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Proximate composition and *in vitro* analysis of antioxidant and antibacterial activities of *Padina boryana* Thivy

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ABSTRACT

Chemical composition, antioxidant and antibacterial properties of the brown seaweed, *Padina boryana* Thivy from Catanauan, Quezon were investigated. The seaweed contains high concentration of carbohydrate (40.81 ± 0.49%), ash (21.80 ± 0.33%) and protein (18.03 ± 0.23%) on dry weight basis. Also, *P. boryana* had a total phenolic content of 1.40 ± 0.02 mg GAE/g. Antioxidant efficiency of *P. boryana* were characterized by having potent DPPH free radical scavenging activity and high copper reducion capacity with IC_{50} value of 31.2 µg GAE/mL and 11.43 µg GAE/mL, respectively, more effective than ascorbic acid. The seaweed extract exhibited potent antibacterial activities against bacterial pathogen such as *Staphylococcus epidermidis*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Aeromonas hydrophila*. The current investigation is a pioneering study in the Philippines showing the potential of *P. boryana* as a source of bioactive substances that can be used for pharmaceutical and food industries.

Keywords: bioactive compound; chemical composition; marine; polyphenols; seaweed

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1. INTRODUCTION

Bioactive substances derived from seaweeds play an important role in the synthesis and development of medically important drugs. Natural products obtained from these organisms contain diverse kinds of secondary metabolites such as terpenoids, halogenated ketones, alkanes, phlorotannins, sterols, acrylic acids, and phenolic compounds that are effective in controlling bacterial infections and other diseases (Arguelles and Sapin, 2020a; 2020b; 2020c). This suggests the potential use of these marine organisms as natural sources of bioactive compounds for the development of novel drugs. In addition, seaweeds are also known as rich sources of biomolecules and nutrients for food and feeds. The nutritional and functional properties of marine macroalgae are not well studied, and they are commonly evaluated from their biochemical composition alone. Generally, seaweeds are abounding in minerals, polysaccharides, dietary fibers, and vitamins (Wong and Cheung, 2000). Recent studies show that utilization of seaweed-derived metabolites can help in lowering the incidence of human diseases, such as heart diseases, obesity, diabetes, and cancers (Shanmuganathan and Devi, 2016; Wong and Cheung, 2000).

The genus *Padina* is a well-defined and widely distributed group of seaweeds found in the subtidal or intertidal zones of tropic coastal areas. These seaweeds are

characterized by having thalli (fronds) that are flabellate, often conspicuously zonate, attached by compacted rhizoidal holdfast. The thalli of *Padina* have thin deposits of lime giving the seaweed a brown or whitish appearance (Trono, 1997). Some species of *Padina* are traditionally being used as food source (such as gelatin-like sweet meat and seasoning in dry flake forms and as salt) with other strains reported to have biological activities, such as antibacterial, antioxidant, anti-diabetic, hypoallergenic, anti-inflammatory, and skin lightening properties (Jayawardena et al., 2020; Salosso et al., 2020; Novaczek and Athy, 2001; Robledo and Pelegrin, 1997). *Padina* is also known to have high carbohydrates, proteins, dietary fibers, vitamins, minerals, and low-calorie content that made this group of seaweeds a significant natural source of food and feed for commercial production (Salosso et al., 2020; Mwalugha et al., 2015).

In spite of the fact that seaweed flora in the Philippines is comparatively rich, these biological resources are considered relatively under-utilized (Arguelles and Sapin, 2020a). In general, most of the Philippine macroalgae are mainly being used as food and animal feeds and only few studies are being done for its pharmacological application (Arguelles, 2020). *Padina boryana* Thivy is a brown seaweed that is classified under the order Dictyotales and class Phaeophyceae commonly observed in coastal areas of the Philippines. Previously, this seaweed was documented to contain bioactive substances, such as fucoxanthin, carotenoids, and polysaccharides that have promising biological properties that can be used as functional food for humans and animals as well as other pharmacological applications (Jayawardena et al., 2020; Salosso et al., 2020). This investigation is a pioneering study in the Philippines highlighting the bioactive properties of brown macroalga, *P. boryana* with potential use as an alternative source of active compounds and biomass for utilization as functional food ingredient and control of bacterial diseases. The current study specifically aimed to determine the proximate composition of *P. boryana* in order to provide an overview of the chemical information of this seaweed. Furthermore, the phenolic content, antioxidant and antibacterial properties of this seaweed were also studied to assess its potential pharmacological use. The antioxidant activities of *P. boryana* were also analyzed. In addition, correlation analysis between antioxidant property and phenolic concentration of the seaweed extract was established.

