Effects of Different Solvents on the Antioxidant Activity of Several Seaweed Species from Semporna, Sabah, Malaysia

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Abstract

Sabah is the main seaweed producer in Malaysia especially red seaweeds which are commercially cultivated for the production of food gum known as carrageenan. Seaweeds are also high in phytochemical such as antioxidant compounds. Three seaweeds species from the Semporna Seawater, Sabah, namely Kappaphycus alvarezii, Caulerpa lentillifera and Sargassum polycystum were chosen for this study for the analysis of their antioxidant activities. K. alvarezii species is commercially cultivated whereas the other two (C. lentillifera and S. polycystum) are wild species. All seaweeds species underwent drying process in a cabinet dryer prior to the analyses. Six types of solvents which were water, ethanol, methanol, chloroform, ethyl acetate and hexane were used for the extraction process. Every concentrated, extracted solutions were then measured for their antioxidant activities based on total phenolic content (TPC), diphenylpicrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) and β-carotene bleaching assay. Results showed that TPC of S. polycystum was significantly higher ($p<0.05$) than K. alvarezii but not significantly different ($P>0.05$) from C. lentillifera. Even though the extraction yield of water was the highest, but the greatest antioxidant activity values were seen in methanolic extraction in comparison to the other solvents in TPC, DPPH and FRAP. This indicated that most of the antioxidant compounds in all the three seaweed samples are polar. However, high antioxidant activity in terms of β-carotene bleaching assay were seen in other solvent extractions, which were chloroform, hexane and ethyl acetate. This indicated that seaweed also contain non-polar antioxidant compounds such as β-carotene. Overall, the best extraction method of antioxidant compounds in seaweed samples was using methanol as the extractant.

Keywords: *polarity, extraction, antioxidant activity, phenolic compound, polar and non-polar compound*

Introduction

Seaweeds are potential renewable resources in the marine environment where about 6,000 species have been identified and grouped into three major classes, namely Rhodophyceae (red), Phaeophyceae (brown) and Chlorophyceae (green) algae where 66% of total seaweeds species are used for food (Bamaniyal *et al.*, 2022). According to FAO (2022), the total world seaweed production was estimated to be 34.7 million metric tons where 88.65% are produced by cultivation while the remaining is exploited from the natural seaweed beds all over the world. The estimated value of wide variety products itself derived from seaweed was USD5.6 billion in 2019. However, the rising commercial demand of seaweeds is causing deterioration of seaweed beds due to over-harvesting of these natural resources (Monagail *et al.,* 2022).

Seaweed is a type of macro algae where it exists in the form of multicellular of great size, in fact some of the algae are organisms that live in complex habitats subjected to extreme conditions such as changes of salinity, temperature, nutrients, ultraviolet irradiation (Salehi *et al.,* 2019), pollution, enrichment of nutrient, pollutions and presence of herbivores (Michalak *et al.,* 2022). Therefore, seaweeds must be flexible to adapt to the conditions of new environment rapidly in order to sustain themselves by producing

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biologically-active secondary metabolites which are not available in any other organism. The state of Sabah, Malaysia which is located in the middle of one of the 12 mega-diversity sites in the world has numerous and varied natural resources with salinity ranges from 28 to 34 ppt and surface water temperature ranges between 27℃ to 29℃ which make it a suitable environment for seaweeds and other marine organisms to grow. As compared to other nations like China and Japan, seaweed is not as normally consumed as a food in Malaysia. Seaweeds collected, including *Gracilaria tenuispitata, Gracilaria changii*, *Kappaphycus alvarezii* under Rhodophytes; and *Caulerpa lentillifera* and *Caulerpa racemosa* under Chlorophytes are usually eaten either raw or mixed with salads. Some species such as *Eucheuma denticulatum*, *Gracilaria* sp., *Hypnes musciformis* and *Sargassum* sp., have long been known for their antibiotic properties (Lovdal *et al.,* 2021).

