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Evaluation of Nutritional Composition and *In vitro* Antioxidant and Antibacterial Activities of *Codium intricatum* Okamura from Ilocos Norte (Philippines)

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Abstract

The nutritional composition of an edible green seaweed *Codium intricatum* Okamura was evaluated, including its potential antioxidant and antibacterial properties. Results showed elemental distribution to be in decreasing order of Na > K > Ca > Mg > Fe > Mn > Pb > Zn > Cu > Cd > Cr. Proximate composition of *C. intricatum* showed high ash and carbohydrate content with estimated value of 37.16 ± 0.21% and 32.16 ± 0.29% respectively. Relative antioxidant efficiency of *C. intricatum* exerted radical scavenging activity in a concentration-dependent manner with EC_{50} value of 40.79 ± 0.015 mg GAE/ml. The tested algal extract exhibited radical scavenging activity that is correlated positively and dose-dependent to its phenolic concentration. Methanol extract of *C. intricatum* showed an extended spectrum of inhibitory activity against Gram-positive drug-resistant bacterium, Methicillin-Resistant *Staphylococcus aureus* (MRSA) with MIC and MBC of 250.00 and 500.00 μg/ml, respectively. It was moderately active against Penicillin–acylase producing *Bacillus cereus* and *Listeria monocytogenes,* each with MIC of 250 μg/ml. Minimum bactericidal concentration of 1000 μg/ml was observed for each of the test organisms. However, no inhibitory effect was observed among the tested Gram-negative bacterial pathogens. This study presented the nutritional profile and functional properties of *C. intricatum*, which make it a good alternative source of compounds possessing diverse bioactivities with prospective usage in the pharmaceutical and food industries.

Keywords: antioxidant activity, biological activity, *Codium intricatum*, Ilocos Norte, polyphenols, proximate analysis, seaweed

1. Introduction

Seaweeds are known in producing several macronutrients (lipids, proteins, carbohydrates, fibers and the like), micronutrients (minerals and vitamins) and other important biologically active compounds (e.g. polyphenols, enzymes, and antibiotics) with potential pharmacological uses (Arguelles *et al.,* 2019a; Ortiz *et al.,* 2006; Muraguri *et al.,* 2016). Analysis of elemental composition and phytochemicals in some seaweed plays a determining role in assessing their nutritional significance. Macroalgae contain high amounts of ash, showing a substantial concentration of macro and micro minerals (Reka *et al.,* 2017). The total elemental composition of seaweed is reported to have an estimated amount of 36% (of its total dried biomass), which is composed mainly of macro-minerals (magnesium, potassium, chloride, sodium, phosphorus, calcium, and sulfur) and micro minerals (copper, iodine, fluoride, zinc, molybdenum, iron, selenium, manganese, nickel, cobalt and boron) (Niranjan & Kim, 2011; Reka *et al.,* 2017). These minerals comprise an addition of up to sixty more trace elements and in much higher amount in comparison to other edible terrestrial plants, thus making these organisms potential dietary food supplements to regulate human nutrition and health (Kannan, 2014; Reka *et al.,* 2017). In the Philippines, few reports are available on the role of micro and macronutrients in edible seaweeds. The relation between elemental content of seaweeds and their curative ability is not yet properly explained in terms of modern pharmacological concepts. Therefore, it is necessary to quantify the concentration of various trace elements in assessing the impact of several edible seaweeds in the treatment of various diseases to understand their pharmacological action.

Public consumers consider seaweed-derived antioxidants to be non-toxic since it is naturally occurring and have been utilized for several centuries (Fu *et al.,* 2015). Seaweed-derived antioxidants showed a significant part in the prevention of several persistent chronic illnesses like cancer and Parkinson's disease (Fu *et al.,* 2015). Several investigations have shown the antioxidant potential of seaweeds from various parts of the world (Muraguri *et al.* 2016). Some identified antioxidant compounds from marine seaweeds include phlorotanins, carotenoids, fucoxanthin, sulphated polysaccharides, catechins, astaxanthin, and sterols (Tenorio-Rodriguez *et al.,* 2017; Muraguri *et al.,* 2016; Shipeng *et al.,* 2015; Sabeena Farvin & Jacobsen, 2013; Cox *et al.,* 2010). In addition, there are reported studies that describe the antibacterial capability (derived from secondary and primary metabolites) of seaweeds against medically - important pathogenic bacteria such as *Pseudomonas aeruginosa*,

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Staphylococcus aureus, Aeromonas hydrophila, Clostridium perfringens, Escherichia coli, Klebsiella pneumoniae, and *Enterobacter aerogenes* (Arguelles *et al.,* 2019b; Liu *et al.,* 2017; Ibtissam *et al.*, 2009; Lima-Filho *et al.,* 2002).

