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Original Article

Chemical composition and *In vitro* study of antioxidant and antibacterial activities of *Sargassum oligocystum* Montagne (Sargassaceae, Ochrophyta)

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Abstract

Seaweeds are marine organisms capable of producing diverse biomolecules and other important bioactive compounds with potential pharmacological uses. Proximate composition analysis of *S. oligocystum* showed high ash, carbohydrate, and protein content with an estimated value of 39.01±0.16%, 21.43±0.37%, and 19.13 ± 0.19% respectively. The macroalga has a phenolic content (TPC) of 30.94 ± 0.06 mg GAE/g. In terms of antioxidant efficiency, high copper reduction capacity (IC_{50} = 6.97 µg GAE/ml) and potent radical scavenging activity (IC_{50} = 28.5 µg GAE/ml) were exhibited by *S. oligocystum* extract, which is more effective than ascorbic acid (control). Also, *S. oligocystum* extract showed potent antibacterial activities towards Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Bacillus cereus* with minimum inhibitory concentration (MIC) of 125 µg/ml and 250 µg/ml, respectively. This investigation is a pioneering study in the Philippines documenting the use of *S. oligocystum* as an alternative source of bioactive substances that can be used as novel therapeutic agents in disease treatment.

Keywords: Biological activity, Marine, Polyphenols, Philippines, Seaweeds

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Introduction

Seaweeds are primary producers and are considered as vital components in the marine ecosystem, providing shelter, nursery grounds, and food sources for various organisms (Moghadamtousi et al., 2014; Cotas et al., 2020; Arguelles and Sapin, 2021). These organisms are commonly vulnerable to unfavorable environmental conditions (e.g. sea mechanical aggression, high light intensity, and herbivory) causing these organisms to produce a wide array of active metabolites for protection (Cotas et al., 2020). These metabolites are beneficial not only to seaweeds but also to humans since they are known to possess biological activities with important health benefits (Moghadamtousi et al., 2014). Moreover, some of these compounds are useful for agricultural animals and crops due to the presence of proteins, minerals, trace elements, and other plant growth regulators (Moller and Smith, 1998). Due to the diverse chemical

compounds reported for seaweeds, more attention has been given to the potential of these organisms as novel sources of bioactive compounds for food, agricultural, and pharmaceutical application.

Sargassum is a widely distributed seaweed in the Philippine coastal waters. It can be recognized by the presence of holdfast, stipes and dark brown to yellowish fronds (Trono, 1997). These organisms are potential sources of various primary and secondary metabolites with potent biological activities. One such metabolite is polyphenols, which is an important constituent in the vegetative thalli of seaweeds. Phenolic compounds (e.g. bromophenols, flavonoids, phlorotannins, and terpenoids) from marine algae are characterized as metabolites possessing hydroxylated aromatic rings (Cotas et al., 2020). These bioactive compounds exhibit diverse chemical forms and structures, from high molecular weight polymers to simple chemical moieties. Polyphenols gained much interest due to their structural diversity and a broad spectrum of functional activities (Cotas et al., 2020). Pharmacological studies revealed that this metabolite present in this genus exhibited anticoagulant, antioxidant, anti-vasculogenic, antimicrobial, and antiproliferative properties (Boonchum et al., 2011; Cotas et al., 2020).

Even though *Sargassum* is reported to have good bioactive properties not all species are well studied and are considered as underutilized marine resources for novel bioactive compounds. To date, there are limited reported studies in the Philippines for bioactive properties in the genus *Sargassum* (Arguelles et al., 2019; Arguelles, 2020). *Sargassum oligocystum* is the dominant seaweed that grows abundantly alongside the coast of Pagkalitan, Batangas in the Philippines. It is a natural renewable resource that was previously reported to contain interesting bioactive compounds such as carotenoids, polyphenols, lipids, chlorophyll, and peptides. These compounds exhibit important biological properties which are useful for pharmacological applications and can serve as a functional food for humans and animals (Arguelles et al., 2019; Arguelles and Sapin, 2021). In the Philippines, no documented studies were conducted regarding the use of *S. oligocystum* as a potential alternative source of bioactive substances that can be used for pharmaceutical application. Documentation of possible other novel pharmacological agents from *S. oligocystum* could elevate their value as important marine resources in Philippine waters. Thus, this study was done as a preliminary investigation to provide baseline information on the proximate composition, total phenolic content (TPC) and assess the antioxidant as well as antibacterial activities of *S. oligocystum.*

