** Obesity has become a global epidemic, causing health problems that need to be addressed immediately. The long-term use of synthetic drugs causes side effects. Therefore, a safe and therapeutically effective anti-obesity agent from natural ingredients is needed. Marine organisms are a potential source of bioactive compounds with anti-obesity activity. Compounds in algae, mainly brown algae, have been proven to have potential anti-obesity activity, including fucoxanthin. The study aims to determine the anti-obesity activity of the fractions and isolates of fucoxanthin from *Sargassum polycystum* as an inhibitor of pancreatic lipase enzyme *in vitro*.

**Methods.** The extraction method uses an ultrasonic with an acetone solvent. Liquid-liquid fractionation with hexane solvent, hexane fraction was separated using TLC with a mobile phase of hexane: ether: acetone (6:3:2) to obtain the TLC isolate. Purity tests include three-phase TLC and two-dimensional TLC, followed by FTIR identification and UV-Vis spectrophotometry. The hexane fraction and fucoxanthin isolate were then subjected to *in vitro* pancreatic lipase inhibition IC50 testing.

**Results.** The study results showed that in the purity test, one orange spot was obtained, with the spectrum of the fucoxanthin isolates at a wavelength of 446.90 nm and had the same pattern as the standard. The FTIR results of fucoxanthin isolates from *Sargassum polycystum* are the same as standard fucoxanthin, having the functional groups OH, CH, allene, C=O, C=C, CH<sub>2</sub>, C-O acetate, C-O-C, and C=C. The pancreatic lipase inhibition activity IC50 values for orlistat, the hexane fraction, and the fucoxanthin isolate were 20.53 ± 1.97; 123.27 ± 1.35, 105.72 ± 1.36 µg/mL, respectively.

**Conclusion.** The fucoxanthin compound from *Sargassum polycystum* has the potential as an anti-obesity agent.

**Keywords:** anti-obesity, fucoxanthin, *in vitro*, pancreatic lipase enzyme, *Sargassum polycystum*

## 1. Introduction

The increasing obesity worldwide has significant impacts on health disorders and a decline in quality of life. Obesity can occur due to an imbalance between energy intake and the energy expended by the body. Thus, obesity contributes significantly to the occurrence of cardiovascular disease, type 2 diabetes mellitus, cancer, and osteoarthritis worldwide (Septiyanti, 2020). According to WHO (2022), cases of obesity amount to 2.5 billion adults (18 years and over) who are overweight, of whom 890 million people live with obesity. Additionally, 37 million children under 5 years of age are overweight, and more than 390 million children and adolescents aged 5-19 years are overweight. In Indonesia, based on the results of the Basic Health Research (Riskesdas) 2018, the prevalence of obesity among the population aged > 18 years increased from 15.4% (2013) to 21.8% (2018). Therapeutic interventions using weight loss medications, accompanied by diet and exercise, are one of the treatment

and management options for obesity. One of the currently approved and available anti-obesity drugs on the market is Orlistat, a synthetic pancreatic lipase inhibitor. Orlistat works by inhibiting the lipase enzyme in the digestive tract, thereby blocking the absorption of fats derived from triglycerides. The use of Orlistat over a certain period can cause side effects (Sharma *et al.*, 2005). Therefore, a safe and therapeutically effective anti-obesity treatment from natural products is necessary (Wan-Loy, 2016). One potential option that can be developed is a marine natural product.

Marine organisms are a potential source of bioactive compounds with anti-obesity activity. Most of the compounds with potential anti-obesity properties are produced by marine algae, especially brown algae. Compounds from algae that have been proven to have anti-obesity activity include phlorotannin, fucoxanthin, algininate, and fucoxanthin, (Wan-Loy, 2016). Fucoxanthin is an orange carotenoid pigment that is abundant in *Sargassum* (Sulistiyani *et al.*, 2021).

