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Phytochemical Contents and Antioxidant Activity of Selected Brown Seaweeds (*Sargassum polycystum* and *Padina minor*) of Sabah, Malaysia

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Seaweed and its derivatives are rich in bioactive components, which have been widely utilized as biostimulants in crop production to increase plant growth and productivity. The current study was conducted to evaluate the phytochemical contents and antioxidant capacity of aqueous seaweed extracts as a potential biostimulant for plant growth. The brown seaweeds studied were *Sargassum polycystum* and *Padina minor*, which were collected from Semporna and Kota Kinabalu, Sabah, respectively. The phytochemical contents namely, total flavonoid content (TFC), total phenolic content (TPC) and total anthocyanin content (TAC); and antioxidant activity (FRAP, DPPH, and ABTS radicals scavenging) of aqueous seaweed extracts were analysed using standard procedures. The results showed that *S. polycystum* has relatively higher TPC and TFC compared to *P. minor*. Meanwhile, the TAC of both seaweeds are not significantly different from one another. The antioxidant activities based on DPPH radical scavenging activity (IC_{50}), ABTS radical scavenging activity (IC_{50}) and ferric reducing power assays of *S. polycystum* were significantly higher than *P. minor*. These findings suggested that both brown seaweeds contain a satisfactory amount of phytochemicals, indicating natural antioxidant potential that might benefit plant growth and productivity.

Key words: Antioxidant; phytochemical; *Padina minor*; *Sargassum polycystum*

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Seaweeds are known as multicellular plants that grow in the sea. Malaysia has a long coastline of 3432 km and a continental shelf area of 418 000 km^2. Several islands along the shores of east and Peninsular Malaysia formed clusters, which include Sarawak and Sabah. Mangroves and mudflats alternate with sandy bays and rocky coastlines, while coral reefs ring most islands. They are potential habitats for the many varieties of seaweed present in the waters of Malaysia [1]. Seaweed comprises nearly 10,000 species, categorized mainly into four broad groups based on their pigmentation, reproduction, and internal and external structures. These broad groups include Phaeophyceae (Brown), Rhodophyceae (Red), Chlorophyceae (Green), and Cyanophyceae (Blue-green) [2,3].

Previous studies reported that brown seaweed species (Phaeophyceae) is the type most used for agriculture and the production of commercial biostimulants for plant growth and productivity because they can reach high biomass levels and are widespread [4,5]. Algae are suitable to be utilized as organic fertilizers as they have effects on biological and biocompatibility because seaweeds have familiar biological substances found in plants. This significant benefit placed seaweeds on the highest list of biostimulants for crops and are used in various plant treatment processes, mainly to support and promote sustainable and organic agriculture [6,7].

In Sabah, Malaysia, *Sargassum* sp. is the major species of brown seaweed found growing along the rocky coast, while *Padina* sp. is the second most

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significant brown seaweed in term of abundance [8]. *Sargassum* sp. had been reported to have a good amount of flavonoids, while *Padina* sp. was found to have a high phenolic content, which may indicate natural antioxidant potential of both seaweed species [9,10]. HPLC profiles of *Padina* sp. was reported to contain phenolic compounds namely ferulic acid, delphinidin-3-O-glucoside, ellagic acid, kaempferol, and naringenin [11]. Meanwhile, the RP-HPLC profiles of *Sargassum* sp. reported to have some phenolic compounds namely tannic acid, vanillic acid, ferulic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, and gallic acid [12]. Both seaweeds were found to have many bioactive compounds that can pose various biological properties such as antiviral, antimicrobial, anti-inflammatory, antifungal, antioxidant, insecticidal, and antibiotics [13-15]. The findings showed that both selected brown seaweeds are the ideal targets for investigating the existence of biomolecules for various applications in industry.

More seaweed species, including brown seaweeds, have been studied and known to provide benefits as biostimulants for plant growth and productivity due to their bioactive compounds, such as macro and micro-element nutrients, vitamins, and plant growth promoters that help to enhance the crop growth and yield [2]. In particular, bioactive compounds in seaweeds that can help to stimulate plant growth are polysaccharide that promotes plants desiccation tolerance [16], betaines which protects plants from drougth stress [2,17], sterols that is involved in membrane homeostasis maintenance in plant cells [18], polyphenols which inhibits total reactive oxygen species (ROS) generation [19], polyunsaturated fatty acids that helps develop abiotic and biotic stresses tolerance [20], minerals which can develop chilling tolerance [21], and plant growth-stimulating hormones which improves the root system [22]. However, there is still little information on the scientific data about the bio-stimulant potential of seaweed, precisely due to their antioxidant activity and phytochemical contents. Hence, this study focused on determining the phytochemical contents (TFC, TPC, and TAC) and antioxidant capacity based on ABTS, DPPH, and FRAP assays of selected brown seaweed species (*Padina minor* and *Sargassum polycystum*) for their potential as biostimulant for the quality and quantity of plants.