Codium intricatum Okamura is a famous edible green seaweed of family Codiaceae commonly called as "pukpuklo" in Ilocos Norte, Philippines. Traditionally, this macroalga is utilized as a vegetable ingredient for food and is noted to have engrossing medicinal attributes such as immunosuppressive, antioxidant, cytotoxic, and anti-metastasis activities (Vasquez & Lirio 2020; Lee *et al.,* 2017; Sanjeewa *et al.,* 2018). However, natural products from edible seaweeds (such as *C. intricatum*) are considered as one of the few studied and underutilized marine resources in the Philippines. The country's coastline has plentiful edible seaweed resources, but only limited effort has been made to examine the nutritional value, antioxidant, and antibacterial potential of these organisms for food and pharmacological applications. In light of the above information, this investigation was undertaken to evaluate the elemental composition and assess the antioxidant capacity as well as antibacterial activity of *C. intricatum* against selected pathogenic strains of bacteria. Also, quantification of the total phenolic content of the macroalga and correlation studies among the phenolic content of the algal extract and antioxidant capacity were examined.

2. Materials and Methods

2.1. Sample Collection and Extract Preparation

Codium intricatum was collected on 03 December 2018, from Pagudpud (Lat. 18^{o} 35' 21.48" N, Long. 120^{o} 47' 11.04" E), Ilocos Norte, Philippines. The macroalga was characterized and identified based on morphological taxonomic features, according to Algae Base (web site: www.algaebase.org) and Trono (2004). The macroalga was rinsed with sterile tap water to remove contaminating epiphytes and salts and further washed with sterilized distilled water. The alga was dried under shade for 10 days and crushed in a mixer grinder until a coarse powder was obtained. The seaweed powder (1 g) was successively treated with 30 mL methanol in a water bath for 30 minutes with stirring (for 1 hour). The extract was then concentrated via centrifugation at 10,000 rpm at 20 ^{o}C for 20 minutes. The liquid extract was collected using a rotary vacuum evaporator and kept under refrigerated conditions at 4 °C until other assays were conducted (Arguelles, 2018).

2.2. Proximate Analysis

The protocol employed for the quantification of percent crude protein, moisture, ash, crude fat, total carbohydrates, and crude fiber were based on AOAC (2011). Proximate composition analysis was performed in triplicates, and biochemical values are presented as means in the results (Arguelles *et al.,* 2018).

2.3. Elemental Composition Analysis

The dried powder of *C. intricatum* was subjected to elemental composition analysis following standard procedures (AOAC, 2011) for the detection of Potassium, Magnesium, Sodium, Manganese, Calcium, Iron, Zinc, Copper, Lead, Chromium and Cadmium using Atomic Absorption Spectrophotometer Perkin Elmer AAnalyst 400.

2.4. Test Microorganisms

Test microorganisms such as Gram-positive bacteria (Penicillin-Acylase producing *Bacillus cereus* BIOTECH 1635, Methicillin-Resistant *Staphylococcus aureus* BIOTECH 10378, *Listeria monocytogenes* BIOTECH 1958, *Streptococcus mutans* BIOTECH 10231) and Gram-negative bacteria (Penicillin-Acylase producing *Escherichia coli* BIOTECH 1634, *Salmonella typhimurium* BIOTECH 1826, *Aeromonas hydrophila* BIOTECH 10089 and *Pseudomonas aeruginosa* BIOTECH 1824) were used in the study. All test organisms were obtained from the Philippine National Collection of Microorganisms (PNCM), National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños.

2.5. Micro-dilution Antibacterial Assay

The minimum inhibitory concentration (MIC) of the algal extract was determined using two-fold serial dilution technique following the protocol done by Arguelles (2018). Bacterial culture suspension (1x 10^{5} cells/ml) was prepared for micro-dilution assay against *C. intricatum* extract. The bacterial suspension (100 µl) was diluted and inoculated to 100 µl of algal extract distributed in a clear 96-well microtiter plate in different dilutions starting from 1000 to 7.8125 µg/ml. In this assay, methanol was also used in the experimental set up as negative control. The seeded plate was kept at 35^{o}C for 12 hours, after which the MIC was noted. The MIC is described as the minimum amount of the tested algal extract that inhibited bacterial growth following a 12-h incubation period. The absence or presence of bacterial growth in microtiter plates was evaluated after the incubation period. The MIC of the algal extract at which no visible bacterial growth was detected was noted as the MIC for the extract–bacteria combination under consideration. As for the controls, the MIC of tetracycline against each bacterial species was also determined.