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Fucoxanthin, as an anti-obesity agent, has been studied from *Sargassum echinocarpum*, showing effects of increasing adiponectin and reducing tumor necrosis factor (Firdaus et al., 2015). Murakami (2021) stated that the compound fucoxanthin in *Sargassum horneri* can inhibit obesity caused by a high-fat (HF) diet and metabolic disorders in rats, with the anti-obesity effect related to the mechanism of inhibiting pancreatic lipase. The anti-obesity activity of fucoxanthin has been proven through tests on mice and rats, where the compound was able to reduce body weight by 5–10% in the test animals (Nurcahyanti, 2007). Miyashita and Hosokawa (2017) reported that fucoxanthin can increase glucose utilization in skeletal muscle by increasing the expression of glucose transporter 4 (GLUT4) and promoting its translocation to the cell membrane from the cytosol. To determine the mechanism of action of a compound, as well as to complete *in vivo* animal data, it is important to conduct *in vitro* testing. This study aims to determine the anti-obesity activity of the hexane fraction and fucoxanthin isolate from *Sargassum polycystum* *in vitro* through the mechanism of pancreatic lipase inhibition.

## 2. Methods

### 2.1. Material

The main material in this study was fresh *Sargassum polycystum* from Montong Village, Sumbawa, West Nusa Tenggara. The chemicals used were 100% acetone (Merck), n-hexane (Merck), ether, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA), methanol (Merck), 1% DMSO (Merck), Porcine pancreatic Lipase (PPL) (Sigma-Aldrich, USA), pNPB (para nitrophenyl butyrate) (Sigma-Aldrich, USA), phosphate buffer pH 7.2, distilled water, silica gel GF 254 (Merck), UV-Vis Spectrophotometer (Shimadzu 1700, Japan), FTIR (Agilent Technologies Carry 630 FTIR, USA).

### 2.2. Extraction of *Sargassum polycystum*

Fresh *Sargassum polycystum* that has undergone wet sorting is extracted using the ultrasonic method. First, weigh 25.0 grams of the sample, cut it into small pieces, and grind it with a mortar and pestle. Then, place it into a beaker, add 100 mL of 100% acetone, and perform extraction using the ultrasonic method for 20 minutes in a dark room protected from light. After that, filter it and perform ultrasonic extraction again by adding 50 mL of solvent until the extract is colorless. The macerate is collected and concentrated using a rotary evaporator at 30°C then evaporated with nitrogen gas (Maulina, 2018; Sibero, 2022).

### 2.3. Preparation of *Sargassum polycystum* Fraction

*Sargassum polycystum* weighing 200.0 mg was added to acetone solvent and placed in a separating funnel, then hexane solvent was added. If necessary, a saturated salt solution and tap water were added to form two layers (Limantara, 2011). Hexane fraction was taken and evaporated with N<sub>2</sub> gas.

### 2.4. Identification pigment

The hexane fraction was used to identify the fucoxanthin pigment by comparing it to standard fucoxanthin using thin-layer chromatography, with a

mobile phase mixture of hexane, ether, and acetone (6:3:2) and a stationary phase of silica gel GF 254 (Kusmita et al., 2023).

### 2.5. Isolation using Preparative Thin Layer Chromatography (PTLC)

Several fractions were dissolved in acetone solvent and spotted as bands on the PTLC plate. Separation was performed using a mobile phase consisting of a hexane, ether, and acetone mixture in a ratio of 6:3:2 (Kusmita et al., 2023). The sample was spotted in the form of a band. After the chamber was saturated with the mobile phase, the PTLC plate was placed into the chamber. Elution was performed up to the elution limit, after which the PTLC plate was removed and air-dried. The formed strip was scraped, and purity tests were conducted using three mobile phases and two-dimensional PTLC, followed by identification using UV-Vis spectrophotometry and FTIR.