EXPERIMENTAL

Materials and Methods

Sample Collection and Preparation

The brown seaweeds, namely *P. minor* (Figure 1) and *S. polycystum* (Figure 2), were collected from Semporna and Kota Kinabalu, Sabah, respectively. The sample collection was carried out between September and November 2021. First, the fresh seaweeds were washed with tap water for the removal of their epiphytes and holdfasts, and then rinsed with distilled water. The cleaned seaweeds were dried by sun drying method, in which the samples were spread evenly on a drying net and placed under direct sunlight until the moisture content is less than 30% which normally took 3 - 4 [23,24]. After drying, the seaweeds biomass were ground using a high-performance grinder and passed through a sieve (0.5 mm) to obtain a uniform size [25]. The seaweed powder were put in an airtight plastic bag and stored in a freezer (-20 °C) if not used immediately [23].

Figure 1. *Padina minor* collected from Semporna, Sabah.

Figure 2. *Sargassum polycystum* collected from Kota Kinabalu, Sabah.

Sample Extraction

The seaweed powder (100 g) was extracted by adding 2000 mL of deionized water with constant stirring at room temperature for about 5 minutes [26]. Then, the mixture was filtered using a muslin cloth to remove solid residues after the extraction process. The filtrate was then centrifuged for about 5 minutes at 5000 rpm, and then filtered through microfilter paper to collect the supernatant [27,28]. The collected filtrate was taken as 10% seaweed extract and preserved in a freezer (-20 ℃) prior to use if not used immediately [29].

Determination of Total Phenolic Content (TPC)

The analysis of TPC in seaweed extracts was performed using a Folin-Ciocalteu reagent as described by Ainsworth and Gillespie [30] with slight modification. Each sample extract (100 µL) was added to 10% (v/v) Folin-Ciocalteu reagent (200 µL), left to stand at room temperature for about 5 minutes and then added with sodium carbonate (800 µL, 700 mM). At room temperature, the solution was incubated and left for 2 hours in the dark. After that, the mixture (200 µL) was loaded into a 96-well plate and then absorbance of the sample solution was measured at 765 nm using a microplate reader. Similarly, the absorbance of gallic acid standard solutions (0-50 µg/mL) was measured and the standard calibration curve was plotted. The TPC of the samples was expressed as milligram gallic acid equivalent (mg GAE/100 g) seaweed extract.

Determination of Total Flavonoid Content (TFC)

The TFC of seaweed extracts was analyzed using Aluminium Chloride Calorimetric Assay, as described by Chang *et al*. [31] with slight modification. A mixture of the sample extract (120 µL), 80% (v/v) methanol (360 µL), 10% (v/v) aluminium chloride (Al_2Cl_3) solution (24 µL), 1.0 M potassium acetate (24 µL), and deionized water (680 µL) was prepared. The mixture was homogenized and kept in the dark at room temperature for about 30 minutes. After that, 302 µL of the mixture was loaded into a 96-well plate. The absorbance of the resulting solution was analyzed at 415 nm using a microplate reader. Standar quercetin, with concentrations within the range of 0-100 µg/mL, was used as standard. For the blank solution, the amount of 10% Al_2Cl_3 solution was replaced by the same amount of distilled water. The standard calibration curve was plotted, and TFC of the samples was expressed as milligram quercetin equivalent (mg QE/100 g) seaweed extract.

