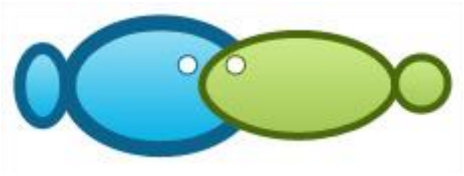

The effect of different drying methods on antioxidant compounds and fucoxanthin content of brown seaweed from the intertidal zone of West Aceh, Indonesia

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Abstract. *Sargassum ilicifolium* and *Padina australis* are two of the brown seaweed found on the coast of West Aceh. This research aimed to determine the antioxidant action and fucoxanthin content of *S. ilicifolium* and *P. australis*. The samples used in this study include fresh, freeze-dried, and sun-dried *S. ilicifolium* and *P. australis*. Each sample was extracted using ethanol 96% for 12 hours. The partition was undertaken using n-hexane and methanol. The antioxidant activity of the extract was analysed using the DPPH radical scavenging activity method. Fucoxanthin content in brown seaweed extract was analysed using high-performance liquid chromatography (HPLC) and mass spectrophotometry. The result revealed that the antioxidant action (IC_{50}) of *S. ilicifolium* extract ranged from 799.00±1.66 ~ 1884.48±425.64 µg mL^{-1}, and *P. australis* extract ranged from 171.50±43.13 ~ 460.88±78.09 µg mL^{-1}. Fucoxanthin of brown seaweeds *S. ilicifolium* and *P. australis* possesses high antioxidant activity in a fresh sample of *S. ilicifolium* (IC_{50} 799.00±1.66 µg mL^{-1}) and the freeze-dried sample of *P. australis* (IC_{50} 171.50±43.13 µg mL^{-1}). The presence of fucoxanthin from brown seaweed *S. ilicifolium* and *P. australis* indicated the presence of a monoisotopic ion molecule at the m/z value of 640.977 [M+H].
Key Words: freeze-drying, *Padina australis,* sample preparation, *Sargassum ilicifolium,* sun-drying.

Introduction. Indonesia has a diverse range of biological resources, including a large amount of brown seaweed. Secondary metabolites such as phenolic compounds, flavonoids, terpenoids, tannin, terpenoids, saponins, alkaloids, and sterols are produced by brown seaweed (Baleta et al 2017). Apart from these components, seaweed also has important bioactive compounds, such as pigments. Seaweed pigments have benefits in the field of health. Brown seaweed contains carotenoid pigment especially fucoxanthin and has a high total phenolic content (Ktari et al 2021; Lailatussifa et al 2017).

Several antioxidants, including fucoxanthin, a carotenoid, have been found in brown seaweed (Tabakaeva & Tabakaev 2019), phlorotannins (Jesumani et al 2019), and tocopherols (Wang et al 2016). Antioxidant activity have been observed in seaweed extracts (Ktari et al 2021). In brown seaweeds, fucoxanthin is the main carotenoid

(Tabakaeva & Tabakaev 2019). The most prevalence carotenoid discovered in seaweeds is fucoxanthin, but research into its role in the antioxidant system is limited (Ktari et al 2021). Although fewer studies have been conducted on carotenoid's physiological effects on seaweeds, fucoxanthin has lately admiring much attention as its strong antioxidant activities, which have shown considerable anti-inflammatory, anti-obesity, and anti-cancer benefits (Ktari et al 2021).

Sargassum ilicifolium and *Padina australis* are two of the brown seaweed found on the coast of West Aceh. *Sargassum* has been shown to have human-beneficial bioactivity. This species has already been utilized by the coastal community as *Sargassum* tea (Pratiwi & Husni 2021). *P. australis* possesses antioxidant activity (Nursid & Noviendri 2017). Therefore, fucoxanthin might be used as an alternative to substitute synthetic antioxidants. The objective of this study was to investigate the antioxidant action and fucoxanthin content of *S. ilicifolium* and *P. australis* from the intertidal zone of West Aceh with different preparations including fresh, freeze-dried, and sun-dried samples.

Material and Method

Materials. The materials used in this research were *S. ilicifolium* and *P. australis* obtained from Lhok Bubon West Aceh, Indonesia. The chemicals used in this study were ethanol (Merck, USA), methanol (Merck, USA), n-hexane (Merck, USA), ethyl acetate (Merck, USA), and DPPH reagent (Merck, USA).