### 2.6. Purity Test with Three Mobile Phases

The obtained isolate was tested for purity using the thin-layer chromatography (TLC) method by spotting the sample on a silica gel GF 254 nm TLC plate. Three TLC plates were placed in three chambers that had been saturated with different mobile phases: a mixture of hexane:ether:acetone (6:3:2); hexane:acetone (6:4); and chloroform:ethanol (7:3). Each plate was eluted until the elution distance was reached, then the color of the spots was observed, and the R<sub>f</sub> value was calculated. If a single orange spot appeared, the sample was positive for containing fucoxanthin. If the purity test was conducted using several mobile phases (at least three) and the elution results still showed one spot, the tested sample was considered pure (Abdullah et al., 2021).

### 2.7. Purity with Two-Dimensional Chromatography

A number of fucoxanthin isolates obtained from the PTLC results were spotted on a 10×10 cm TLC plate and then eluted with a mobile phase mixture of hexane:ether:acetone (6:3:2). The second elution was performed by rotating the plate 90° counterclockwise with a hexane:acetone (6:4) mobile phase mixture (Salatiana et al., 2023).

### 2.8. Identification of fucoxanthin using UV-Vis Spectrophotometry.

A number of fucoxanthin isolates obtained from PTLC results were subjected to spectral absorption analysis to determine the spectral profile and standard absorption of reference fucoxanthin by diluting the PTLC isolates in 3 mL of acetone within a wavelength range of 300–600 nm (Sulistiyani et al., 2021).

### 2.9. Identification of Fucoxanthin using FTIR Spectrophotometry

A quantity of 0.5–1.5 mg of the substance (isolate) was placed into the sample holder and then scanned with FTIR. The results obtained were analyzed based on functional groups at specific wave numbers and compared with the standard reference of fucoxanthin (Riwant, 2019).

### 2.10. Lipase Inhibition Activity Test and IC<sub>50</sub> of Fucoxanthin Fractions and Isolates

The anti-obesity activity test as a lipase inhibitor on the hexane extract and fraction of *Sargassum polycystum* was conducted in 96-well plates using an ELISA reader. The

enzyme stock concentration was set at approximately 0.1 mg/mL for every 1.0 mg of solid PPL powder dissolved in 1 mL of buffer solution (a). Hexane fractions and isolates were prepared at concentrations of 9.375, 18.75, 37.5, 75, and 150 µg/mL (b). p-NPB was dissolved in 1% DMSO (c) and then diluted with a 50 mM phosphate buffer (pH 7.2) at a concentration of 0.5% to obtain a final concentration of 2.5 mM in 100 µL (d). Solutions (a) + (b) + (d) were mixed and incubated at 37°C for 10 minutes. Orlistat reference standards at concentrations of 40, 20, 10, 5, and 2.5 µg were used as positive controls, while a negative control without an inhibitor was prepared using 1% DMSO. One unit of activity is defined as the reaction rate that produces µmol p-NPB at 37°C. The inhibition of anti-obesity activity by lipase inhibitors is expressed as the percentage decrease in activity when PPL is incubated with the test compound. Lipase inhibition is calculated based on the formula equation (Abd-Rahman, 2017, Babu et al., 2021).

$$\% \text{ Lipase Inhibitory Activity} = 100 - \left( \frac{B-a}{A-a} \right) \times 100$$