The minimum bactericidal activity (MBC) was noted by inoculating a loop of the sample in microtiter plate wells that exhibited no evident growth from the MIC assay onto a tryptic soy agar (non-selective, rich culture media) plates. The plates were stored at 35°C for 24 hours and were evaluated for visible colony growth or lack of bacterial growth for each dilution subculturing being considered. Lack of growth confirms that the seaweed extract was bactericidal at that particular dilution. Bacterial growth on agar plates shows that the sample was bacteriostatic at that dilution. The lowest concentration of the algal extract exhibiting no visible growth of the bacterial pathogen on agar was noted as the minimum bactericidal activity (MBC) value (Arguelles & Sapin, 2020).

2.6. Determination of Total Polyphenolic Content

The total phenolic compound present in *C. intricatum* extract was calculated by Folin-Ciocalteu method following the protocol done by Nuñez Selles *et al.,* (2002). *C. intricatum* extract was diluted with sterile distilled water. Aliquot of about 0.5 ml of Folin-Ciocalteu's reagent and 0.5 ml of 10% Na_2CO_3 solution was placed to 0.5 ml of the diluted seaweed extract and were thoroughly mixed in a test tube using a vortex mixer. The mixture was kept for 5 minutes, and 5 mL of sterile water was added to each of the solutions. Spectrophotometric readings were done using a Shimadzu UV-1601 spectrophotometer at 720 nm wavelength with reagent plus water as the blank sample (Arguelles & Sapin, 2020). Construction of the calibration curve was done using gallic acid as the standard, and gallic acid equivalent (GAE) was utilized to show the total phenol content in *C. intricatum* extract.

2.7. DPPH Radical Scavenging Assay

The test for antioxidant activity of *C. intricatum* was done following the method proposed by Ribeiro *et al.,* (2008) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) with few modifications. Aliquot of 100 µl of *C. intricatum* extract was mixed with 5.0 ml of 0.1 mM DPPH methanolic solution. The solution was thoroughly mixed by a vortex mixer and set aside at 30 ^oC for 20 minutes. The absorbance readings of the tested sample solutions were done using a Shimadzu UV-1601 spectrophotometer at 517 nm wavelength. The amount of inhibition was evaluated using the mathematical expression used by Ribeiro *et al.,* (2008).

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where: A_{sample} = absorbance reading of the seaweed extract (DPPH solution and test extract) and $A_{control}$ = absorbance reading of the control (DPPH solution only). In this investigation, gallic acid served as a positive control. The antioxidant activity of the seaweed extract was determined and noted as EC_{50} value (the amount of the seaweed extract showing 50% scavenging activity of DPPH radical expressed in µg/ml).

2.8. Statistical analyses

The data obtained from the experimental set-ups were given as means ± standard deviations (mean ± SD) of three simultaneous experimental readings. Estimation of the linear correlation coefficient and evaluation of the correlation analysis were done using MS Office Excel 2007.

3. Results And Discussion

3.1. Proximate Composition Analysis

Biochemical composition of *C. intricatum* collected from Pagudpud, Ilocos Norte, showed that the biochemical composition of this seaweed displayed a good nutritional profile (Table 1). In this study, *C. intricatum* exhibited high ash and carbohydrate content with 37.16±0.21% and 32.16±0.29% respectively. The total amount of ash observed in the seaweed sample was almost similar to ash content reported for *Codium geopiorum* (37.96 ± 0.05) but is higher to *Codium fragile* (21.79 ± 0.52). On the other hand, other species, such as *Codium dwarkense* have relatively higher crude ash content (in comparison to *Codium intricatum*) with an estimated value of 69.94 ± 0.11% (Mwalugha *et al.,* 2015; Turan *et al.,* 2015). High concentration of ash would mean presence of considerable concentration of macro (such as magnesium, phosphorus, potassium, sodium, chloride, calcium, and sulfur) and micro minerals (such as copper, iodine, zinc, molybdenum, iron, selenium, manganese, cobalt, nickel and boron) present in *C. intricatum* biomass (Niranjan & Kim, 2011; Reka *et al.,* 2017). Carbohydrates are regarded as the most significant biochemical component in seaweeds for the reason that it serves as the primary source of energy to execute important metabolic processes in the alga (El-Manawy *et al.,* 2019). The carbohydrate content obtained from *C. intricatum* is significantly higher compared to some of the seaweeds (*Hormophysa cuneiformis* and *Padina boergesenii*) obtained from the Red Sea coast in Egypt but is lower than *C. fragile* obtained from the coastline region of Northern Chile (El-Manawy *et al.,* 2019; Ortiz *et al.,* 2008). On the other hand, Akhtar & Sultana (2002) observed similar carbohydrate content range for other known seaweeds such as *Caulerpa* sp. (32.9%) and *Sargassum* sp. (32.3%).