Preparation of sample. The brown seaweeds were collected from the coast of West Aceh, Aceh Province, Indonesia in August 2018. The seaweed samples were amassed by carving below thallus near holdfast with scissors. For morphological identification, the samples were cleaned and divided. Then the remaining samples were deposited in a coolbox to be delivered to the laboratory. Brown seaweed identification was conducted at the Faculty of Fisheries and Marine Science, Teuku Umar University, Aceh. The samples that were used in this study include fresh *Sargassum ilicifolium*, freeze-dried *Sargassum ilicifolium*, sun-dried *Sargassum ilicifolium*, fresh *Padina australis*, freeze-dried *Padina australis*, and sun-dried *Padina australis*. Freeze-dried samples were prepared by freeze-drying (Martin Christ, Germany) for 10 hours, and dried samples were prepared by sun drying for 3-4 days.

Extraction and partition. Each sample (500 g) was minced little by little and extraction took place using ethanol 96% for 12 hours. The macerated samples were filtered with filter paper then evaporated with a vacuum-rotary evaporator (Buchi Korean Inc). The crude extract was freeze-dried. The partition was undertaken using n-hexane and methanol. Firstly, the ethanol extract was diluted in methanol, then homogenized in a separating funnel, and methanol-n-hexane was added with a ratio of 1:1. Mixed dilutions were shaken for 3-4 minutes. Then, incubation took place for 12-14 hours. There were two layers formed: the upper layer was n-hexane fraction, and the bottom layer was methanol fraction that contained fucoxanthin and polyphenol compounds. Methanol extract was obtained using column chromatography of normal phase with the stationary phase (SiO_2) and mobile phase hexane-ethyl acetate-methanol. The fraction of methanol used was approximately 1 g. Elution was conducted with a ratio of 1:9 (n-hexane-acetone), 2:8 (n-hexane-acetone). Elution with two solvents was conducted repeatedly until target fraction yellow color eluted.

DPPH free radical scavenging assay. The antioxidant evaluation was carried out using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) technique refer to Sachindra et al (2007) with several adjustments. Three mg were diluted in 1 mL MeOH (p.a.) to prepare DPPH reagent (Merck). A total of 160 µL ethanol diluted dry brown seaweed extract and was transferred into micro-plate, and then DPPH reagent of 40 µL was added (A). Sample control (comprising 160 µL of seaweed extract and 40 µL of MeOH (B), negative control 40 µL of DPPH reagent and 160 µL MeOH) (C) and a blank (200 µL MeOH) (D) were

further utilized in this measurement. DPPH free radical inhibition percentage was determined using the subsequent equation:

$$[(C-D)-(A-B)]/(C-D) \times 100\%$$

where:
A = 160 µL of extract + 40 µL of DPPH reagent
B = 160 µL of extract + 40 µL of MeOH
C = 160 µL of MeOH + 40 µL of DPPH reagent
D = 200 µL of MeOH

The absorbance of the sample and control solution was measured at a wavelength of 517 nm using a UV-VIS spectrophotometer (Thermo Scientific). Inhibition concentration 50 (IC_{50}) value was determined by probit analysis. In this analysis, ascorbic acid was used as a positive control in this experiment and formulated at concentrations ranging from1-10 µg mL^{-1}.

Fucoxanthin content analysis. High-performance liquid chromatography (HPLC) was used to evaluate fucoxanthin content in brown seaweed extract as described by Nursid et al (2013). Fucoxanthin levels are expressed in units of mg per 1 gram of extract. Identification of fucoxanthin was carried out by comparing it with standard fucoxanthin (Sigma-Aldrich, USA) based on retention (Rt) and ultraviolet (UV) chromatogram of fucoxanthin. The concentration of fucoxanthin was calculated based on the peak area of the fucoxanthin extract chromatogram using the linear regression equation generated by the fucoxanthin standard. As much as 1 mg of seaweed extract was mixed in 1 mL of methanol so that the concentration became 1000 ppm. The HPLC conditions used were as follows: Shimadzu LC–10AD instrument, Diode Array Detector (SPD-M20A Shimadzu) detector, stationary phase C_{18} Zorpax 150 x 2.0 mm, mobile phase gradient acetonitrile to water 10–100%, flow rate 0.2 mL min^{-1}, and injection volume of 10 µL. Pearson correlation analysis was used to examine the link between fucoxanthin content and antioxidant activity.