Material and Methods

Collection of seaweed

Sargassum oligocystum was collected on January 2020 during low tide condition alongside the coast of Pagkalitan (Lat. 13° 38’ 4.2” N; Long. 121° 2’ 39” E), Batangas, Philippines (Figure 1). The collected seaweeds were initially rinsed several times with seawater to separate the sand particles and other epiphytes followed by sterile water. The washed seaweed was sun-dried for six days and pulverized before solvent extraction (Arguelles et al., 2019). The brown seaweed was identified using reference taxonomic works and monographs of Trono (1997) and Algae Base (Guiry and Guiry, 2021).

Figure-1: Thallus morphology of *Sargassum oligocystum* Montagne.

Preparation of seaweed extract

Powderized biomass of *S. oligocystum* (1 gram) was extracted using 30 ml acidified methanol (1 HCl: 80 CH_3OH: 10 H_2O) placed for 1 hour in an ultrasonic bath with continuous stirring (Gao et al., 2002; Arguelles and Sapin, 2020a). The supernatant was separated from the algal biomass by centrifugation at 10,000 rpm for 20 minutes at 20°C. Using a rotary evaporator, the collected algal supernatant was further

concentrated under reduced pressure set at 40 °C. The concentrated *S. oligocystum* extract was stored in a refrigerator (4 °C) to preserve its biological activity until used in the different biological assays (Gao et al., 2002).

Proximate composition analysis

Proximate composition analysis of *S. oligocystum* was carried out following the procedure of AOAC (2011). The moisture content of *S. oligocystum* was determined by subjecting the algal biomass (2 g) to dryness using an oven set at 105°C for 8 hours until a constant and uniform weight was noted (Arguelles and Sapin, 2020a). Crude fiber and protein content of *S. oligocystum* was obtained using the Weende and Kjeldahl method. On the other hand, ash content was analyzed by subjecting the algal biomass in complete burning using a muffle furnace set at a temperature of 550°C for 4 h until a grayish powder (ash) was obtained. Lastly, crude fat content (%) was analyzed gravimetrically using the Bligh and Dyer method with 1:2 chloroform/methanol as the extraction solvent. The total carbohydrate content was calculated by subtraction of the sum of the crude fat, fiber, protein, ash, and moisture.

Total phenolic content (TPC)

The TPC of *S. oligocystum* extract was determined using Folin-Ciocalteu assay done by Nuñez-Selles et al. (2002). The algal extract (0.5 ml) was mixed with 10% sodium carbonate solution and Folin-Ciocalteau's reagent in equal volume for 1 minute and was kept at 22 °C for 5 minutes. The volume of the reaction solution was further adjusted to 5 ml using distilled water. The absorbance readings of the sample mixtures were noted using an Ultraviolet-Visible (UV-VIS) spectrophotometer at 720 nm. The phenolic content of *S. oligocystum* is expressed as milligram (mg) of gallic acid equivalent (GAE) per gram of the algal sample (Nuñez-Selles et al., 2002).