Determination of Total Anthocyanin Content (TAC)

The TAC of seaweed extracts was analyzed using the pH differential method as described by Rafi *et al*. [32] with slight modification. This method refers to the structural changes in anthocyanin chemical form and the measurements of absorbance at pH 1.0 and 4.5. The anthocyanin colour changes with the pH i.e. colourless at pH 4.5 and reddish at pH 1. The samples were diluted separately in buffers at pH 1 (0.025 M hydrochloric acid-potassium chloride buffer) and pH 4.5 (0.4 M sodium acetate buffer). Sample extract (100 µL) was added to 1400 µL of each buffer and was vortexed. Then, the absorbance of the mixture was measured using a microplate reader at 515 and 700 nm. The TAC of the extracts was determined according to equation (1).

$$\text{Anthocyanin (mg/L)} = \frac{A \times 1000 \times MW \times DF}{\varepsilon \times l} \qquad (1)$$

Where A = (A510 nm – A700 nm at pH 1) – (A510 nm – A700 nm at pH 4.5), MW = molecular weight (449.2 g/mol), DF = the dilution factor, ε = extinction molar coefficient (26,900 L cm^{-1} mol^{-1}), l = the cell path length (1 cm). The results were expressed as milligram cyanidin-3-glucoside equivalent (mg C-3-GE/100 g) seaweed extract.

$$\text{Radical scavenging activity (\%)} = \left[\frac{(absorbance\ ctrl - absorbance\ sample)}{absorbance\ ctrl} \right] \times 100\% \quad (2)$$

DPPH Free Radical Scavenging Assay

The scavenging activity of the seaweed extract was determined by using 2,2-diphenyl-1-pycryl-hydrazil (DPPH) as a free radical [33] with slight modifications. Seaweed extract or standard (50 µL) with different concentration was reacted with an aliquot of freshly prepared 0.1 mM DPPH methanolic solution (195 µL) in a 96-well plate. Five different concentrations of the sample extract were assayed to determine the anti-oxidant activity values in an appropriate linear range and check the response linearity. The mixtures were then swirled gently for about 1 minute and left in the dark at room temperature for 60 minutes. The absorbance of the mixtures was analyzed using a microplate reader at 519 nm. The free-radical scavenging activity was determined according to equation (2). The results were expressed as the 50% inhibition (IC_{50}) value, which represents the sample concentration that scavenges 50% of DPPH free radical. A standard calibration curve of Trolox was plotted using different concentrations, and the results were expressed as mg TE/g.

ABTS Free Radical Scavenging Assay

ABTS (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) assay was performed based on the reaction between samples and ABTS radical cation [34]. The working solution for ABTS was prepared by mixing 7.4 mM ABTS solution (15 mL) with 2.6 mM potassium persulfate (264 µL) and was then left in the dark for 12 hours. The working solution was diluted to obtain an absorbance of 0.7 ± 0.05 units at 734 nm. The seaweed extracts or standard solution was mixed with the ABTS working solution in a ratio of 1:2 in the dark room and then the mixture's absorbance was measured using a microplate after 30 minutes at 734 nm. The radical scavenging activity was calculated according to equation (2). The results were expressed as the 50% inhibition (IC_{50}) value, which represents the sample concentration that scavenges 50% of free radicals. A standard calibration curve of Trolox was plotted, and the results were expressed as mg TE/g.

Determination of Ferric Reducing Antioxidant Power (FRAP)

This procedure was carried out according to the method described by Russo *et al*. [35] with slight modifications. The working FRAP reagent was prepared by mixing 300 mM of sodium acetate anhydrous buffer in distilled water (pH 3.6) with 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in distilled water in a ratio of 10:1:1. The mixture of reagent was then put in a water bath for 30 minutes at 37 °C. Sample extract (20 µL) and FRAP reagent (180 µL) were mixed in a 96-well plate and then heated for 40 minutes at 37°C in the dark. The absorbance of the resulting solution was measured using a microplate reader at 593 nm. Trolox with concentrations within the range of 0-100 µg/mL was used as an antioxidant standard and for calibration. The results were expressed as mg TE/100 g seaweed extract.

Statistical Analysis

All the analyses were performed in triplicates. All the experimental data were reported as mean ± standard deviation (SD) using Statistical Product and Service Solutions (SPSS) software version 28.0. The data were analyzed by one-way analysis of variance (ANOVA). The differences were considered significant at a probability below 5% (p<0.05). The correlations among data were determined using Pearson's correlation coefficient (r).

RESULTS AND DISCUSSION

Phytochemical Contents of Selected Brown Seaweeds

The aqueous extracts of selected brown seaweed were analyzed for their TPC, TFC, and TAC and the results are summarized in Table 1. The results show that *S. polycystum* has a higher TPC (107.35 ± 0.73 mg GAE/100 g) and TFC (87.60 ± 1.54 mg QE/100 g) compared to *P. minor*. (84.07 ± 0.93 mg GAE/100 g; 65.01 ± 1.19 mg QE/100 g, respectively). However, both *S. polycystum* and *P. minor* have a lower TAC value with 26.26 ± 5.40 and 22.69 ± 2.29 mg C-3-GE/100 g, respectively. Statistically, the differences in TPC and TFC between both seaweeds were significant ($p < 0.05$), meanwhile, the TAC value was not significantly different from one another ($p > 0.05$). Among the phytochemicals content that was analyzed, TAC showed very little content for both seaweeds.