2. MATERIALS AND METHODS

2.1 Materials

The brown macroalga, *P. boryana* was collected in November 2019 during low tide condition along the coast of Catanauan (Lat. 13° 36' 20.88" N; Long. 122° 14' 18.24" E), Quezon, Philippines. During the collection period, the mature seaweed thalli is attached in rock surfaces, light brown in color and smooth to touch with very few epiphytes. The thalli of *P. boryana* measures 5.1-6.5 cm, broadly flabellate, prostrate rhizoid with calcified inner and outer thallus surfaces (Figure 1). The seaweed thalli was gently scrubbed using brush bristles and rinsed several times with tap water to eliminate epiphytes, necrotic parts, and sand particles attached to the seaweed. The cleaned samples were sun-dried for six days, cut into small pieces and pulverized before solvent extraction (Arguelles and Sapin, 2020a; Arguelles et al., 2019b). The macroalga was identified using relevant morphotaxonomic features and identification key of Trono (1997) and Algae Base (website: www.algaebase.org).

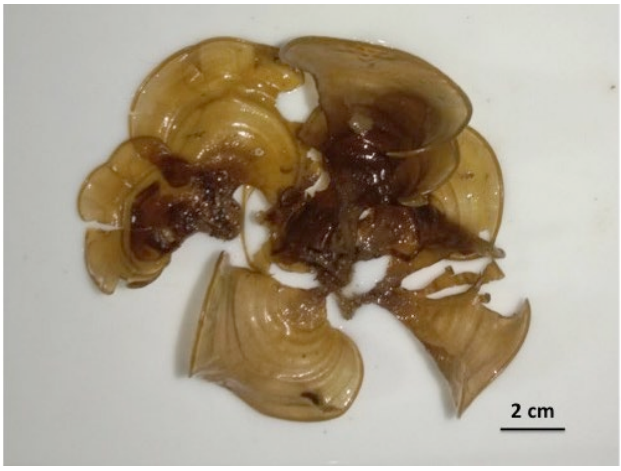

Figure 1. Thallus morphology of *P. boryana* from the coast of Catanauan, Quezon

2.2 Preparation of seaweed crude extract

The preparation of the algal crude extract was done following the extraction protocol of Gao et al. (2002) with some modification. Briefly, 1 gram of powdered *P. boryana* was extracted using 30 mL acidified methanol (1 HCl: 80 methanol: 10 water) placed in an ultrasonic bath for 30 minutes with stirring for 1 hour (Arguelles and Sapin, 2020c). The extraction mixture was centrifuged at 10,000 rpm set at 20°C for 20 minutes. The extract was further concentrated using a rotary evaporator under reduced pressure set at 40°C. The crude extract was stored at 4°C in a refrigerator to preserve its activity until used in the different test assays (Arguelles, 2021).

2.3 Proximate nutritional composition analysis

Crude fiber content of *P. boryana* was obtained using the Weende method (Horwitz and Latimer, 2011). Briefly, 0.3 g of the seaweed biomass was digested using 1.25% HCl

followed by 1.25% NaOH. The reaction residue acquired after digestion of the algal biomass was oven-dried for 3 h at 105°C and weighed. On the other hand, ash content was analyzed by subjecting the dried seaweed biomass in complete burning using an oven set at a temperature of 550°C for 4 h until a grayish powder (ash) was obtained (Arguelles, 2021). The crude fat concentration of *P. boryana* was estimated using the Bligh and Dyer method (Horwitz and Latimer, 2011). Dried seaweed biomass (10 g) was mixed with 1:2 chloroform/methanol (extraction solvent) and set aside overnight after addition of calcium chloride. The algal biomass was separated from the chloroform layer (Arguelles et al., 2019a). Using an evaporator, the chloroform layer was evaporated to dryness and placed in an oven set at 105°C for 30 min. The crude lipid concentration of the algal biomass was determined by calculating the weight difference between flasks. Determination of the moisture content of *P. boryana* was done by subjecting the algal biomass (2 g) to complete dryness using an oven set at 105°C until a constant weight was noted (Arguelles, 2020). The protein content of *P. boryana* was determined using Kjeldahl method. The amount of nitrogen in crude protein of the algal extract was determined using 6.25 as the conversion factor. The total carbohydrate content was obtained via difference method following the equation below:

$$\%Carbohydrates = 100 - (\%Moisture\ Content + \%Protein + \%Fat + \%Ash)$$

2.4 Total phenolic content (TPC)

The TPC of *P. boryana* crude extract was analyzed following the Folin-Ciocalteu assay done by Nuñez Selles et al. (2002). Briefly, 0.5 mL of *P. boryana* crude extract was mixed with 10% sodium carbonate solution and Folin-Ciocalteau's reagent in equal volume for 1 minute. The mixture was set aside at room temperature for 5 minutes and the final volume was adjusted to 5 mL using distilled water. The absorbance reading of the mixture was noted at 720 nm using an ultraviolet-visible (UV-VIS) spectrophotometer. The TPC is expressed as milligram of gallic acid equivalent (GAE) per gram of the algal sample.

2.5 Antioxidant activities

The antioxidant property of *P. boryana* crude extract was analyzed using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay as well as copper reduction antioxidant capacity (CUPRAC) assay. These antioxidant assays were used in the study to evaluate the antioxidant activity of *P. boryana* crude extract.

2.5.1 DPPH radical scavenging assay

The scavenging activity of *P. boryana* crude extract against DPPH free radical was analyzed using the protocols done by Ribeiro et al. (2008). Briefly, 100 µL of *P. boryana* crude extract prepared at different phenolic concentrations (10.0, 20.0, 30.0, 40.0, and 50.0 µg/mL) were mixed in 5.0 mL of 0.1 mM methanolic solution with DPPH free radical. The mixture was homogenized and kept at ambient temperature for 20 minutes. The absorbance of the crude extract and control was measured at 517 nm using a UV-VIS spectrophotometer. The free radical scavenging activity was calculated as:

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

where $A_{control}$ is absorbance of the control (DPPH solution without algal crude extract) and A_{sample} is absorbance of the algal sample (DPPH solution plus algal crude extract). Ascorbic acid was used as the control in this assay. Standard curve of the crude extract concentration versus % DPPH was generated to determine the effective concentrations (IC_{50}) of *P. boryana* crude extract and the control. IC_{50} is the concentration of the algal crude extract needed to have a 50% reduction of the initial DPPH concentration and is expressed in µg/mL (Arguelles et al., 2017).

2.5.2 CUPRAC assay

The copper reduction antioxidant capacity of *P. boryana* crude extract was analyzed following the method done by Alpinar et al. (2009). Briefly, 1 mL each of 0.0075 M neocuproine, 0.01 M copper chloride solution, and 1 M ammonium acetate buffer (pH 7) were mixed in sterile test tubes containing 0.5 mL of crude extract (2.5, 5.0, 7.5, 10.0 and 12.50 µg GAE/mL) and standard antioxidant (ascorbic acid) (Arguelles and Sapin, 2020c). The volume for each prepared reaction mixtures were adjusted to 4.1 mL using distilled water. The mixtures were kept for 30 minutes at ambient temperature condition. The absorbance readings of the crude extract concentrations and control were noted at 450 nm wavelength (Arguelles and Sapin, 2020c). IC_{50} of *P. boryana* crude extract was calculated by interpolation. It is the concentration of the crude extract needed to give a CUPRAC value of 0.5 absorbance reading at 450 nm (Arguelles et al., 2017).