Mass spectrophotometry analysis. The extract was analyzed using Liquid Chromatography Ion Trap Time of Flight Mass Spectrophotometer (LC-IT-ToF-MS) (Shimadzu) (Nursid et al 2016a) equipped with photodiode array (PDA) detector detected peaks. Chromatography was conducted on a Phenomenex Luna C_{18} column (2.0 x 100 mm, particle size: 5-µm). Before to the injection, a 5 mg crude extract specimen was cleaned up using a C_{18} flash column (0.5 cm x 2.0 mm). HPLC grade methanol was used to dilute the sample. The filtrate was concentrated below the nitrogen gas flow after being filtered using a 0.45-mm filter. The sample was injected into a Shimadzu automated sampler and cleaned for 30 minutes using a water-acetonitrile gradient system (20-100 percent water).

Data analysis. The data compiled was detailed in mean ± standard deviation (n=3) and analyzed using Statistical Package for Social Sciences (SPSS) software.

Results and Discussion

Antioxidant activity. The DPPH radical scavenging activity of different extracts of *Sargassum ilicifolium* and *Padina australis* are presented in Table 1. The antioxidant activity (IC_{50}) of *S. ilicifolium* extract ranged from 799.00±1.66 ~ 1884.48±425.64 µg mL^{-1}, where the extract from the fresh sample had the highest antioxidant activity (IC_{50} value 799.00±1.66 µg mL^{-1}), whereas the dried sample was the lowest (IC_{50} value 1884.48±425.64 µg mL^{-1}). meanwhile, the antioxidant activity (IC_{50}) of *P. australis* extract ranged from 171.50±43.13 ~ 460.88±78.09 µg mL^{-1}. Moreover, the *P. australis*

extract from freeze-dried sample possesses the highest antioxidant activity (IC_{50} value 171.50±43.13 μg mL^{-1}), and the dried sample was the lowest antioxidant activity (IC_{50} 460.88±78.09 μg mL^{-1}). According to Badarinath et al (2010), the antioxidant standard is very strong (<50 μg mL^{-1}), strong (50-100 μg mL^{-1}), moderate (100-150 μg mL^{-1}), and weak (>200 μg mL^{-1}).

The species with the highest antioxidant action is the freeze-dried sample from *P. australis* (IC_{50} value 171.50±43.13 μg mL^{-1}). The result showed that sun-dried *S. ilicifolium* and *P. australis* have lower than fresh and freeze-dried samples. The poor antioxidant activity induced by a variety of conditions such as sample preparation and extraction procedure. In this investigation, the freeze-dried sample showed stronger scavenging activity followed by the fresh, and sun-dried samples, respectively. The lowest values of antioxidant action were detected in the sun-dried samples. These methods are strongly influenced by the weather and the duration of the day. As result of the extended drying duration (3 to 4 days in direct sunlight) and desiccation throughout the drying procedures, the antioxidant activity in these drying procedures was influenced (Roshanak et al 2016; Ling et al 2015). Its more gradual drying rate likely exacerbated the leaching impact and the seaweed will be more exposed to air. Corresponding to Ling et al (2015), desiccation, whether caused by the sun or artificial heat causes, would influence not just ascorbate, however also carotenoids and tocopherols as a product of UVA-UVB light, heat, and air exposure. Sun drying is regarded as the most affordable and available method of food care. Nonetheless, it has been linked to a decrease in unstable antioxidants like GSH and L-ascorbate (Ling et al 2015).

Table 1

Effect of different extracts from *S. ilicifolium* and *P. australis* on antioxidant activity (IC_{50} value of DPPH scavenging activity)

Species	*Extract*	*Antioxidant activity (μg mL^{-1})*
Sargassum ilicifolium	Fresh	799.00±1.66
	Freeze-dried	807.00±15.55
	Sun-dried	1884.48±425.64
Padina australis	Fresh	265.00±28.85
	Freeze-dried	171.50±43.13
	Sun-dried	460.88±78.09
Vitamin C		9.27±0.69

According to several findings, freeze-drying is the best drying process for preservation the nutritional content and anti-inflammatory properties of seaweed polysaccharides (Ling et al 2015). Amorim et al (2020) studied the response of oven-drying, silica-drying, and freeze-drying on the antioxidant activities of four Brazilian macroalgae (*Gracilariopsis tenuifrons*, *Sargassum stenophyllum*, *Pterocladiella capillacea*, and *Ulva fasciata*) and discovered that freeze-drying has the least effect on antioxidant potentiality in examined algae. According to Deng et al (2017), high-temperature processing, as well as the length of the drying cycle reduce antioxidant potential in dried samples of red pepper.