From the aspect of nutrition, edible seaweeds have low calories and are rich in various vitamins, minerals, proteins low in lipid. Seaweeds are particularly high in vitamins B_1, B_2, B_3, B_5, B_9, B_{12}, A,C, D, E and minerals like K, P, Ca and Na Ca (Peñalver *et al.,* 2020). Seaweeds or their extracts have been researched for several decades as unique sources that have been demonstrated to create a wide range of compounds, some of which have been shown to produce biological activity with potential therapeutic benefit (Lomartire and Goncalves, 2022). Currently, it is a new trend that pharmaceutical firms are focusing on the utilization of marine organisms, including the utilization of seaweeds as a source of new drug (Alves *et al*., 2018). One crucial factor needed to consider is the potential of antioxidant compounds in the seaweeds. Antioxidants are known to be helpful in protecting the body against damage by reactive oxygen species (ROS). Recently, there is an increasing interest in natural antioxidants due to the safety and toxicity problems of synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) that are commonly used in lipid-containing food (Lourenco *et al.,* 2019). It was identified that antioxidants in seaweeds are due to the presence of some pigments such as fucoxantin, astaxantin, carotenoid and etc., and polyphenols such as phenolic acid, flavonoid, tannins, etc. (Gomes *et al*., 2022). In this study, three different types of seaweeds were studied for their antioxidant properties by subjecting them to total phenolic content (TPC), diphenylpicrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) and β-carotene bleaching assay.

Materials and Methods

The three edible seaweeds, *S. polycystum, C. lentillifera* and *K. alvarizii* were harvested from Semporna seawater area. After harvesting, seaweeds samples were then washed and packed properly in polyethylene boxes to be transferred to laboratory. Their holdfasts and epiphytes were removed when running tap water was used to clean them. The seaweeds were subsequently dried in a cabinet dryer, followed by grinding into powder form (125µm). The extraction protocol was modified slightly from that of Norra *et al.* (2017) where six types of solvents, which were water, methanol, ethanol, chloroform, ethyl acetate and hexane were used for the extraction process. Ground samples were transferred into separating funnels after being weighed, followed by the addition of solvent at the ratio of 1:10 (w/v) and the extraction mixture was placed in a shaker for 24 h in a dark room. All collected mixtures were then concentrated by using a rotary evaporator and analyzed for their total phenolic content (TPC), diphenylpicrylhydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and β-carotene bleaching assay.

Total phenolic content (TPC) of the extract in each solvent were measured using Folin Ciocalteu method as demonstrated by Neoh *et al.* (2021) with slight modification where 2.0 $mg.ml^{-1}$ phloroglucinol standard solution was prepared by dissolved 0.1 g of phloroglucinol in 50 ml water. A 40 µg of seaweed extract at the concentration of 10 $mg.ml^{-1}$ from each solvent and phloroglucinol were added with 1.8 ml of Folin reagent. Mixture was allowed to stand for 5 min at room temperature before being added with 1.2 ml of 7.5% sodium bicarbonate ($NaHCO_3$). Absorbance was measured after 30 min at 765 nm. TPC content of sample was calculated by following formula 1 and expressed in mg $PGE.g^{-1}$.

$$C = \frac{cv}{m} \qquad (1)$$

Note: C = Total phenolic content; c = Concentration of Phloroglucinol acid; V = Volume of seaweed extract; m = Weight of seaweed extract

DPPH radical scavenging assay was modified slightly from Chakraborty *et al.* (2015) and was used to measure the radical scavenging activity of the seaweed extracts from each solvent. A 0.5 mM solution of DPPH (1,1-diphenyl-2-picrylhdrazol) in methanol and 0.05M acetate buffer at pH 5.5 were prepared. Seaweed extract (0.1 ml) at the concentration of 10 $mg.ml^{-1}$ was added to 2 ml acetate buffer, and a mixture of 1.9 ml methanol and 1 ml DPPH solution was prepared and used as the sample. The same mixture without DPPH was prepared as the blank sample. Meanwhile, the control sample without seaweed was prepared by mixing 2 ml of acetate buffer with 1 ml of DPPH and 2 ml of methanol. The mixture was vortexed immediately after adding DPPH and allowed to stand at room

temperature in the dark and the decrease in absorbance at 517 nm was measured after 30 min until the reaction reached a plateau. The inhibitory percentage of DPPH was calculated by using formula 2.

$$\text{Scavenging effect (\%)} = \frac{A_0-(A-A_b)}{A_0} \times 100\% \quad (2)$$