#### Information:

A = absorbance of the negative control with the addition of enzyme and substrate

a = absorbance of the negative control without addition of enzyme and substrate

B = absorbance of the inhibitor with addition of enzyme and substrate

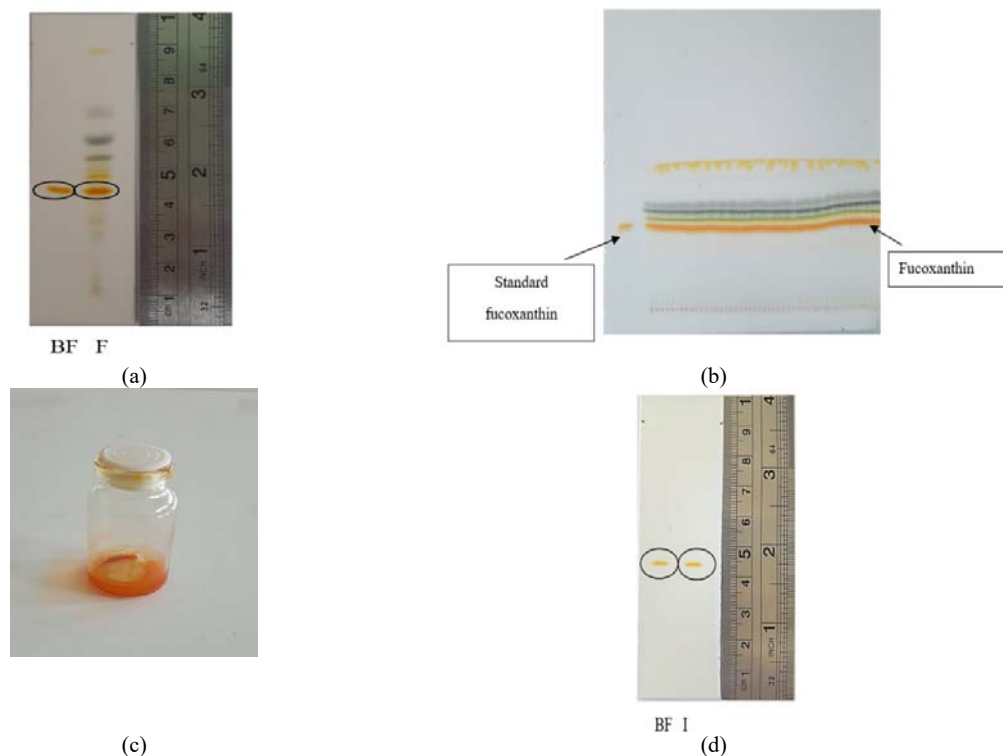
b = absorbance of the inhibitor without addition of enzyme and substrate

Inhibitor = fraction samples, isolates, and positive control.

### 3. Results

#### 3.1. Extraction, identification and isolation of fucoxanthin

The yield of the ultrasonic extraction method was  $1.30 \pm 0.11\%$ . In this study, a fraction yield percentage obtained was  $55.58 \pm 2.22\%$ . The hexane fraction was tested using TLC with a mobile phase mixture of hexane:ether:acetone (6:3:2) to determine the presence of the target compound, fucoxanthin. The results showed the presence of fucoxanthin, indicated by an orange spot with an R<sub>f</sub> value of 0.56, compared to the standard fucoxanthin (Figure 1a). The concentrated hexane fraction was then used for the isolation process using the PTLC method (Figure 1b). Before use, PTLC plates were activated by oven-drying at 105°C for 30 minutes. After activation, the fraction dissolved in acetone and the standard fucoxanthin dissolved in acetone were spotted. The standard was applied as a single spot, while the fraction was applied in the form of a band and subsequently eluted using a mobile phase of hexane:ether:acetone (6:3:2). After elution, the separation resulted in bands of orange, green, gray, and yellow colors. The standard fucoxanthin on PTLC showed an orange color, so the corresponding orange band was collected. From the PTLC isolation of 0.1682 grams of the fraction, 0.0264 grams of pigment isolate was obtained, yielding 15.70% (Figure 1c).

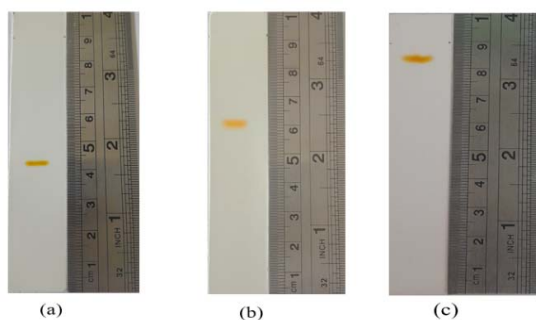


**Figure 1.** Identification and isolation fucoxanthin, (a) TLC Results of Fucoxanthin Standard (BF) Comparison Hexane Fraction (F); (b) Isolation fucoxanthin with Preparative-TLC; (c) Fucoxanthin isolate obtained from PTLC; and (d) TLC results of the fucoxanthin standard (BF) compared to the fucoxanthin isolate (I).