2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay

The DPPH free radical scavenging ability of *S. oligocystum* extract was analyzed by the method of Ribeiro et al. (2008). Briefly, 100 µl of different prepared concentrations of *S. oligocystum* extract (10.0, 20.0, 30.0, 40.0, and 50.0 µg/ml) were added in 5.0 ml of 0.1 mM methanolic solution with DPPH free radical. The reaction solution was mixed for 1 minute and kept at 22 °C for about 30 minutes. The absorbance readings of all the sample solutions and control were taken at 517 nm using a spectrophotometer. DPPH scavenging activity was calculated as follows:

$$\textbf{\textit{Inhibition}}\ (\%) = \left(\frac{\boldsymbol{A_{control} - A_{sample}}}{\boldsymbol{A_{control}}}\right) \times \mathbf{100}$$

where: $A_{control}$ represents the absorbance reading of the control (DPPH solution without algal extract), and A_{sample} is the absorbance reading of the algal sample (DPPH solution with algal extract). A standard curve of *S. oligocystum* extract concentration against % DPPH inhibition was prepared to determine effective concentrations (IC_{50}) of algal extract and the control. IC_{50} is defined as the amount of *S. oligocystum* extract that effectively causes a 50% reduction of the initial concentration of DPPH and is expressed in µg/ml (Arguelles et al., 2017).

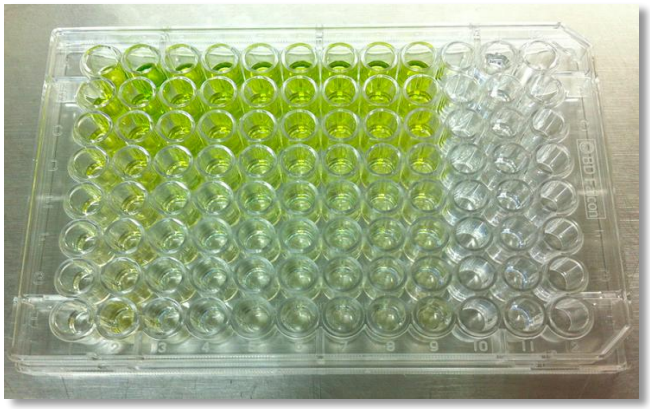


Figure-2: Microtitre plate dilution assay set-up.

Copper reduction antioxidant capacity (CUPRAC) assay

Measurement of the cupric ion (Cu^{2+}) reduction capacity of *S. oligocystum* extract was analyzed following the procedures previously done by Alpinar et al. (2009). Aliquots (0.5 ml) of the prepared concentrations of *S. oligocystum* extract (2.5, 5.0, 7.5, 10.0 and 12.50 µg GAE/ml) and standard antioxidant (ascorbic acid) were added with 1 ml each of 0.0075 M neocuproine, 1 M ammonium acetate ($C_2H_4O_2.H_3N$) buffer (pH 7), and 0.01 M $CuCl_2$ solutions (Arguelles and Sapin, 2020b). The volume of each reaction mixture were adjusted to 4.1 ml using distilled water and was kept for 30 minutes in ambient temperature condition. The absorbance readings of the control and *S. oligocystum* extract concentrations were

taken at 450 nm and the IC_{50} of *S. oligocystum* extract was determined by interpolation. IC_{50} is defined as the amount of seaweed extract needed to give an absorbance reading of 0.5 at 450 nm wavelength (Arguelles et al., 2017; Arguelles and Sapin, 2020b).

Antibacterial Activity

Three Gram-positive bacteria (*Bacillus cereus* BIOTECH 1509, Methicillin-resistant *Staphylococcus auresus* BIOTECH 10378, and *Listeria monocytogenes* BIOTECH 1958) and four Gram-negative bacteria (*Klebsiella pneumoniae* BIOTECH 1754, *Pseudomonas aeruginosa* BIOTECH 1824, *Aeromonas hydrophila* BIOTECH 10090, and *Enterobacter aerogenes* BIOTECH 1145) were tested against *S. oligocystum* extract using microtiter plate dilution assay (Arguelles et al., 2017). The test organisms were provided by PNCM, BIOTECH-UPLB. The purity of each bacterial test organisms were regularly monitored via biochemical tests and morphological characterization done every week. The antibacterial assays were conducted following appropriate guidelines and regulations set for Biosafety Level 2 (BSL-2) on the use and handling of pathogenic microorganisms in the laboratory.