Note: A_0 = A517 of DPPH without sample; A = A517 of sample and DPPH; A_b = A517 of sample without DPPH (blank)

FRAP assay was also carried out according to Chakraborty *et al.* (2015) with some modifications.2.5 ml of 0.1M potassium phosphate buffer (pH 6.6) and 2.5 ml of 1% w/v potassium ferricyanide were mixed with 1 ml of seaweed extract in each solvent. The reaction mixture was then incubated at 50 ℃ for 20 min after adding 2.5 ml of 10% (w/v) trichloroacetic acid. Later, 2.5 ml of water and 0.5 ml of 0.1% (w/v) ferric chloride ($FeCl_3$) were then added to 2.5 ml of the reaction mixture. The solution was then incubated at ambient temperature for 30 min for the development of colour. The absorbance was then measured at 700 nm. The reducing power was expressed in mg PGE.g^{-1} dried seaweed sample.

β-carotene bleaching assay was carried out according to Aydın and Mammadov (2017), with some modifications. β-carotene solution (1 ml, 0.2% in chloroform) was pipetted into 50-ml round-bottom flask containing 0.02 ml of linoleic acid and 0.2 ml of 100% Tween 20. The mixture was then evaporated at 40 ℃ for 10 min by using a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 ml of distilled water. The distilled water was added slowly to the mixture with vigorous agitation to form an emulsion. Then, 5 ml aliquots of the emulsion were transferred into different test tubes containing 0.2 ml of samples in different solvent. The tubes were then be gently mixed and placed at in a water bath at 45℃ for 2 h. Absorbance of the samples were measured at 470 nm using a UV-Vis spectrophotometer at initial time against a blank consisting of an emulsion without β-carotene. Antioxidant activity (AA) was measured in term of successful bleaching of β-carotene by using formula 3 as shown.

$$\text{Antioxidant activity (\%)} = \left[1 - \frac{A0-At}{A0_0-At_0} \times 100\%\right] \quad (3)$$

Note: A_0 = Initial absorbance of sample; At = Initial absorbance of control; AO_0 = Sample's absorbance after 2 h; At_0 = Control's absorbance after 2 h

Result and Discussion

Percentage yield recovery

In general, water extraction yield for green seaweed (*C. lentillifera*) was the highest at 15.97% compared to brown seaweed (*S. polycystum*) (13.62%) and red seaweed (*K. alvarezii*) (11.85%). The second highest yield was methanolic extraction with *S. polycystum* (6.94%) having the highest yield, followed by *C. lentillifera* (6.63%) and *K. alvarezii* (4.63%). Hexane extraction recorded the lowest yield for all samples. All extraction yields were shown in descending order of solvents' polarity as depicted in Table 1. Based on overall recovery, *C. lentillifera* had the highest yield at 30.76%, followed by *S. polycystum* and *K. alvarezii* with 29.46% and 26.17%, respectively. Among the *C. lentillifera* extracts, most of the extracts were the highest among these three seaweed species with the highest polar components that can be extracted through conventional extraction methods. The high polar yield of *C. lentillifera* might be due to its high chlorophyll content. It is found by Matanjun *et al.* (2008) that the total chlorophyll content in *C. lentillifera* was 546.8-786.8 μg.g^{-1}, approximately three times higher than brown (241.6 μg.g^{-1}) and red species (107-211.6 μg.g^{-1}). Additionally, the higher polar group is also attributed to the highest protein content in *C. lentillifera* (10.41%) in comparison to *K. alvarezii* (9.76%) and *S. polycystum* (5.40%). Green seaweed also gave a higher yield in non-polar extract as observed in hexane (0.87%) when compared with brown and red seaweeds with 0.56% and 0.68% of yields, respectively. Green seaweed also gave the highest yield in non-polar extraction based on hexane extraction (0.87%) compared to red (0.68%) and

Table 1. Extraction yield of *C. lentillifera, S. polycystum* and *K. alvarezii* by using different solvent extraction