The pigment isolate from PTLC was subjected to TLC and compared with the standard fucoxanthin, as shown in Figure 1d. The isolate from the preparative thin-layer chromatography produced an orange spot with an Rf value of 0.55, and the standard fucoxanthin comparison also showed an orange spot with an Rf value of 0.55.

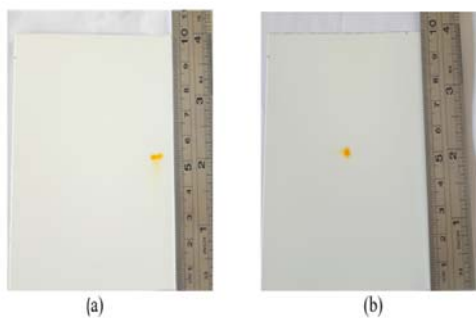
### 3.2. Purity Test with Three Different Mobile Phase and Two-Dimensional Chromatography

Fucoxanthin isolate was tested for purity using the TLC method with three different mobile phases: mobile phase (a), a mixture of hexane:ether:acetone (6:3:2), yielded an Rf value of 0.54 with one orange spot; mobile phase (b), a mixture of hexane:acetone (6:4), yielded an Rf value of 0.77 with one orange spot; and mobile phase (c), a mixture of chloroform:ethanol (7:3), yielded an Rf value of 0.98 with one orange spot (Figure 2).



**Figure 2.** Results of the Purity Test of Fucoxanthin Compound. Stationary phase: Silica Gel GF254, mobile phase (a) hexane : ether : acetone (6:3:2), (b) hexane : acetone (6:4), (c) chloroform : ethanol (7:3).

Purity test results were conducted using two-dimensional TLC with two different mobile phases. The PTLC isolate was spotted on a 10x10 TLC plate and eluted with a mobile phase of hexane:ether:acetone (6:3:2) until the elution limit was reached, resulting in an Rf value of 0.66 with one orange spot (Figure 3).

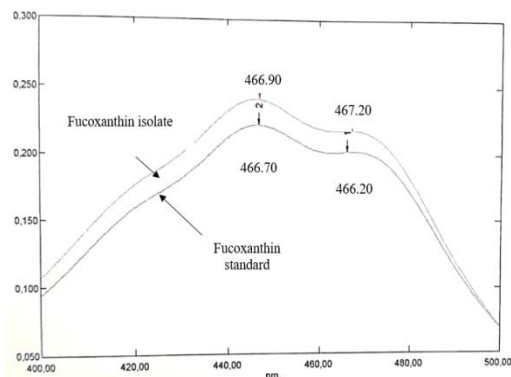


**Figure 3.** Results of Two-Dimensional TLC of Fucoxanthin Isolate, First Elution (a) and Second Elution (b)

The second elution used a hexane:acetone (6:4) mobile phase, performed by rotating the plate 90° counterclockwise, resulting in an Rf value of 0.66 with a single orange spot.