Initially, test bacterial pathogens were cultivated using Luria Bertani (LB) broth medium and incubated 37 ^{o}C with shaking for 24 hours (Arguelles et al., 2017). MIC of *S. oligocystum* extract was assessed using the microtitre plate dilution (two-fold serial dilution) technique (Arguelles et al., 2019). Briefly, 100 µl of the bacterial culture (cell density of 1x 10^6 cells/ml) were mixed with 100 µl of *S. oligocystum* extract at different dilutions (1000 µg/ml-7.8125 µg/ml). The antibacterial assay was conducted in triplicate using a 96-well microtiter plate and was incubated at 35°C incubator for 12 hours (Figure 2). MICs of *S. oligocystum* extract against bacterial pathogens were observed after the incubation period. The minimum bactericidal concentration (MBC) of *S. oligocystum* was assessed by inoculating a loopful of the samples in the microtiter plate wells that did not show bacterial growth (turbidity) from the MIC assay into freshly prepared tryptic soy agar (TSA) plates and was placed in an incubator set at 35°C for 24 hours (Arguelles, 2018; Arguelles et al., 2017). The inoculated TSA plates were evaluated for any bacterial growth and the absence of growth in TSA plates would indicate that the *S. oligocystum* extract was effective in killing (bactericidal) the organism at that specific dilution (Arguelles, 2018).

Statistical analysis

The data obtained from the different assays are given as means ± standard deviation (mean ± SD) of the three experimental replicates. The Pearson's linear correlation coefficient (R) was calculated using the MS Office Excel 2007 for the statistical test of the correlation between antioxidant activities and the *S. oligocystum* extract concentration (Arguelles and Sapin, 2020a).

Results and Discussion

Proximate composition

Several species of the genus *Sargassum* (Ochrophyta) are known food ingredients and are widely consumed in several countries (Arguelles et al., 2019; Arguelles and Sapin, 2021). *Sargassum oligocystum* has high carbohydrates, proteins, dietary fiber, vitamins, and minerals, making this group of seaweeds a potential natural food source for commercial production (Peng et al., 2012; Reka et al., 2017; Arguelles and Sapin, 2021). However, the chemical composition of this seaweed has not been fully studied since only a few studies on the proximate composition of these *Sargassum* species have been conducted in the Philippines (Arguelles et al., 2019; Arguelles and Sapin, 2021). Thus, the study was done to contribute to this limited information as baseline data that will be useful to other researchers (especially in the Philippines). The proximate composition of *S. oligocystum* is shown in Table 1. In this study, *S. oligocystum* exhibited high ash, carbohydrate, and protein content with 39.01 ±0.16%, 21.43±0.37%, and 19.13 ± 0.19% respectively. Ash content of *S. oligocystum* is greater than those reported from other *Sargassum* species such as *S. naozhouense* (35.18%), and *S. vulgare* (27.09%) (Arguelles et al., 2019). A high concentration of ash would indicate the presence of high amounts of micro- (eg. copper, iodine, zinc, molybdenum, iron, selenium, manganese, cobalt, nickel, and boron) and macro-minerals (eg. magnesium, phosphorus, potassium, sodium, chloride, calcium, and sulfur) in *S. oligocystum* biomass (Peng et al., 2012; Reka et al., 2017). The protein concertation of *S. oligocystum* is greater than those observed from *Sargassum ilicifolium* (8.9 ± 0.94%), *Colpomenia sinuosa* (9.2 ± 1.78%), *Hypnea valentiae* (16.5 ± 2.78%), *Gracillaria corticata* (19.3 ± 2.19%), *Enteromorpha intestinalis* (10.5 ± 1.02%), and *Ulva lactuca* (17.1 ± 1.59%) obtained from the Persian Gulf of Iran but is lower in carbohydrate content (with an

estimated value range of 31.8% - 59.1% of the total algal biomass) (Rohani-Ghadikolaei et al., 2012). Carbohydrates and proteins are considered important biochemical components in seaweeds since it serves as a source of energy in carrying out some important metabolic processes in the macroalga (Arguelles, 2020; Arguelles and Sapin, 2020c).