Table 1. Proximate composition of *Codium intricatum* Okamura.

Proximate Composition	Percent Composition (%)*
Moisture Content	12.34±0.12
Ash Content	37.16±0.21
Crude Protein	5.03±0.05
Crude Fat	2.17±0.17
Crude Fiber	11.14±0.32
Carbohydrate	32.16±0.29

* All values are reported as mean ± standard deviation (n = 3)

Proteins are also considered as one of the principal constituent in the proximate analysis of *C. intricatum* showed in the study. In collation with other *Codium* species, the protein content of *C. intricatum* (5.03±0.05%) is within the reported range (Mwalugha *et al.,* 2015; Ortiz *et al.,* 2008). However, the protein concentration of *C. intricatum* is relatively lower than the reported protein contents of other brown (*Dictyota cervicormis, Dictyota bartaynesiana, Sargassum cristaefolium,* and *Spatoglossum asperum*) and red (*Acanthophora spicifera, Hypnea musciformis,* and *Gracilaria arcuata*) seaweeds (Mwalugha *et al.,* 2015). The total amount of protein in macroalga changes not only among species of certain genera of seaweeds but also between habitats and maturity level (Fathy, 2007). Variations in the biochemical constituents of seaweeds are attributable to environmental factors (salinity, temperature, and dissolved oxygen) and seasonal differences. Generally, seaweeds contain a low concentration of lipids ranging from 0.92 to 5 % of the total algal biomass (Schmid *et al.,* 2014; El-Manawy *et al.,* 2019; Arguelles & Martinez-Goss, 2020). The lipid content obtained for *C. intricatum* is within the reported range, with an estimated value of 2.17±0.17%. On the other hand, the average amount of dietary fiber in seaweeds varies from 9 to 21% of the total dried algal biomass (Mwalugha *et al.,* 2015). *C. intricatum* showed a total crude fiber content of 11.14±0.32%, which is within the range of those reported crude fiber content of red, brown, and green seaweeds. Moisture content gives information about the storage/shelve life of food products. The moisture content for commercially dried seaweeds should be maintained between 15 to 35% and must remain stable even below 15% (Blakemore, 1990). The result of the proximate analysis for moisture content of *C. intricatum* (12.34±0.12%) suggests its stability during storage and marketing. This study presents preliminary data regarding the biochemical composition of *C. intricatum* obtained from the coast of Ilocos Norte, Philippines. Results of the proximate composition of *C. intricatum* suggest that it could be an excellent source of carbohydrates, ash, and crude fiber.