### 3.3. Identification of fucoxanthin using UV-Vis Spectrophotometry.

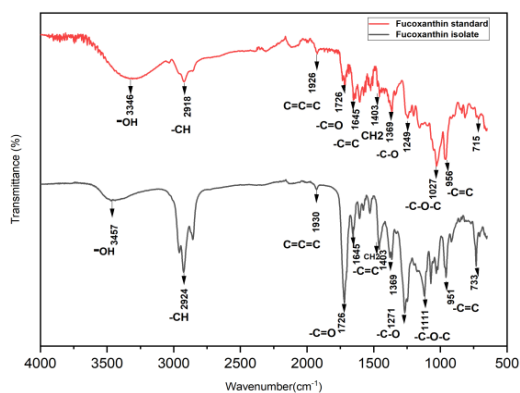
The fucoxanthin compound from the preparative thin layer chromatography (PTLC) was identified using UV-Vis spectrophotometry at a wavelength of 400–500 nm, with minimal absorption in other regions or the presence of two peaks. The fucoxanthin compound, when compared with the standard fucoxanthin, showed a similar spectrophotometric profile. The maximum wavelength for the fucoxanthin isolate was 466.90 nm, while the standard fucoxanthin was 466.70 nm as shown in Figure 4.



**Figure 4.** Spectrum of the fucoxanthin isolate and the fucoxanthin standard.

### 3.4. Identification of Fucoxanthin using FTIR Spectrophotometry

Spectrum identification was also performed using FTIR spectroscopy to observe the absorption bands characteristic of the functional groups of the fucoxanthin compound (Figure 5). The functional groups of the fucoxanthin isolate and the fucoxanthin standard are shown in Table 1.



**Figure 5.** Standard absorption spectrum of fucoxanthin with the *Sargassum polycystum* fucoxanthin isolate.

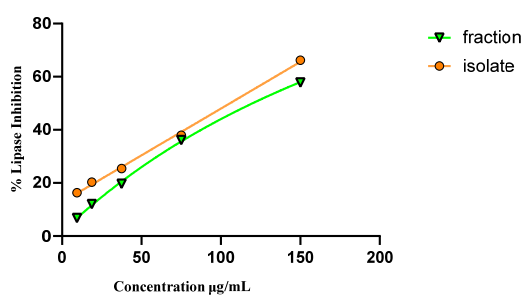
**Table 1.** FTIR Results of Fucoxanthin standard (A) with *Sargassum polycystum* Fucoxanthin Isolate (B).

| No. | Functional group          | Vmax (cm <sup>-1</sup> ) (A) | Vmax (cm <sup>-1</sup> ) (B) | Vmax (cm <sup>-1</sup> ) fucoxanthin standard (Rajauria et al., 2013) |
|-----|---------------------------|------------------------------|------------------------------|---|
| 1.  | OH                        | 3346                         | 3457                         | 3441  |
| 2.  | CH                        | 2918                         | 2924                         | 2918  |
| 3.  | Allene                    | 1926                         | 1930                         | 1929  |
| 4.  | C=O, ester                | 1726                         | 1726                         | 1732  |
| 5.  | C=C conjugated            | 1674                         | 1674                         | 1649  |
| 6.  | CH <sub>2</sub>           | 1403                         | 1403                         | 1403  |
| 7.  | C-O, acetat               | 1369, 1249                   | 1369, 1271                   | 1362  |
| 8.  | C-O-C                     | 1027                         | 1111                         | 1172  |
| 9.  | C=C, trans disubstitution | 956, 715                     | 951, 733                     | 958   |

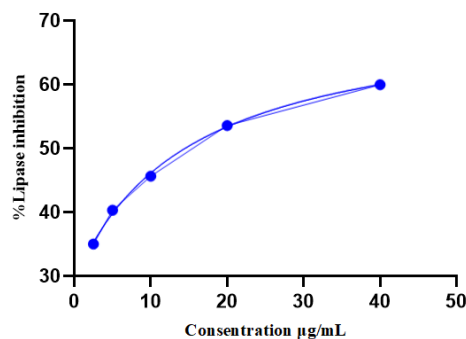
### 3.5. Lipase Inhibition Activity Test and IC50 of fractions and fucoxanthin Isolate.

The inhibition test of the pancreatic lipase enzyme was conducted in vitro using the p-NPB substrate. The principle of this method is that the lipase enzyme hydrolyzes the p-NPB substrate into p-nitrophenol and butyrate. The produced p-nitrophenol exhibits a yellow color, and its absorbance can be measured at a wavelength of 405 nm. A higher absorbance indicates higher pancreatic lipase activity in the sample. The test was performed on the hexane fraction and fucoxanthin isolate, along with the orlistat reference standard at various concentrations.