Table-1: Proximate composition of *Sargassum oligocystum*.

Proximate composition	Percent composition (%)
Ash Content	39.01 ± 0.16
Moisture Content	8.97 ± 0.64
Crude Fat	1.03 ± 0.67
Crude Protein	19.83 ± 0.19
Crude Fiber	9.73 ± 0.28
Carbohydrate	21.43 ± 0.37

The majority of the seaweeds contain low concentration of lipids ranging from 0.92 to 5 % of the algal biomass (Schmid et al., 2014; Arguelles et al., 2018; Arguelles and Martinez-Goss, 2021). The crude fat content observed for *S. oligocystum* is within the reported range, with a computed value of 1.03±0.67%. *S. oligocystum* showed a total crude fiber content of 9.73±0.28%, which is also within the range of those reported crude fiber content of other seaweeds (9-21% of the algal biomass) (Schmid et al., 2014; Arguelles, 2020). Variations in the proximate composition within different species of seaweeds are affected by several environmental and ecological factors like concentration of aquatic nutrients, water temperature, geographical location, depth of the waters, and seasonal variation (Rohani-Ghadikolaei et al., 2012; Schmid et al., 2014). The results of this investigation showed that *Sargassum oligocystum* is a good alternative source of minerals, carbohydrates, and proteins that can be utilized for food, agricultural and industrial applications.

Total phenolic content (TPC)

Polyphenolic compounds are a group of bioactive metabolites that are famous for their antioxidant activities that act as terminators and scavengers of free radicals. The TPC present in *S. oligocystum* extract was analyzed using Folin-Ciocalteu reagent. *Sargassum oligocystum* extract showed a TPC of 30.94 ± 0.06 mg GAE/g which is greater than those obtained from other brown seaweeds such as *Padina antillarum* (12.4 mg GAE/g), *Spatoglossum schroederi* (11.75 mg GAE/g), *Zonaria tournefortii* (0.78 mg GAE/g), *Sargassum binderi* (0.267 mg GAE/g), *Sargassum fusiforme* (26.9 mg GAE/g), *Sargassum muticum* (10.73 mg GAE/g), and *Sargassum plagiophyllum* (7.48 mg GAE/g) (Chew et al., 2008; Plaza et al., 2010; Machu et al., 2015; Júnior et al., 2015; Del Pilar Sánchez-Camargo et al., 2016; Fellah et al., 2017). Generally, TPC is highly influenced by the extraction procedure used in the assay, sample particle size, as well as the existence of interfering compounds in the sample extract such as fats, pigments and waxes. In addition, these biologically active compounds are difficult to quantify because of their large molecular mass, resemblance in chemical structure, and their vulnerability to react chemically with another compound (Mekinić et al., 2019).