Sun-drying is the familiar and least expensive procedure. However, according to Fudholi et al (2014), microbial attack and weather, dust contamination, birds and insects, action control difficulties, and poor odor impact product quality. This issue can be avoided if seaweeds are dried in an isolated controlled circumstance such as by using freeze-dryers. Freeze-drying is often considered the most effective process for producing high-quality dried products. However, there are certain drawbacks, such as high manufacturing costs, high levels of energy usage, and limited throughputs (Uribe et al 2019).

Analysis of fucoxanthin. Fucoxanthin content of brown seaweeds was determined. According to the retention duration of fucoxanthin standard, the peak of fucoxanthin compounds were detected at 23.51 min. On the other hand, it also used a spectrum of fucoxanthin standard UV that has optimum absorbance with wavelengths of 226, 267, and 447 nm. The peak of fucoxanthin was clear to display in *S. ilicifolium* and *P. australis* extracts (Figure 1). The result showed the fucoxanthin content used fucoxanthin standard curve exhibited that *S. ilicifolium* and *P. australis* with different extractions including dried, freeze drying, and fresh possesses high fucoxanthin content but it is not different. In comparison, the fucoxanthin of *P. australis* in this study is higher than the fucoxanthin of *P. australis* from Seremban, Negeri Sembilan Malaysia (0.43 ± 0.07 mg/g dry wt), thus, *P. australis* has a major amount of fucoxanthin (Jaswir et al 2011). Furthermore, the fucoxanthin content of *S. ilicifolium* is higher than that of Sargassaceae such as *Sargassum thunbergia*, *S. confusum,* and *S. fusiforme* (1.8 ± 1.0, 1.6 ± 0.8, and 1.1 ± 0.6, respectively) from Hakodate, Japan (Terasaki et al 2009). According to Nursid et al (2013), the level of fucoxanthin in seaweed affects antioxidant activity, the higher the level of fucoxanthin is, the higher the antioxidant activity is. It is indicated that the fucoxanthin from brown seaweed has important role as a shield for protection from oxidative stress such as highly sunlight intensity. Gupta & Abu-Ghannam (2011) reported that as well as terrestrial photosynthesis plants, seaweed also has an oxidative mechanism that protects itself from environmental stress.