Samples	Yield (%) of solvent extract					
	Water	Ethanol	Methanol	Chloroform	Ethyl Acetate	Hexane
C. lentillifera	15.97±0.35^{a}	3.54±0.02 ^{a}	6.94±0.03^{a}	1.69±0.02^{b}	1.75±0.10^{b}	0.87±0.01^{a}
S. polycystum	13.62±1.22^{ab}	3.51±0.01^{a}	6.63±0.02^{a}	3.38±0.01^{a}	1.76±0.01^{c}	0.56±0.01^{a}
K. alvarezii	11.85±1.56^{b}	2.95±0.04^{b}	4.63±0.03^{b}	3.10±0.01^{a}	2.96±0.01^{a}	0.68±0.02^{a}

Values in presented as mean (n= 3)
Values in the same column followed by different letters comparing same solvent in different seaweed species as significantly different (P< 0.05)

brown seaweed (0.56%). *S. polycystum* had the second highest yield in most of the solvents, except for hexane.

The overall trend for extraction yields followed the descending order: water > methanol > ethanol > chloroform > ethyl acetate > hexane for all seaweed species. This result resembles the research by Wo *et al.* (2022) where the extraction yields of three seaweeds was in the following order: methanol > chloroform > acetone > ethyl acetate > diethyl ether > n-hexane. Castejón *et al.* (2022) also found that water extraction was the most effective extraction and had a 70% higher extraction than that of acetone extraction for eight Icelandic seaweed samples. High level of water-soluble compounds can be extracted from seaweed such as soluble polysaccharides, proteins and peptides, which contribute to the high weight of yield from polar solvent extractions (Echave *et al.,* 2021).

Antioxidant activity (AA)

Total phenolic content (TPC) of these three seaweeds showed a good relation with the percentage of yield recovery. The polarity of solvent and solubility of compounds of interest play a vital role in determining the yield of polyphenol (Wakeel *et al*., 2019). Overall, the TPC of methanolic extraction showed the highest values, ranging from 30.54 – 50.67 mg PGE.g^{-1} sample with *S. polycystum* extract being the one with the highest TPC content (50.67 mg PGE.g^{-1}) (Table 2). This result was supported by the finding from several studies which showed that brown seaweed has the highest TPC as compared to another two seaweed species due to the presence of a compound known as phlorotannin which is mostly found in brown seaweed (Ford *et al*., 2020). However, the value in this study was slightly lower in comparison to that of Matanjun *et al.* (2008) and this can be due to the different drying methods employed, where cabinet drying was used in this study whereas freeze-drying was employed in the former study. Methanolic extract of *C. lentillifera* (35.77 mg PGE.g^{-1} sample) in this study showed a moderately high value and this was close with the data found by Wo *et al.* (2022), which was 34.2mg PGE.g^{-1}, but slightly lower in comparison to the value found by Rosni *et al*. (2015), which was 48.98 mg PGE.g^{-1}. The TPC recovery for ethyl acetate extract was the lowest.

In general, the DPPH values ranged from 15.25-64.83% (Table 3.) with methanolic extract of brown seaweed recording the highest inhibition percentage and followed by red seaweed. Most of the extracts showed a higher scavenging effect in polar solvent extractions with brown seaweed exhibiting the highest effect, followed by red and green seaweed. According to Rattaya *et al*. (2015), it was found that *S. polycystum* in all extraction at the concentration of 1 mg.ml^{-1} was able to act as an excellent radical scavenger. The fact that water extraction gave the highest yield suggested that *C. lentillifera* possessed the highest content of polar compounds, which are effectively extracted by water since water is the most polar extraction solvent used in this study. This data is further supported by Yap *et al.* (2019) which showed that water extraction of *C. lentillifera* was about 2.3 times greater than that of methanolic extraction of *C. lentillifera*. However, fucoxanthin (fx), the predominant carotenoid found in the brown seaweed, particularly *S. polycystum* with approximately 2.76-3.01 mg.g^{-1} (Mikami and Hosokawa, 2013; Sabdono *et al*., 2021) is best extracted by methanol (Savira *et al*., 2021) and it is totally insoluble in water (Wang *et al*., 2023). Carotenoids and phenolics in macroalgae have been proven to be associated with antioxidant activity, with fucoxanthin being the lead compound responsible for this (Foo *et al.,* 2017). Thus, this possibly explains why methanolic extract of *S. polycystum* showed the greatest scavenging capability in this study. The fact that all the methanolic extracts showed a better scavenging capability than that of water can be explained by its lower polarity than water and the presence of non-polar methyl group which can possibly contribute to the extraction of less polar compounds. Furthermore, Tobgay *et al*. (2020) mentioned that the surface tension of methanol is comparatively lower than water, thereby enabling methanol to extract significantly higher content of flavonoids. Flavonoids can contribute to the conversion of purple DPPH solution to yellow since they can act as antioxidants, too.