DPPH Radical scavenging activity

DPPH is a widely used free radical in checking reducing substances and is a recommended reagent in analyzing free radical scavenging activities of the sample extract (Arguelles, 2020; Arguelles and Sapin, 2020c). Table 2 presents the scavenging activity of *S. oligocystum* on DPPH free radicals. The algal extract showed a concentration-dependent DPPH scavenging activity. This observation shows that greater antioxidant activity was noted in *S. oligocystum* extract with higher phenolic concentration which is similar to other seaweeds reported from previous studies such as *Sargassum siliquosum* and *Codium intricatum* found in the Philippines (Arguelles, 2020; Arguelles and Sapin, 2020b). The antioxidative activity of *S. oligocystum* extract obtained from this study have an IC_{50} value of 28.50 µg GAE/ml and is considered more potent than ascorbic acid with IC_{50} of 59.60 µg/ml (Table 2 and Figure 3). Also, the antioxidant activity of *S. oligocystum* is more effective as compared to some known commercially available edible seaweeds such as *Porphyra* sp. (0.67.01±0.06 mg/ml), *Laminaria* sp. (0.86±0.33 mg/ml), *Undaria* sp. (0.42±0.00 mg/ml), and *Hijkia* sp. (0.47±0.06 mg/ml) (Ismail and Hong, 2002). The potent antioxidant activity of *S. oligocystum* extract can be ascribed to the possible existence of polyphenolic compounds in the extract such as phlorotannins, gallic acid, and terpenoids that can be utilized as a bioactive ingredient for pharmacological application (Ismail and Hong, 2002). Correlation analysis between phenolic concentration and antioxidant activity of *S. oligocystum* using DPPH radical scavenging assay is

presented in Figure 5. A significant positive correlation for DPPH assay (R = 0.9972) exists between antioxidant activity and phenolic concentration of the *S. oligocystum* extract, indicating the significant role of phenolic compounds to scavenging activity against free radical of the algal extract.

Table-2: DPPH radical scavenging activity and IC_{50} value of phenolics from *Sargassum oligocystum* and ascorbic acid.

Sample	Extract concentration (µg GAE/ml)					IC_{50}*
	10.0	20.0	30.0	40.0	50.0	
	DPPH inhibition (%)					
Sargassum oligocystum	20.52 ± 0.23	37.12 ± 0.12	52.29 ± 0.52	65.45 ± 0.17	76.78 ± 0.12	28.5 µg/ml
	Concentration (µg/ml)					
	20.0	40.0	60.0	80.0	100.0	
	DPPH inhibition (%)					
Ascorbic Acid	16.64 ± 0.00	34.11 ± 0.61	50.34 ± 0.00	67.32 ± 0.61	83.58 ± 0.67	59.6 µg/ml

*IC_{50} is the effective concentration that inhibits DPPH free radical by 50%. Computed by interpolation.

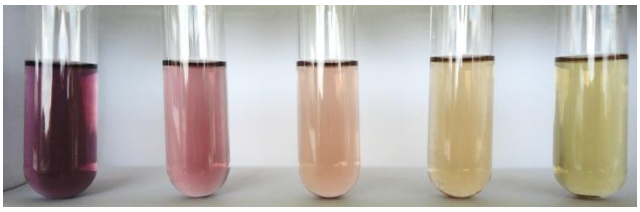


Figure-3: The DDPH free radical scavenging activity of *S. oligocystum* extract in increasing phenolic concentration.

Copper reduction antioxidant capacity (CUPRAC)

The CUPRAC assay was used to evaluate the capability of the antioxidants present in *S. oligocystum* extract to reduce cupric copper to the cuprous form. This method gives distinct advantages over DPPH assay due to its applicability to both lipophilic and hydrophilic antioxidants as well as its insensitivity to parameters (such as light, relative humidity, and pH) that adversely affects electron transfer based antioxidant assays (Rico et al., 2012). Similarly, with the results obtained from DPPH radical scavenging assay, *S. oligocystum* extract exhibited a dose-dependent copper reduction capacity. The seaweed extract is considered more potent as compared to ascorbic acid with an IC_{50} values of 6.97 µg/ml and 23.15 µg/ml, respectively (Table 3 and Figure 4). Also, the reduction capacity of *S. oligocystum* extract is more potent than those observed from other brown seaweeds such as *Turbinaria ornata* (24.34 µg/ml), and *Sargassum siliquosum* (18.50 µg/ml) respectively (Arguelles and Sapin, 2020b,c).

Table-3: Copper reduction antioxidant capacity (CUPRAC) and IC_{50} value of phenolics from *Sargassum oligocystum* and ascorbic acid.