2.6 Antibacterial Activity

The test organisms were provided by the Philippines National Collection of Microorganisms, University of the Philippines Los Baños (UPLB). Four Gram-negative bacteria (*Klebsiella pneumoniae* BIOTECH 1754, *Aeromonas hydrophila* BIOTECH 10090, *Pseudomonas fluorescens* BIOTECH 1123, and *Pseudomonas aeruginosa* BIOTECH 1824) and four Gram-positive bacteria (*Micrococcus luteus* BIOTECH 1793, *Staphylococcus epidermidis* BIOTECH 10098, *Staphylococcus aureus* BIOTECH 1823 and *Bacillus cereus* BIOTECH 1509) were tested against *P. boryana* crude extract using microtiter plate dilution assay (Arguelles, 2020). The bacterial pathogens were cultivated using Luria Bertani (LB) broth medium and incubated at 37°C with shaking for 24 hours. Viability and purity of each test organisms are regularly monitored via morphological characterization and biochemical tests done every week (Arguelles, 2018).

Two-fold serial dilution technique was used to evaluate the minimum inhibitory concentration (MIC) of *P. boryana* crude extract (Arguelles et al., 2019b). In this method, 100 µL of test bacterial culture (cell density of 1 x 10^6 cells/mL) were mixed with 100 µL of the crude extract prepared at different dilutions (1000 µg/mL - 7.8125 µg/mL). The experimental assay was done using a 96-well microtiter plate in triplicate and were incubated at 35°C for 12 hours. MICs of *P. boryana* crude extract against the tested bacterial pathogens were noted after incubation period. Minimum bactericidal concentration (MBC), a lowest crude extract

concentration displaying no visible growth (total kill) of bacteria on agar subculture, was determined by inoculating a loopful of the samples in microtiter plate wells that did not exhibit any bacterial growth from the MIC assay onto tryptic soy agar plates. The inoculated plates were kept at 35°C for 24 hours (Arguelles et al., 2017). The petri plates were evaluated for bacterial growth for each dilution subculturing. Absence of bacterial growth in petri plates would mean that *P. boryana* crude extract was bactericidal at that specific dilution.

2.7 Statistical analysis

The experimental data obtained from the different assays were presented as mean ± standard deviation (SD) of three replicates. The statistical test for the correlation between antioxidant activities and the crude extract concentrations was assessed by calculating the Pearson's linear correlation coefficient (R) using the MS Office Excel 2007.

3. RESULTS AND DISCUSSION

3.1 Proximate nutritional composition analysis

Macroalgae are important alternative sources of macronutrients like fiber, protein, lipids, and carbohydrates with direct relevance to pharmaceutical and food industries (Goecke et al., 2012; Arguelles, 2020). The proximate composition of *P. boryana* from Catanauan, Quezon is presented in Table 1. Among the nutrient compounds, carbohydrates obtained the highest content in collation to ash and protein. The typical carbohydrates that are present in brown algae are laminaran, fucoidan, alginates and cellulose (Goecke et al., 2012). The carbohydrate content observed in this study falls within the usual concentration range of *Padina* spp. from earlier studies (Salosso et al., 2020; Goecke et al., 2012). Generally, brown seaweeds generate acidic polysaccharides (such as alginic acids) as well as heterofucans with variable ratio of galactose, glucuronic acid, mannose, glucose, fucose, and xylose together with a protein moiety and sulfate (Goecke et al., 2012).

Table 1. Proximate nutritional composition of *P. boryana* from the coast of Catanauan, Quezon in the Philippines

Proximate composition	Percent composition (%)
Moisture Content	9.79 ± 0.71
Ash Content	21.80 ± 0.33
Crude Protein	18.03 ± 0.23
Crude Fat	1.27 ± 0.18
Crude Fiber	8.30 ± 0.15
Carbohydrate	40.81 ± 0.49