3.2. Elemental Composition Analysis

Seaweeds contain a high concentration of minerals because of the diverse kinds of substances they absorb from the marine habitat where they grow and proliferate (MacArtain *et al.,* 2007). Ash content of edible seaweeds is usually considered as a benchmark of quality for the evaluation of the nutritional and bifunctional properties of the edible alga (Reka *et al.,* 2017). *C. intricatum* possesses a high amount of ash, up to 37.16±0.21%, in the dried algal biomass. The average concentration of some important minerals in *Codium intricatum* is presented in Table 2. The elemental distribution in *C. intricatum* was observed to be in decreasing order of Na > K > Ca > Mg > Fe > Mn > Pb > Zn > Cu > Cd > Cr. High concentration of sodium (174,900 ± 0.88 ppm) followed by potassium (75,680 ± 4,187 ppm), calcium (55,081 ±4,289 ppm) and magnesium (13,941 ± 861 ppm) were observed in the algal biomass. The high level of sodium in *C. intricatum* biomass (Table 2) is similar to the findings reported in seaweeds such as *Fucus vesiculosus*, *Halimeda opuntia, Gracillaria corticata,* and *Turbinaria artriquetra* (Balina *et al.,* 2016; Omar *et al.,* 2013). The current consumption of sodium in some of the local foods in the Philippines is far beyond the recommended concentration levels. Thus, consumption of this edible seaweed (with high sodium concentration) can contribute to a greater intake of sodium, thus causing health-associated diseases such as hypertension. This attribute may, nevertheless, be favorable if considering *C. intricatum* as a replacement of salt in several processed meat foods, for the reason that its rich mineral content can help in maintaining the salty taste of food without adding table salt (NaCl) (Circunsisão *et al.,* 2018). Recent studies show the formulation of processed meat products (such as frankfurters and meat patties) fortified with seaweeds (such as *Porphyra umbilicalis, Himanthalia elongata* or *Undaria pinnatifida*) targeting the decrease of the use of traditional salt (NaCl) and enhancing its mineral composition and content (López-López *et al.,* 2011; López-López *et al.,* 2009). Potassium is the second most abundant mineral element in *C. intricatum,* which is higher than that obtained by Omar *et al.,* (2013) from seaweeds (*Halimeda opuntia, Gracillaria corticata,* and *Turbinaria artriquetra*) at the Southern coast of Jeddah in Saudi Arabia. The abundance of potassium in this edible macroalgae is good in preventing potassium related diseases such as hypokalemia. The third most abundant element in the algal biomass is calcium. *C. intricatum* can be regarded as a suitable mineral source of calcium for the maintenance of the proper functional role of some important processes in the human body (such as glandular secretion, muscle contraction and nerve transmission) as well as resolve vascular vasodilation and contraction (Ooi *et al.,* 2012). High concentration of calcium was also noted by MacArtain *et al.,* (2007) in *Ulva lactuca* (a green seaweed) and proved to have higher concentration as compared to calcium concentration present in other terrestrial foodstuffs (MacArtain *et al.,* 2007). Furthermore, *C. intricatum* can also be considered as a good alternative source of magnesium since high concentration of the element was also observed in the macroalga. Magnesium supports the proper functioning of the immune, muscular, and nervous system. This trace element fortifies bones, control blood pressure and sugar as well as protein synthesis. However, high magnesium intake can cause a lowering of blood pressure, reduced kidney function, diarrhea and cardiac arrest (Jahnen-Dechent & Kettleler, 2012).

Trace microelements are metal constituents that are divided into two main subclasses. The first group includes cobalt, copper, iron, manganese, and zinc, which are important to the human body for the proper functioning of biochemical processes but can be toxic at very high concentrations. On the other hand, the second group includes mercury, cadmium, chromium, and lead, which are metals that do not have a biological function and includes critical metallic chemical contaminants in the marine ecosystem (Omar *et al.,* 2013). Manganese was noted in the biomass of *C. intricatum* (32.40 ±1.67 ppm) in significant amount but is lower than that obtained by Balina *et al.,* (2016) from *Fucus vesiculosus* (1680 ppm). Based on the result, this edible seaweed can also be used to supplement manganese in food which is important in controlling blood sugar levels, formation of bone and tissues as well as prevention of diseases (diabetes, osteoporosis, arthritis and epilepsy) (Nielsen, 1999).

Iron deficiency is a frequent problem affecting several people globally. Limitation of iron can result in chronic infections and bleeding, as well as a deficiency in folic acid, vitamin A and vitamin B12. The high concentration of iron observed in *C. intricatum* (290.53 ±24.74 ppm) suggests that the edible seaweed can serve as an excellent alternative source of dietary iron to address iron deficiency (Ooi *et al.,* 2012). Zinc and copper are important metallic chemical elements that are required at most in minimal concentration by humans for the proper function of some important biochemical processes in the body. Zinc acts as a stimulus for the formation of metallothionein that possesses a strong affinity for copper, which hampers systemic assimilation of copper inside the cells of the intestine (Ooi *et al.,* 2012). *C. intricatum* contains minimal amounts of copper and zinc that can be used as a source of food to address copper-zinc deficiency and disparity. The amount of copper and zinc obtained in this study is lower than that obtained from *F. vesiculosum* with copper and zinc concentration of 12.7 ppm and 89 ppm, respectively (Balina *et al.,* 2016). Chromium, cobalt, and copper are three microelements that support important biological processes in the human body. Chromium is a mineral that helps in the regulation of blood sugar and pressure levels as well as the normal development of body muscle (Nriagu, 1988). On the other hand, cobalt is an important microelement needed for vitamin C and vitamin B12 synthesis. This mineral helps in the normal cardiac functioning of the human body and facilitates iron absorption within the body (Kazantzis, 1981).