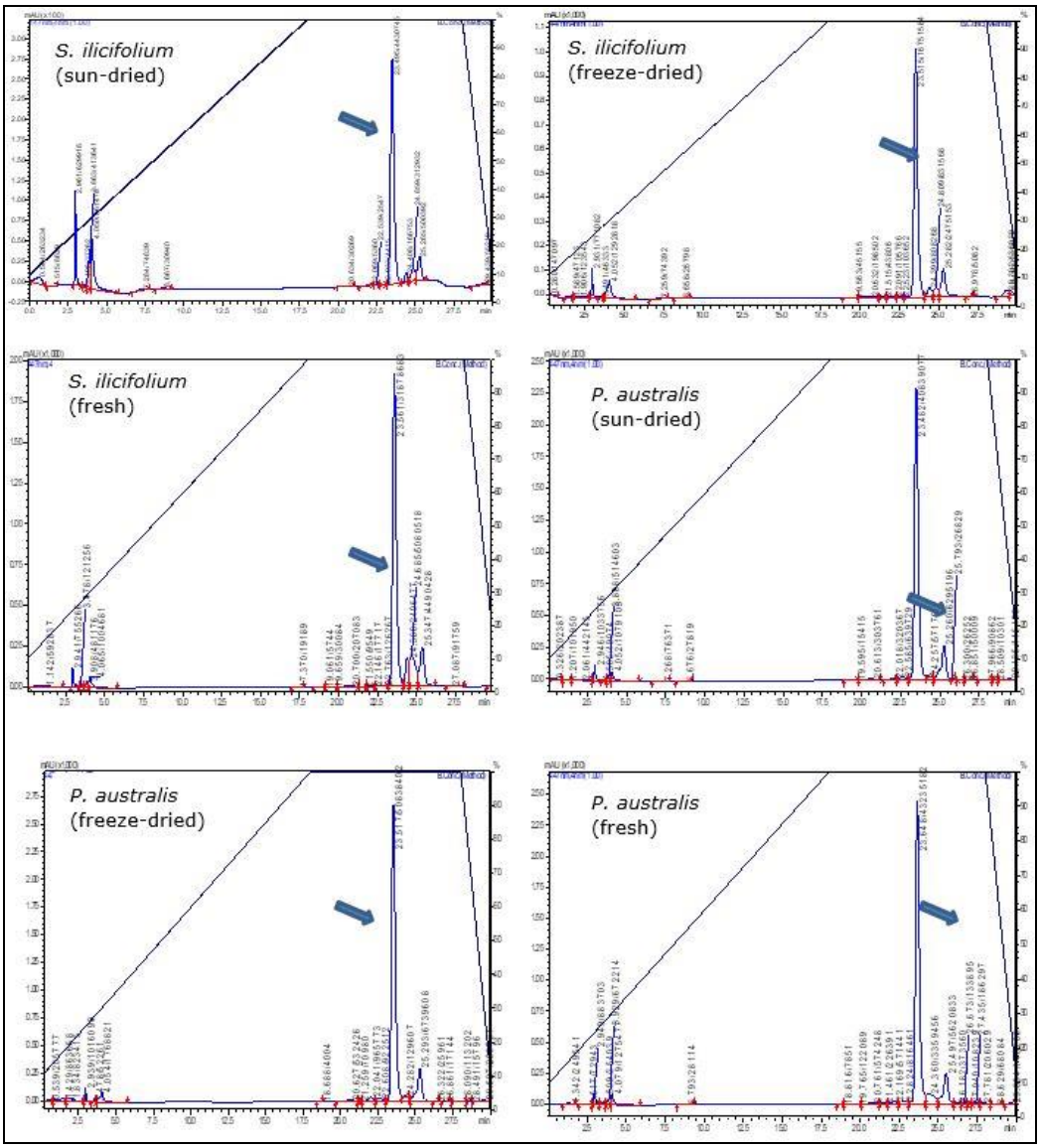

Figure 1. Fucoxanthin peak (arrow) of *S. ilicifolium* and *P. australis* extracts.

According to Nursid et al (2013), the most fucoxanthin content of *Hormophysa triquetra* extract was 88.5 mg g^{-1}, followed by *Turbinaria decurrens* and *Padina australis* with concentrations of 86.9 mg g^{-1} and 77.8 mg g^{-1}, respectively. In contrast, the fucoxanthin of *Sargassum binderi, S. ilicifolium,* and *Turbinaria ornata* extracts were low (<20 ppm).

Mass spectra analysis. The LC/MS analysis results showed that *S. ilicifolium* and *P. australis* extract showed fucoxanthin molecule. This analysis depicted a molecular ion peak of m/z 640.9 [M+H] in brown seaweed *S. ilicifolium* with different drying methods including fresh, freeze-dried, and sun-dried, showed in Figure 2. The presence of the ion peak m/z 640.9 [M+H] indicates that the fucoxanthin molecule is fragmented into 2 H atoms and 1 O atom.