The amount of protein presented in *P. boryana* was greater than those obtained by Balasundari et al. (2017) from different brown seaweeds from the Gulf of Mannar in India, including *Padina gymnospora*, *Turbinaria ornata*, *Sargassum tennerimum*, *Sargassum longifolium*, *Sargassum ilicifolium* and *Padina tenuis*. Generally, seaweeds are not considered a good source of lipid since majority of seaweed possess not more than 4% of lipid at dry biomass weight basis (Arguelles, 2018; Robledo and Pelegrin, 1997). Low crude fat content was observed for *P. boryana,* which is similar to those observed form other species of seaweeds, such as *Hypnea charoides* and *Eucheuma cottonii* with crude fat content of 1.48% and 1.10%, respectively (Wong and Cheung, 2000; Matanjun et al., 2008). High ash content was also observed in *P. boryana*, indicating the appreciable amount of diverse mineral components in macroalga. This parameter was highly influenced by existing minerals in tissues and salts attached to *P. boryana*. Crude fiber content of *P. boryana* showed the amount of indigestible component of the seaweed. These indigestible components include insoluble and soluble fibers. The concentration of crude fiber observed in *P. boryana* was greater than those obtained from other species of seaweeds such as *Gracilaria cervicornis*, *Sargassum filipendula*, *Gracilaria cornea*, and *Sargassum vulgare* (Marinho-Soriano et al., 2006; Robledo and Pelegrin, 1997). Variation on the proximate composition among seaweeds could be due to factors such as geographical difference, habitat, nutrient concentration in the environment, and climate on growth and development of seaweeds (Yaich et al., 2011; Matanjun et al., 2008). Thus, differences in the chemical composition of *P. boryana*, as compared to other seaweeds, were observed in the study. *P. boryana* proved to be an engrossing alternative source of dietary fiber, proteins, carbohydrates, and other minerals. High concentration of these chemical substances shows the potential of this seaweed for utilization as a functional ingredient in food industry.

3.2 TPC

Phenolic compounds are natural antioxidants that are capable of inhibiting enzymes involved in the formation of free radicals as well as exhibit antibacterial, anticancer, anti-allergic, anti-diabetes, and anti-aging activities (Sameeh et al., 2016). In this investigation, the TPC of *P. boryana* crude extract was 1.40 ± 0.02 mg GAE/g. The TPC observed in this investigation was greater than that obtained by Boonchum et al. (2011) and Fu et al. (2015) from ethanol extracts of *Sargassum binderi* and *Sargassum polycystum*. Furthermore, TPC of *P. boryana* is comparable to those observed from other brown algal species, such as *Saccharina japonica* and *Lessonia trabeculata* obtained from Japan and China (Machu et al., 2015; Yuan et al., 2018). The amount of phenolic compounds extracted from the algal biomass was influenced by the type of solvent used in the extraction. Polar organic solvents (such as acidified methanol) exhibited greater capacity of extracting phenolic compounds in seaweed samples, as compared to non-polar solvents (Wang et al., 2009; Arguelles, 2020).

3.3 Antioxidant activities

In this investigation, antioxidant activities of *P. boryana* crude extract were analyzed using DPPH radical scavenging assay and CUPRAC assay.

3.3.1 DPPH radical scavenging activity

Antioxidants capable of free radical scavenging activity are useful in prevention and control of many diseases (Al-Enazi et al., 2018). DPPH assay is a popular method being used to assess the free radical scavenging activity of different plant, algal, or fungal extracts and fractions. The scavenging activity of *P. boryana* crude extract against free radical was assessed using DPPH assay. Results of the analysis suggested that the scavenging activity of *P. boryana* crude extract against DPPH free radicals increased when the phenolic concentration of the crude extract (10 - 50 µg/mL) increased (Table 2).

Table 2. DPPH free radical scavenging activity of phenolics from *P. boryana* and ascorbic acid

Sample	Phenolic concentration (µg GAE/mL)				
	10.0	20.0	30.0	40.0	50.0
Padina boryana	22.73± 0.35	36.51± 0.06	48.73± 0.12	58.95± 0.12	61.28± 0.06
	Concentration (µg/mL)				
	20.0	40.0	60.0	80.0	100.0
Ascorbic acid	16.64± 0.00	34.11± 0.61	50.34 ± 0.00	67.32± 0.61	83.58± 0.67

Note: IC_{50} the effective concentration that inhibits DPPH free radical by 50%, as computed by interpolation, was 31.2 µg/mL for *Padina boryana* and 59.6 µg/mL for ascorbic acid