Copper is important in the synthesis of phospholipids and hemoglobin as well as helping in the production of melanin pigment for the skin (Uauy *et al.,* 1988). *C. intricatum* contains these microelements in considerable amounts (Table 2) and thus serves as a potential natural source of these important nutrients.

Toxic microelements such as cadmium and lead, if present at minimal concentrations, can be detrimental to human health. These elements are not needed in the proper functioning of the body. Thus, non-inhalation and non-ingestion of these microelements are encouraged for the reason that serious toxic poisoning can result in death (Kisten *et al.,* 2017). Cadmium, although not considered essential, helps in the proper functioning of important internal organs such as kidney and liver. However, it is also considered toxic in high amounts, causing damage to important organs (kidneys, liver, and lungs), diarrhea, cardiovascular problems, anemia, and severe damage to the brain (Bernard & Lauwerys, 1986). On the other hand, lead is noted for its importance in the normal brain, bone, and muscle functions but has also been known to cause seizures, kidney failure, comas, anemia, and heart attacks (Schroeder & Tipton, 1968). *C. intricatum* revealed the presence of these microelements but in amounts that are not toxic and confirms the nutritional potential and benefit of this edible seaweed.

Table 2. Concentrations of macro and micro-elements of *Codium intricatum* Okamura.

Seaweed	Elemental Parameter*										
	Ca (ppm)	Mg (ppm)	Na (ppm)	Mn (ppm)	K (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Pb (ppm)	Cr (ppm)	Cd (ppm)
Codium intricatum	55,081 ±4,289	13,941 ± 861	174,900 ±0.88	32.40 ±1.67	75,680 ±4,187	290.53 ±24.74	4.65 ±0.25	2.27 ±0.08	6.47 ±0.25	0.84 ±0.03	1.70 ±0.04

* All values are reported as mean ± standard deviation (n = 3)

C. intricatum is potentially a good alternative source of microelements needed for human health and nutrition. Differences in the concentration of trace element content in *C. intricatum* in comparison to other seaweed species are highly influenced by several environmental and cultural factors including: pH, interactions, salinity and interactions among elements, light intensity, amount of trace elements in water, and other metabolic influences like dilution of elemental composition caused by growth and development of seaweed (El-Said & El-Sikaily, 2013; Zbikowski *et al.,* 2006). The result of this study provides baseline information on the nutritional value of *C. intricatum* that can be used in the food industry for human consumption to address nutrient deficiency in the world.

3.3. Total Phenolic Content

Several studies have demonstrated that polyphenols are the primary compounds responsible for the antioxidant activities of diverse kinds of marine macroalgae (Zhang *et al.,* 2007; Shipeng *et al.,* 2015). The total polyphenolic content (TPC) in the algal extract was evaluated using Folin-Ciocalteu method and is given as mg GAE/g of the dried seaweed biomass (Table 3).

Table 3. Phenolic content and DPPH free radical scavenging activity of methanol extract of *Codium intricatum* Okamura.

Seaweed	Solvent Extractant	Total Phenolic Content (mg GAE/g)	EC_{50} (mg GAE/ml)
Codium intricatum	Methanol	0.934±0.02	40.79 ±0.015

The TPC in the algal biomass is 0.934±0.02 mg GAE/g which is greater than that obtained from the methanol extracts of other seaweeds such as *Gracilaria bursa-pastoris* from Marmara Sea coast of Turkey with 0.35 ± 0.05 mg GAE/g and *Sargassum horneri* from the seacoast of Bijin-do, South Korea with 0.51 ± 0.02 mg GAE/g (Shipeng *et al.,* 2015; Yildiz *et al.,* 2011). However, higher phenolic concentrations were observed by Ismail (2017) from three Egyptian seaweeds (*Sargassum linifolium, Corallina officinalis* and *Ulva fasciata*) with maximum value of 10.35 mg GAE/g, 4.89 mg GAE/g, and 11.95 mg GAE/g dry weight respectively. Variations in the total polyphenolic contents among species of seaweeds are caused by several influencing environmental factors like seasonal variation, physiological condition, and geographical origin (Machu *et al.,* 2015).