Table 1. Phytochemical contents of *S. polycystum* and *P. minor* seaweeds.

Sample	TPC (mg GAE/100 g)	TFC (mg QE/100 g)	TAC (mg C-3-GE/100 g)
S. polycystum	107.35 ± 0.73	87.60 ± 1.54	26.26 ± 5.40
P. minor	84.07 ± 0.93	65.01 ± 1.19	22.69 ± 2.29

Over the past few years, seaweed extracts have gained a lot of interest as biofertilizers for numerous plants to increase productivity and growth of plants [36]. The impacts of seaweed extracts on plants primarily depend on the solubility, biological activity, and avail-ability of the biomolecules in seaweeds [37]. Based on the results obtained in this current study, both seaweeds showed a high amount of TPC and TFC but relatively low amount of TAC. This result is in agreement with a previous study on TPC and TFC of a sargassum seaweed but different species (i.e. *Sargassum subrepandum*) as reported by Abdelaal and co-workers [38] where they found TPC and TFC of 122.67 ± 20.34 mg GAE/g and 29.31 ± 5.67 mg CE/g, respectively, and a lower amount of TAC (3.65 ± 0.44 mg C-3-GE/100 g). Another study on *Sargassum* sp. and *Padina* sp. harvested from Persian Gulf as reported by Ebrahimi *et al.* [39] also showed that both seaweeds have a high TPC with 28 ± 2.7 mg GAE/g and 19 ± 3.9 mg GAE/g, respectively. The recent finding also stated high phenolic and flavonoid contents in *Padina* sp. with the value 69.5 ± 1.74 mg GAE/g and 38.4 ± 1.64 mg QE/g, respectively [40]. However, no previous study reported the TAC of *Padina* sp.

The phenolic compounds are known to possess the capacity to reduce oxidative damage and function as antioxidants. They can either directly trap or scavenge the free radicals using a sequence of coupled reactions with antioxidant enzymes [10]. Flavonoids are known as natural phenolic substances which have unique characteristic in their structures that results in a variety of biofunctional properties, such as free radical scavenging and antioxidant activities [41]. Anthocyanin is known as a family of natural pigments which belongs to the class of flavonoids. It was scientifically reported to have antioxidant effects because of their phenolic compounds' chromophores structure [42]. It was reported that phenolic, flavonoid, and anthocyanin compounds were associated with antioxidant activity [43-45].

Antioxidant Activity of Selected Brown Seaweeds

The seaweed aqueous extracts were analysed for their capability in scavenging the ABTS and DPPH free radicals. These assays refer to the measurement of DPPH and ABTS free radicals lost after reaction with samples. The value of IC_{50} is a parameter commonly used to determine samples' antioxidant activity. It represents the concentration of antioxidants needed to lessen the initial concentration of ABTS and DPPH by 50% [46]. Thus, the lower IC_{50} value indicated a higher antioxidant capacity. Based on the results in Table 2, *S. polycystum* has relatively lower IC_{50} value for ABTS and DPPH assays (2.87 ± 0.01 µg/mL; 3.21 ± 0.01 µg/mL) than *P. minor*. (3.01 ± 0.05 µg/mL; 3.44 ± 0.01 µg/mL). However, overall, the ABTS and DPPH IC_{50} values for both seaweeds indicated they have higher scavenging activities against the free radicals. Both seaweed extracts showed a lower inhibition activity against DPPH radicals as compared to ABTS radicals. The differences in scavenging activity values between DPPH and ABTS might be due to the different mechanisms for both radicals. It has been reported that DPPH radicals have a slower reaction with most biomolecules than ABTS radicals [47].

The FRAP assay is based on the antioxidant's capability in seaweed extract, which acts as a reductant through electron donation that causes the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) [41]. Based on Table 2, *S. polycystum* (203.44 ± 0.79 mg TE/100 g) has a higher FRAP value than *P. minor*. (152.02 ± 0.77 mg TE/100 g). These results showed significant differences in antioxidant activities based on ABTS, DPPH, and FRAP assays between both seaweeds ($p < 0.05$).

Table 2. Antioxidant activity of the selected brown seaweeds.