Sample	Extract concentration (µg GAE/ml)					IC_{50}*
	2.5	5.0	7.5	10.0	12.5	
	CUPRAC value (Absorbance at 450 nm)					
Sargassum oligocystum	0.187 ± 0.001	0.369 ± 0.001	0.535 ± 0.004	0.635 ± 0.016	0.860 ± 0.002	6.97 µg/ml
	Concentration (µg/ml)					
	5.0	10.0	15.0	20.0	25.0	
	CUPRAC value (Absorbance at 450 nm)					
Ascorbic acid	0.112 ± 0.002	0.213 ± 0.007	0.328 ± 0.004	0.429 ± 0.012	0.542 ± 0.011	23.15 µg/ml

***IC_{50} is defined as the effective concentration of seaweed extract needed to give an absorbance reading of 0.5 at 450 nm wavelength.

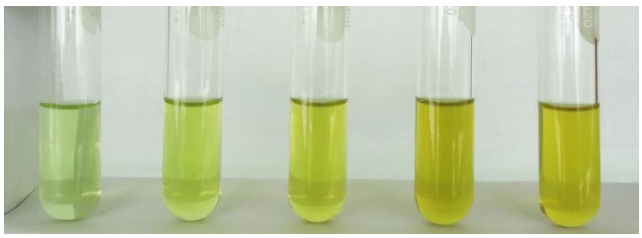
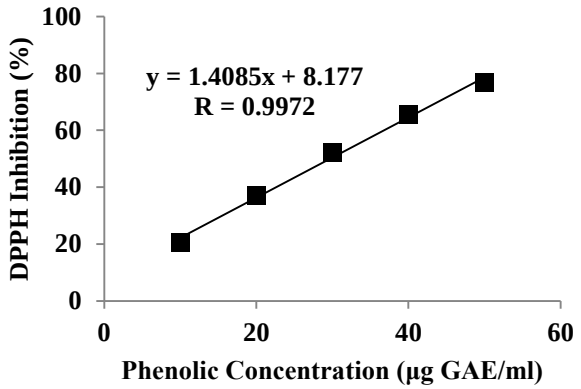


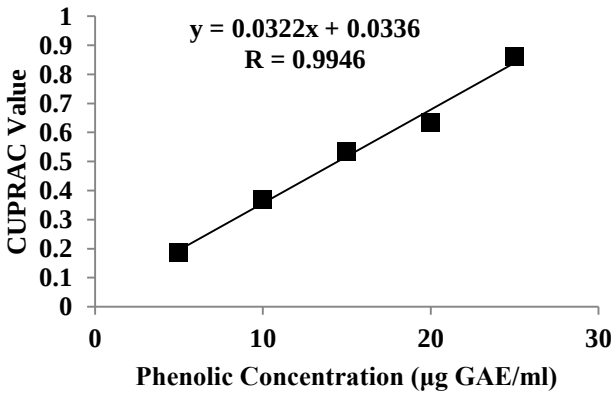
Figure-4: The copper reduction antioxidant capacity of *S. oligocystum* extract in increasing phenolic concentration.

A positive correlation for CUPRAC assay was also observed in the study with Pearson's correlation coefficient (R) value of 0.9946 proving the potential role of polyphenols in the antioxidant activity of the seaweed extract (Figure 5). Metal accumulation in cells can induce reactive oxygen species (ROS) formation that leads to the peroxidation of membrane lipid. In addition, Cu ions accelerate the formation of hydroxyl radical (using Fenton-like reaction), which reacts with DNA and proteins causing damage and functional abnormalities (Corsetto et al., 2020). Seaweeds have polyphenols that have several sites for metal complexation capable of metal ion chelation. Thus, the potent copper reduction activity of *S. oligocystum* extract is considered a good Cu chelating agent because of its capability to form chemically

inert complexes with metal ions (Corsetto et al., 2020).