3.4. Antioxidant Activity

Seaweeds are often exposed to stressful conditions because of exposure to high light and oxygen that results in the emergence of free radicals; thus, seaweeds form a defense mechanism and produce substances such as antioxidants (Cox *et al.,* 2010). In this study, assessment of the antioxidant capacity of the solvent extract of *C. intricatum* was established by assessing the DPPH radical scavenging activity at different prepared concentrations of seaweed extract, and EC_{50} value of the seaweed extract was calculated and presented in Table 3. DPPH is a quick method for evaluating the antioxidant activity by measuring the DPPH scavenging capability of the extracts. The seaweed extract was capable of reducing DPPH free radical into diphenyl picryl hydrazine. DPPH scavenging activity of the extract of *C. intricatum* showed that at 53.2 mg GAE/ml concentration, the maximum antioxidant activity was found to be 63.21 ± 0.21%. The results of the analysis exhibited that the DPPH scavenging activity increases when the amount of the seaweed extract is increased (Figure 1).

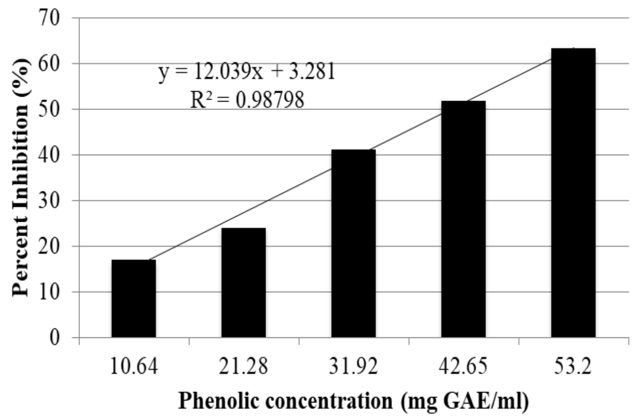

Figure 1. Correlation between total phenolic content and total antioxidant activity (DPPH radical scavenging assay) of *Codium intricatum.*

The results suggested that the algal extracts could inhibit oxidation through free radical scavenging, which can be attributed to phenolic compounds that can act as natural antioxidants. The computed EC_{50} value of the algal extract is 40.79 ± 0.015 mg GAE/ml. Lower EC_{50} concentrations are indicative of high antioxidant activity. In collation to the EC_{50} of other edible seaweed such as *Enteromorpha spirulina* (50.00 ± 0.04 mg GAE/ml), *C. intricatum* had a comparatively greater antioxidant activity (Cox *et al.,* 2010). Polyphenolic compounds are largely distributed in seaweeds, and several factors such as extraction methods and polarity of solvents used for polyphenol extraction must also be considered in order to obtain the target polyphenols in the sample. Generally, polyphenols are usually soluble in polar organic solvents than in aqueous mixtures of ethanol, acetone and methanol (Aili Zakaria *et al.,* 2011; Arguelles *et al.,* 2017).

3.5. Correlation Study Between Antioxidant Activity And Total Polyphenolic Content

Several studies suggested a direct relationship between the antioxidant activity and total phenolic content in several species of seaweeds (Shipeng *et al.,* 2015; Ismail, 2017; Arguelles *et al.,* 2019b). Nevertheless, other studies show the absence of such a relationship (Tenorio-Rodriguez *et al.,* 2017). In this study, antioxidant activities of the seaweed extract tested follow a dose-dependent manner and increased with the increase in the concentration extract. A strong and direct correlation between DPPH assay and TPC (R^2=0.988) indicated that polyphenols present in *C. intricatum* crude extract are involved in antioxidant activity by scavenging DPPH (Figure 1). Several species of seaweeds contain active antioxidant compounds such as flavonoids and polyphenols from which the antioxidant activity of *C. intricatum* extract tested in the study could be attributed to (Farasat, 2013; Fu *et al.* 2015). Phenolic profiling of the crude extract is essential to identify the components of active substances to get a more appropriate correlation among the role of polyphenols and antioxidant activity (Arguelles *et al.*, 2017).