The lipase enzyme inhibition activity exhibited varying effects across five different concentration variants (9.375, 18.75, 37.5, 75, and 150 µg/mL) of the hexane fraction, fucoxanthin isolate, and orlistat, which served as the standard or positive control. This can be seen in Figure 6a, which shows that the inhibitory activity increases with the addition of concentration. The highest lipase inhibition activity was observed at a concentration of 150 µg/mL in the hexane fraction at 57.86 ± 0.68% and in the fucoxanthin isolate at 66.23% ± 1.29%, while orlistat exhibited an inhibition activity of 59.97 ± 2.00% at a lower concentration of 40 µg/mL in the hexane fraction and fucoxanthin isolate, as shown in Figure 6b.



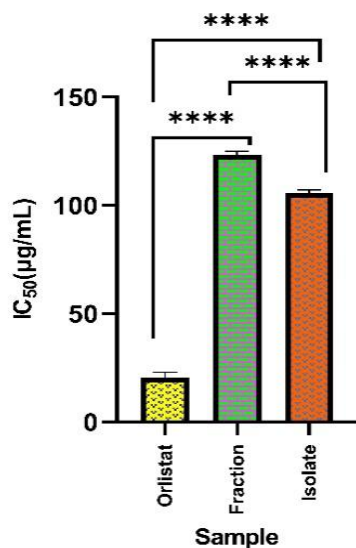
(a)



(b)

**Figure 6.** Lipase inhibition hexane fraction and fucoxanthin isolate (a) and orlistat standard (b).

The IC<sub>50</sub> value represents the concentration of a sample required to inhibit 50% of pancreatic lipase enzyme activity. The smaller the IC<sub>50</sub> value, the greater is the inhibition activity against pancreatic lipase enzyme. The IC<sub>50</sub> values of the hexane fraction, fucoxanthin isolate, and the reference standard orlistat as a positive control can be seen in Figure 7.



**Figure 7.** IC<sub>50</sub> values of orlistat, fractions, and fucoxanthin isolate.

The IC<sub>50</sub> of the fucoxanthin isolate ( $105.72 \pm 1.36$  µg/mL) demonstrates better pancreatic lipase inhibition activity compared to the hexane fraction ( $123.27 \pm 1.35$  µg/mL).

#### 4. Discussion

The ultrasonic extraction method enhances mass transfer, which damages the cell walls of the material, leading to increased release of dissolved substances in the liquid-liquid extraction process by generating cavitation within the material. During sonication, numerous bubbles form and eventually collapse. As the number of cavitation bubbles increases, the release of dissolved substances also rises (Oliyaeei, 2021). The extract of *Sargassum polycystum* was fractionated using hexane as the solvent. The purpose of fractionation is to separate compounds based on the polarity of the solvent used. Hexane solvent will attract fucoxanthin compounds that have non-polar properties (Britton, 1995). The hexane phase was concentrated using N<sub>2</sub> gas to prevent oxidation (Rodriguez-Amaya, 2004).

Identification of the hexane fraction by TLC showed that the spots and R<sub>f</sub> were the same as the standard. Sulistiyani et al. (2021) showed that the fucoxanthin compound has yellow spots and the same R<sub>f</sub> value as the standard. Fucoxanthin isolation was carried out using the PTLC method which produced the same spots as the standard. Based on the TLC results, the pigment isolate from PTLC exhibited the same orange color and R<sub>f</sub> value as the standard fucoxanthin. Therefore, the isolate from the preparative TLC indicates the presence of fucoxanthin.

The obtained fucoxanthin isolate was then tested for its purity. Purity test with three mobile phase different and

two-dimensional chromatography. The TLC results using three different mobile phases each yielded one orange spot, so the TLC results with the three different mobile phases can be considered pure. Based on the analysis results using two-dimensional TLC, a single orange spot was produced, indicating that the isolate can be considered pure (Ningrum, 2018).