(A)



(B)

Figure-5: Correlation analysis between extract concentration and antioxidant activity via DPPH free radical scavenging (A) and copper reduction antioxidant capacity (B) assay of *S. oligocystum*.

Antibacterial activity

Phenolic compounds derived from seaweeds are considered attractive bioactive compounds in treating infectious diseases because of their potent antibacterial properties (Arguelles, 2020; Arguelles and Sapin, 2020a,b,c). The findings of the antibacterial assay of *S. oligocystum* extract as opposed to some common pathogenic bacteria are presented in Table 4. Out of the seven bacterial pathogens tested, two medically-important bacteria were effectively inhibited by the algal extract by exhibiting pronounced antibacterial activity towards *Bacillus cereus* (MIC value = 250 µg/ml) and Methicillin-resistant *Staphylococcus aureus* (MRSA) (MIC value = 125 µg/ml). The antimicrobial activity of *S. oligocystum* extract against *B. cereus* is more potent than that obtained from the n-hexane extract of *Padina australis* from Cape Rachado (Malaysia) with an MIC value of 0.365 mg/ml (Chiao-Wei et al., 2011). In addition, anti-MRSA activity of *S. oligocystum* extract is comparable to those observed from *Codium intricatum* (MIC value = 250 µg/ml) and *Turbinaria decurrens* (MIC value = 125 µg/ml) (Arguelles, 2020; Arguelles and Sapin, 2020a). However, *S. oligocystum* showed no antibacterial activities against *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae*. Minimum bactericidal concentration (MBC) is the least concentration of the algal crude extract that is required to kill the test pathogenic bacteria. In this study, *S. oligocystum* extract is observed to be more potent in inhibiting the growth of Methicillin-resistant *Staphylococcus aureus* (MBC value = 250 µg/ml) than *Bacillus cereus* (MBC value = 500 µg/ml).

Table-4: The antibacterial activities of *Sargassum oligocystum* extract.

Bacterial Pathogen	Minimum inhibitory concentration (µg/ml)	Minimum bactericidal concentration (µg/ml)
Gram-positive bacteria		
Methicillin-Resistant *Staphylococcus aureus* BIOTECH 10378	125.00	250.00
Bacillus cereus BIOTECH 1509	250.00	500.00
Listeria monocytogenes BIOTECH 1958	>1000.00	ND
Gram-negative bacteria		
Enterobacter aerogenes BIOTECH 1145	>1000.00	ND
Pseudomonas aeruginosa BIOTECH 1824	>1000.00	ND
Aeromonas hydrophila BIOTECH 10090	>1000.00	ND
Klebsiella pneumoniae BIOTECH 1754	>1000.00	ND

*ND = None detected

Sargassum oligocystum extract is observed to be more effective in inhibiting Gram-positive bacteria as compared to Gram-negative bacteria. This result can be ascribed to differences in the bacterial cell wall structure. The gram-negative bacteria possess a thinner cell wall and peptidoglycan layer with an outer membrane composed of lipopolysaccharides and proteins, which serve as a barrier protecting the bacterial cells against antibiotics (Tuney et al., 2006). The findings of the current study documented that *S.*

oligocystum contain bioactive compounds with promising antibacterial properties against medically important pathogenic bacteria. Additional studies that will focus on the identification and mass production of these bioactive compounds should be conducted to know the characteristics and mechanisms involved in this biological activity.

Conclusion

From the result above, we concluded that *S. oligocystum* has important macromolecules and minerals that can be used in the food and feed industry. Also, the seaweed contains promising biologically active compounds that could be used as a valid candidate for the synthesis and development of novel therapeutic agents in disease treatment, with markedly high potency, compared to the commercially available standard antioxidant and antibacterial compounds. Additional studies targeting the identification and chemical structure elucidation of the active compounds in *S. oligocystum* extract are needed to better understand the mechanisms involved in its biological activities which are important in large-scale and product development studies.

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