FRAP assay is based on the colour changes of the test solution into various shade of green and blue, relying on the reducing power of each compound. The presence of reducing agent, such as antioxidant causes the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form (Gülçin, 2015). Generally, the FRAP values ranged from 15.86 to 297.65 mg PGE.g^{-1} sample with methanolic extract of brown seaweed exhibiting the highest reducing power and the ethanolic extract of the same sample came in the second (Table 4.). This finding resembles that of Matanjun *et al.* (2008) which found that the same extract solvent had the highest FRAP value, which was 366.69 µM Trolox.mg^{-1} sample. It can also be seen that brown seaweed (*S. polycystum*) generally showed the highest FRAP values in all the solvent extractions. This is in line with the statement from Nazarudin *et al.* (2020) that the highest antioxidant activity is seen in brown seaweed in comparison to green and red seaweed. This can be due to the presence of phlorotannin, a compound found more commonly in brown seaweeds, which possesses strong antioxidant activity due to its unique molecular

Table 2. Total Phenolic Content (TPC) of *C. lentillifera, S. polycystum* and *K. alvarezii* Species by using Different Solvents Extraction

Samples	mg PGE.g^{-1}					
	Water	Ethanol	Methanol	Chloroform	Ethyl Acetate	Hexane
C. lentillifera	17.34±0.31^{a}	33.76±1.35^{b}	35.77±1.27^{b}	6.27±0.05^{ab}	5.87±0.37^{a}	6.61±0.55^{a}
S. polycystum	15.87±0.23^{a}	45.18±0.95^{a}	50.67±0.87^{a}	7.15±0.13^{a}	5.87±0.50^{a}	6.32±0.47^{a}
K. alvarezii	16.98±0.36^{a}	29.66±1.13^{c}	30.54±1.03^{b}	6.02±0.66^{b}	4.11±0.39^{b}	5.75±0.29^{a}

Values in presented as mean (n= 3)
Values in the same column followed by different letters comparing same solvent in different seaweed species as significantly different (P< 0.05)

Table 3. DPPH Radical Scavenging Effect (%) of *C. lentillifera, S. polycystum* and *K. alvarezii* Species by Using Different Solvents Extraction

Samples	% of Inhibition					
	Water	Ethanol	Methanol	Chloroform	Ethyl Acetate	Hexane
C. lentillifera	53.77±1.82^{a}	45.13±3.00^{a}	60.01±3.80^{a}	18.22±1.52^{b}	18.01±2.41^{a}	16.85±2.22^{a}
S. polycystum	48.25±2.08^{a}	45.10±1.08^{a}	64.83±4.21^{a}	23.85±3.42^{a}	19.27±1.82^{a}	17.59±2.40^{a}
K. alvarezii	48.55±1.57^{a}	35.75±3.33^{b}	63.98±2.29^{a}	29.65±3.18^{a}	15.25±3.51^{b}	16.18±3.78^{a}

Values in presented as mean (n= 3)
Values in the same column followed by different letters comparing same solvent in different seaweed species as significantly different (P< 0.05)

Table 4. Reducing Power *C. lentillifera, S. polycystum* and *K. alvarezii* Species by Using Different Solvents Extraction

Samples	mg PGE.g^{-1}					
	Water	Ethanol	Methanol	Chloroform	Ethyl Acetate	Hexane
C. lentillifera	251.71±7.27^{a}	261.73±9.31^{a}	275.51±6.35^{a}	20.73±0.73^{b}	16.53±1.76^{a}	15.97±0.93^{a}
S. polycystum	253.65±9.31^{a}	276.66±9.11^{a}	279.65±7.77^{a}	25.08±1.31^{a}	16.11±2.01^{a}	16.35±0.31^{a}
K. alvarezii	218.65±8.93^{a}	253.11±8.22^{b}	269.51±5.53^{b}	26.15±1.23^{a}	15.86±2.01^{a}	16.29±0.76^{a}

Values in presented as mean (n= 3)
Values in the same column followed by different letters comparing same solvent in different seaweed species as significantly different (P< 0.05)

skeleton as it has eight interconnected benzene rings, way more than many polyphenols obtained from terrestrial plants, for example catechins with only three to four interconnected benzene rings (Sathya *et al.,* 2017). Honold *et al.* (2016) stated that this unique chemical structure allows phlorotannin to act as a stronger antioxidant than many monophenols and polyphenols due to the presence of a greater number of functional –OH groups.