3.6. Antibacterial Activity

Seaweeds are potential renewable resources of bioactive compounds with diverse beneficial effects. In the Philippines, several studies report that bioactive secondary metabolites isolated from various seaweed exhibit potential for being used as antimicrobial molecules (Arguelles *et al.*, 2019b; Jerković *et al.*, 2019; Vasquez & Lirio, 2020; Moubayed *et al.*, 2017). Nevertheless, the possible use for the biomedical application of species belonging to the genus *Codium* has been the least investigated for their biological activities among all members of Chlorophyceae (Jerković *et al.*, 2019; Vasquez & Lirio, 2020). Methanol extract of *C. intricatum* was subjected to antibacterial assay against a wide array of bacterial pathogens. *Codium intricatum* showed an extended spectrum of inhibitory activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA) with MIC and MBC of 250 and 500 µg/ml, respectively (Figure 2). It was moderately active against Penicillin–acylase producing *Bacillus cereus* and *Listeria monocytogenes,* each with MIC of 250 µg/ml. MBC of 1000 µg/ml was observed for each of the test organisms. Moreover, no inhibitory effect was observed in *S. mutans* and among the Gram-negative bacterial pathogens used in the study, which is similar to that observed by Lavanya & Veerappan (2011) as well as Ibtissam *et al.*, (2009) against *Escherichia coli, Pseudomomas aeruginosa* and *Klebsiella pneumoniae*. However, other studies such as that of Koz *et al.*, (2009), antibacterial activity of *C. fragile* extract against *E. aerogenes, E. coli* and *B. subtilis* was observed. Antibacterial activity of *C. intricatum* extract in opposition to *S. aureus,* and MRSA is similar to that reported from methanol extracts of three *Codium* species (*C. tomentosum, C. dichotomum,* and *C. fragile*) where high inhibitory activity were noted (Ibtissam *et al.*, 2009; Koz *et al.*, 2009). However, when *S. aureus* was tested against *C. bursa*, no antibacterial activity was observed (Ibtissam *et al.*, 2009; Jerković *et al.*, 2019). As previously shown in several studies, antibacterial activity of marine seaweeds inhibited the growth of a wide array of bacteria. Also, variations in the inhibitory activity of seaweed extracts against microorganisms are highly dependent on several factors like habitat and place of collection, physiological phenomena (developmental stage of the macroalga), ecological parameters (irradiance and nutrients) as well as seasonality variations (Salvador *et al.*, 2007). The findings of the present investigation are analogous to those observed by Tuney *et al.*, (2006) and Taskin *et al.*, (2001) where Gram-positive bacteria are, to a greater extent, vulnerable to seaweed extracts used in their investigation compared to Gram-negative bacteria. The sensitivity of a specific kind of bacteria to the activity of bioactive substances found in the algal extract is attributed to the difference in structure and composition of the cell walls (Arguelles & Sapin, 2020). Gram-positive bacteria are marked by dense peptidoglycan in the outer layer of its cell wall, while Gram-negative bacteria have composite, multilayered cell wall structure that makes the entry of bioactive compounds more difficult (Arguelles & Sapin, 2020).

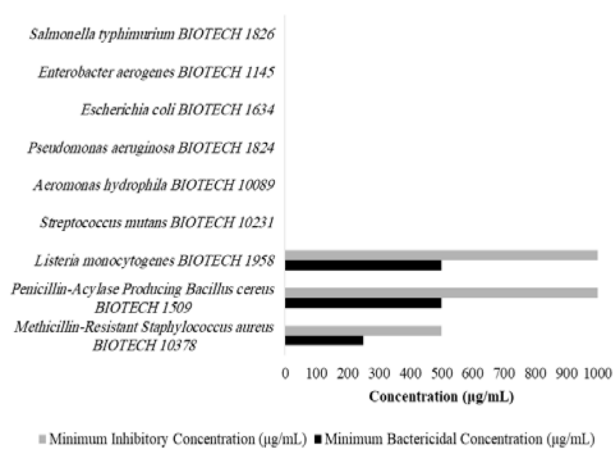

Figure 2. Antibacterial activity of *Codium intricatum* extract.

In the Philippines, no extensive and individual scientific studies are available, showing the potential antibacterial property of *C. intricatum*. This study is the first investigation in the country about the antibacterial property of *C. intricatum* against Methicillin-resistant *Staphylococcus aureus,* Penicillin-acylase producing *Bacillus cereus,* and *Listeria monocytogenes*. In light of the recent findings in this study, *C. intricatum* extract exhibited the existence of bioactive compounds with promising antibacterial property against these important pathogenic bacteria. Further study should be conducted to purify and identify these bioactive substances.

4. Conclusion

Seaweeds are potential renewable resources that have been reported to provide several beneficial effects. The elemental composition and proximate composition of *C. intricatum* suggest that the alga has potential food value that can be use in the food industry. Also, this study concludes that *C. intricatum* represents an alternative natural source of polyphenols and other bioactive compounds for the development and production of novel antibiotics and natural antioxidants. Further studies should be conducted to identify the structure and elucidate the mechanism of action of different biologically active substances present in the algal extract that showed promising antibacterial activity.

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