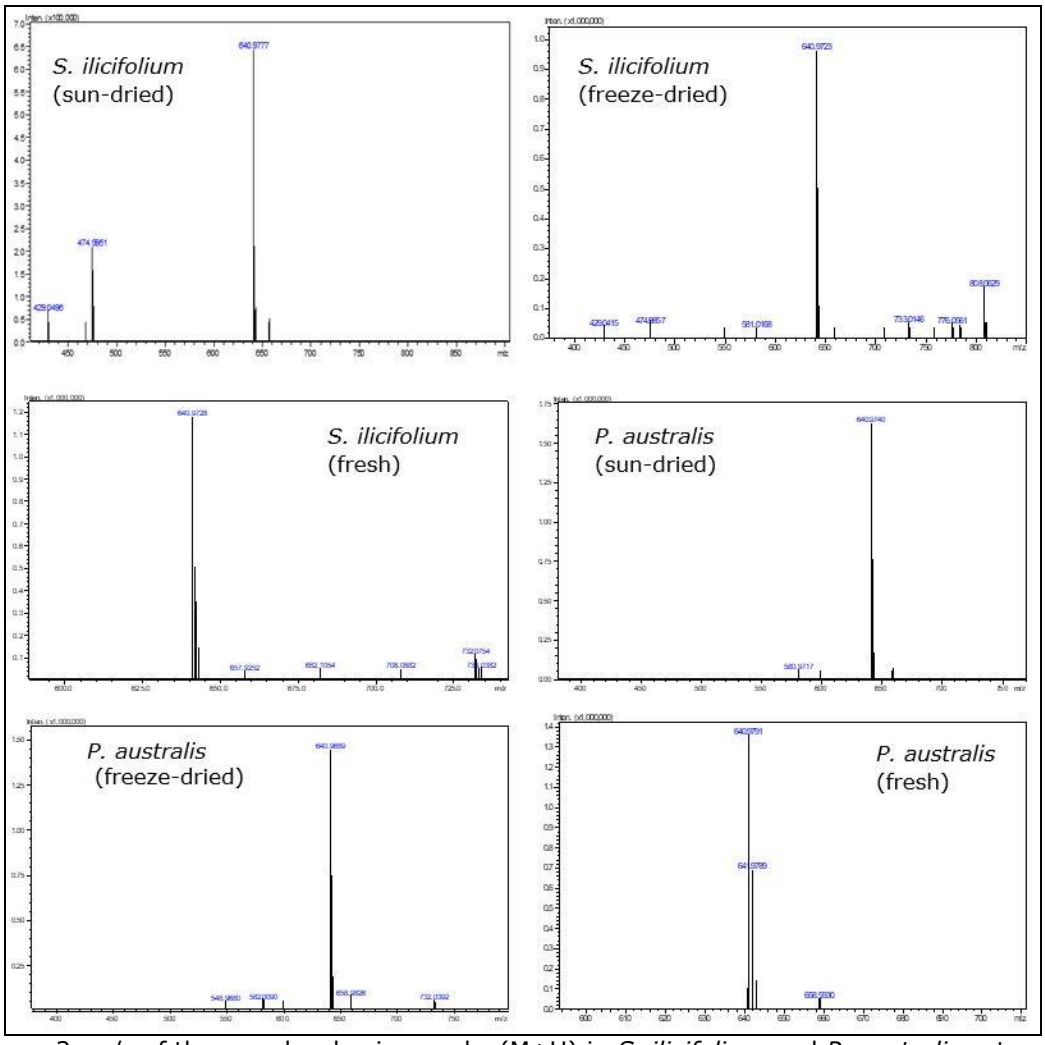

Figure 2. m/z of three molecular-ion peaks (M+H) in *S. ilicifolium* and *P. australis* extracts.

Ion peak of fucoxanthin molecule ($C_{42}H_{58}O_6$, molecule weight 658.92 g mol^{-1}) of fresh and freeze-dried *P. australis* described in m/z 658.9. Ion peak of m/z 640.9 was not found in *P. australis* samples. This can affect the antioxidant activity. Nursid & Noviendri (2017) reported that the fucoxanthin content of *P. australis* decreased in line with drying time. Thus, the different drying methods affected the fucoxanthin content that determined the antioxidant activity.

Nursid et al (2016b) mentioned that high antioxidant activity of *P. australis* from the Binuangeun Coast is most likely because of the existence of fucoxanthin and phenolic compounds. The combination of the particular two active compounds resulted in *P. australis* extracts having significant antioxidant substances, making it suitable for use in pharmaceutical and nutraceutical applications.

Conclusions. Fucoxanthin of brown seaweeds *Sargassum ilicifolium* and *Padina australis* possesses high antioxidant activity in a fresh sample of *S. ilicifolium* (IC_{50} 799.00±1.66 μg mL^{-1}) and the freeze-dried sample of *P. australis* (IC_{50} 171.50±43.13 μg mL^{-1}). Fucoxanthin from brown seaweed *S. ilicifolium* and *P. australis* eluted at 23.51 min in HPLC system detected molecule ion monoisotopic of fucoxanthin with m/z value 640.977 [M+H].

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Conflict of interest. The authors declare that there is no conflict of interest.

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