Sample	DPPH, IC_{50} (µg/mL)	ABTS, IC_{50} (µg/mL)	FRAP (mg TE/100 g)
S. polycystum	3.21 ± 0.01	2.87 ± 0.01	203.44 ± 0.79
P. minor	3.44 ± 0.01	3.01 ± 0.05	152.02 ± 0.77

Based on the results obtained in this study, both *S. polycystum* and *P. minor* have a higher radical scavenging activity against ABTS and DPPH free radicals as they showed a lower IC_{50} value. The findings is in agreement with a recent study on *Sargassum* sp. and *Padina* sp. that showed a lower DPPH IC_{50} value with 0.611 ± 0.02 mg/mL and 0.649 ± 0.03 mg/mL, respectively. The study also reported a lower ABTS IC_{50} value for *Sargassum* sp. and *Padina* sp. (0.849 ± 0.02 mg/mL and 1.392 ± 0.01 mg/mL, respectively) [48]. The FRAP values for both seaweeds in this study also reported a higher antioxidant capacity. *Sargassum* sp. and *Padina* sp. had been reported to have a high FRAP value with 221.43 ± 4.55 µM TE/g and 537.95 ± 9.11 µM TE/g, respectively [24,49]. The high antioxidant activity shown by both seaweeds are possibly due to the existence of phytochemical substances in the samples and their high amount of flavonoid and phenolic contents.

Each of the phytochemical compound has its role in plant growth and productivity. For example, flavonoid is very effective in the interactions of plant microbes in the rhizosphere and may have an impact on increasing biotic and abiotic stress tolerance. Besides, they also act as an antioxidant, antibacterial, insect repellent, and ROS scavengers in plants [50]. Phenolic compounds were scientifically claimed to have good effects on seed germination processes and plant development [51]. It was reported that the application of polyphenolic substances on crops as aqueous extract showed an auxinic effect. At lower concentrations, they help stimulate plant growth and yield [52].

Correlation Between Phytochemical Contents and Antioxidant Activity

This study determined the correlation between antioxidant activity (DPPH, ABTS, and FRAP) and phytochemical contents (TFC, TPC, and TAC) of the studied seaweeds using Pearson's correlation coefficient. As demonstrated in Table 3, there was a significant positive correlation between TPC and TFC with FRAP value (r = 0.998, p<0.001) and (r = 0.996, p<0.001), respectively. This is implying that the higher the total flavonoid and phenolic contents, the higher the antioxidant capacity of seaweed extracts. At the same time, there is a strong negative correlation between TFC and TPC with IC_{50} values in DPPH and ABTS assays. The higher TPC and TFC in seaweed extract samples resulted in a lower sample concentration needed to scavenge the free radicals. However, TAC showed a moderate correlation with all the antioxidant capacity assays (DPPH, ABTS, and FRAP).

A previous study had reported a significant correlation between TPC with the FRAP and DPPH values of the sample, with (r = 0.9474, p<0.0012) and (r = 0.9832, p<0.0001), respectively. The study had shown a significant correlation between TFC with FRAP and DPPH values (r = 0.9521, p<0.0009) and (r = 0.9885, p<0.0001), respectively [53]. Another research also reported a significant correlation between TFC and TPC with ABTS radical scavenging activity (r = 0.903) and (r = 0.876), respectively [54]. Thus, this study suggested that phenolic and flavonoid compounds could be significant contributors to the high antioxidant capacity of the seaweed samples.

CONCLUSION

The aqueous extracts of *S. polycystum* and *P. minor* were analysed for their phytochemical contents and antioxidant activities. Both brown seaweeds were found to have a satisfactory amount of phytochemicals (TPC and TFC) which translated in their strong antioxidant activities. This may indicates that these seaweeds possess high natural antioxidant that would be beneficial for agricultural applications, such as biostimulant, which hold huge potential to serve and facilitate organic and sustainable agricultural practices. For further analysis of the phytochemicals in these seaweeds aqueous extracts, chemical analyses using LC-MS are currently being conducted in our lab.

Table 3. Correlation between phytochemical contents and antioxidant activity of selected brown seaweeds.

Phytochemical Contents	**Antioxidant Activity**		
	DPPH IC_{50}	ABTS IC_{50}	FRAP
TPC	-0.997**	-0.925**	0.998**
TFC	-0.996**	-0.907*	0.996**
TAC	-0.450	-0.461	0.445

[a] *, P < 0.05, **, P < 0.01

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