β-carotene bleaching (BCB) measures the ability of antioxidants in hindering the extent of β-carotene discoloration by scavenging the linoleic hydroperoxyl radicals formed due to the oxidation of linoleic acid that can lead to oxidative discoloration of highly-unsaturated β-carotene (Kulisic *et al.,* 2014). The capability of seaweed extracts as antioxidants in this assay was expressed as percentage of antioxidant activity (AA). In general, more than 66% of extracts possessed high or intermediate antioxidant activity. The antioxidant capacity was categorized as high (>70%), intermediate (40-70%) or low (<40%) (Corsetto *et al*., 2020). The highest AA was obtained by the non-polar solvent extract (ethyl acetate extract) of *K. alvarezii* (89.57%) followed by *S. polycystum* (82.34%) (Table 5). Most of the polar solvent extracts had lower values of AA for β-carotene bleaching assay since the compound was dissolved in non-polar solvent (Muller and Bohm, 2011). β-carotene bleaching assay can screen both lipophilic and hydrophilic sample (Danet, 2021). Being a semi-polar solvent, ethyl acetate is capable of extracting both lipophilic and hydrophilic antioxidants from the samples (Sulmartiwi *et al.,* 2017). Even though the total phenolic content was lower in all the nonpolar extracts as compared to extracts derived from solvents of a higher polarity, but Belkacemi *et al.* (2020) mentioned that antioxidant activity of seaweeds is not only contributed solely by phenolic compounds, but also non-polar compounds, including steroids, fatty acids, tocopherols, terpenes, glycolipids, phospholipids. Thus, this explains the reason why extracts derived from this solvent showed the greatest antioxidant activity in this assay. Olszowy and Dawidowicz (2016) stated that this technique is appropriate for researching the capacity of lipophilic antioxidants in protecting β-carotene from being bleached. Thus, the results here indicates that an abundant amount of lipophilic antioxidant compounds are present in the seaweed samples.

Table 5. β-Carotene Bleaching Assay of *C. lentillifera, S. polycystum* and *K. alvarezii* Species by Using Different Solvents Extraction

Samples	% Antioxidant Activity					
	Water	Ethanol	Methanol	Chloroform	Ethyl Acetate	Hexane
C. lentillifera	34.76±2.31^{a}	35.54±1.33^{a}	40.25±0.89^{a}	69.12±2.31^{a}	89.57±4.44^{a}	64.35±7.36^{ab}
S. polycystum	41.24±2.23^{a}	37.84±0.85^{a}	41.39±1.23^{a}	72.64±3.37^{a}	82.34±3.31^{ab}	67.32±6.37^{a}
K. alvarezii	35.87±0.91^{a}	32.87±0.88^{b}	39.15±2.08^{a}	68.05±2.47^{a}	81.27±4.11^{b}	63.73±7.31^{b}

Values in presented as mean (n= 3)
Values in the same column followed by different letters comparing same solvent in different seaweed species as significantly different ($P< 0.05$)

Conclusion

Generally, solvents of a higher polarity act as better extractants in extracting antioxidant compounds than non-polar solvents from seaweed samples. The data collected from all the three seaweed samples indicated that majority of the antioxidants present in seaweeds are polar compounds, with methanolic extraction typically showing the highest values in TPC, DPPH and FRAP assays. This means that methanol, being a highly-polar solvent with a non-polar methyl group was considered the most suitable solvent in extracting antioxidative compounds from the samples. However, the data obtained from β-carotene bleaching assays also indicates that an abundant amount of non-polar antioxidant compounds are present in the seaweeds. Nevertheless, among all the tested samples, *S. polycystum*, in general showed the highest AA values as compared to *C. lentillifera* and *K. alvarezii* in both polar and non-polar solvent extractions.

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