Pure fucoxanthin isolates were identified using UV-Vis and FTIR spectrophotometers. Fucoxanthin is a group of carotenoids that has absorption at a wavelength of 400-600 nm (Britton, 1995). The isolate obtained has the same pattern and maximum wavelength as the standard. The resulting spectrum pattern is consistent with the research of Sulistiyani et al. (2021) and the literature by Jeffrey (1997). The FTIR results of the fucoxanthin isolate from *Sargassum polycystum*, when compared with the standard fucoxanthin, exhibit the same wave number range as reported in previous studies. The FTIR analysis results are in accordance with the findings of Rajauria et al. (2013) which shows that fucoxanthin has OH, CH, allene, C=O, C=C, CH<sub>2</sub>, C-O acetate, C-O-C, and C=C functional groups.

After ensuring that the isolate obtained was fucoxanthin, pancreatic lipase inhibition activity testing was performed. According to Ado et al. (2013) and Fernando et al. (2019), pancreatic lipase inhibition activity is classified as weak (<40%), moderate (41%–80%), or strong (>80%). Based on Figure 6, the hexane fraction and fucoxanthin isolate fall into the moderate category, while the reference standard orlistat, as a positive control, falls into the strong category due to its lower test concentration. Based on Figure 7, the fucoxanthin isolate exhibits better lipase inhibition activity than the hexane fraction. Natural inhibitors of pancreatic lipase for obesity treatment include several classes of compounds that have been proven to exert anti-obesity effects through the lipase inhibition pathway, such as polyphenols, flavonoids, proanthocyanidins, catechins, saponins, and triterpenoids. Statistical analysis show that the activity of fucoxanthin is different or significant. These results suggest that, in addition to phenolic compounds, flavonoids, proanthocyanidins, catechins, saponins, and triterpenoids, fucoxanthin isolate also possesses lipase inhibitory activity (De La Garza et al., 2011).

Orlistat has a mechanism of action that reversibly inhibits gastric and pancreatic lipase. This lipase plays an important role in the digestion of dietary fats. Lipase works by breaking down triglycerides into free fatty acids and monoglycerides that can be absorbed by the body. Orlistat covalently binds to the serine residue and active site of lipase and inactivates it. Inactivation of lipase prevents the hydrolysis of triglycerides, so free fatty acids are not absorbed. The majority (more than 99%) of the drug binds to plasma proteins (lipoproteins and albumin being the main binding proteins (Bansal, 2020).

Additionally, orlistat has an ester group that contributes to the chemical and physical properties of the drug, such as solubility and distribution in the gastrointestinal tract. The long aliphatic chain in orlistat helps it interact with lipid components in food, allowing the drug to be close to the lipase enzyme substrate and enhancing inhibition efficiency. In comparison, fucoxanthin acts as an anti-obesity agent by inhibiting the pancreatic lipase enzyme, thereby preventing the breakdown of triglycerides into

fatty acids and glycerol. Fucoxanthin has a key structural feature, namely the carotenoid end of the polyene chromophore, which contains an allenic bond and two hydroxyl groups (Gammone, 2015).

## 5. Conclusion

Fucoxanthin isolate from *Sargassum polycystum* has the same characteristics as standard fucoxanthin. The IC50 values for pancreatic lipase inhibition as an anti-obesity agent of orlistat, hexane fraction, and fucoxanthin isolate were  $20.53 \pm 1.97$ ;  $123.27 \pm 1.35$ ;  $105.7 \pm 1.36$   $\mu\text{g/mL}$ , respectively, showing significant differences. This study only looked at the anti-obesity activity of fucoxanthin through pancreatic lipase enzymes. It is necessary to continue this study on the activity of fucoxanthin as an anti-obesity agent through other mechanisms.

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