The IC_{50} value of *P. boryana* crude extract (31.2 µg/mL) showed more potent antioxidant activity than that obtained from ascorbic acid (control) with IC_{50} value of 59.6 µg/mL. Comparison of the IC_{50} of *P. boryana* and other brown seaweeds obtained from other studies such as *Padina tetrastomatica* (45.57 µg/mL), *Padina concrecens* (50.0 µg/mL), *Macrocystis pyrifera* (227.2 µg/mL), *Dictyopteris delicatula* (71.9 µg/mL), *Cystoseira osmundacea* (69.0 µg µg/mL), and *Eisenia arborea* (277.9 µg/mL), *P. boryana* had a comparatively potent DPPH antioxidant activity (Chia et al., 2015; Tenorio-Rodriguez et al., 2017). The results confirmed that phenolic compounds contribute to the DPPH radical scavenging activity of *P. boryana* crude extract. Antioxidant properties of seaweeds may be associated to polyphenols (such as flavonoids, dieckol, phloroglucinol, and vanillic acids) present in *P. boryana* crude extract. Polyphenols are known antioxidants and are considered potent free radical scavenger. These substances are capable of ceasing autoxidation of free radicals by hydrogen atom donation from several hydroxyl (OH) bases present outside the benzene rings of the compound. In addition, pigments present in seaweeds also play a significant effect to the increase in antioxidant activity of *P. boryana* crude extract. Previous studies show that pigments from seaweeds such as fucoxanthin, phycoerythrobilin, chlorophyll a, and chlorophyll b exhibited antioxidant activities thus, contributing to the overall synergistic antioxidant capacity of the algal extract (Pangestuti and Kim, 2011).

3.3.2 CUPRAC

The CUPRAC assay is a method used to evaluate the antioxidant activity of a sample by assessing the ability of the extract to reduce cupric ion to cuprous ion (Arguelles and Sapin, 2020a). This assay was used in this study to evaluate the potential of *P. boryana* to inhibit oxidation via metal chelation mechanism. Like the result obtained in DPPH radical scavenging assay, the copper reduction antioxidant capacity of *P. boryana* increases when the concentration of the algal crude extract increases. The extract exhibited a dose-dependent copper ion reduction activity (Table 3).

Table 3. Copper reduction antioxidant capacity (CUPRAC) of phenolics from *P. boryana* and ascorbic acid

Sample	Phenolic concentration (µg GAE/mL)				
	2.5	5.0	7.5	10.0	12.5
Padina boryana	0.125 ± 0.004	0.310 ± 0.007	0.437 ± 0.001	0.465 ± 0.010	0.526 ± 0.00
	Concentration (µg/mL)				
	5.0	10.0	15.0	20.0	25.0
Ascorbic acid	0.112 ± 0.002	0.213 ± 0.007	0.328 ± 0.004	0.429 ± 0.012	0.542 ± 0.011

Note: IC_{50} the effective concentration that gives CUPRAC value of 0.5 absorbance reading at 450 nm, as computed by interpolation, was 11.43 µg/mL for *Padina boryana* and 23.15 µg/mL for ascorbic acid

The IC_{50} value of *P. boryana* crude extract was 11.43 µg/mL and was considered more potent than that obtained from ascorbic acid with IC_{50} = 23.15 µg/mL. This result is relatively comparable to that observed by Arguelles and Sapin (2020a; 2020c) for *Sargassum siliquosum*, and *Turbinaria decurrens* from Luzon, Philippines. The results showed that *P. boryana* crude extract contained bioactive substances (such as polyphenols) that have good copper reducing antioxidant property. Seaweeds are known to have excellent antioxidant systems as a coping mechanism against environmental stresses, and synthesizes biologically active substances, including alkaloids, polyphenols, carotenoids, terpenoids, and other enzymes (Tenorio-Rodriguez et al., 2017). Polyphenolic compounds from seaweeds are considered more effective than their chemical analogues from land plants and exhibit potent antioxidant activities due to the existence of up to eight linked phenol rings in the compound (Tenorio-Rodriguez et al., 2017; Nagayama et al., 2002). The presence of natural antioxidants in *P. boryana* crude extract suggested the potential of this seaweed as an inexpensive alternative source of antioxidants that can be utilized for pharmaceutical industry.

3.4 Correlation analysis

The *P. boryana* crude extract showed significant correlations with DPPH and CUPRAC assays. It was observed that the TPC of the seaweed crude extract exhibited strong positive correlation with antioxidant activities using DPPH and CUPRAC assays having Pearson's linear correlation coefficient (R) of 0.9763 and 0.9502, respectively (Figures 2 and 3). This correlation was similar to those observed from previous studies from other seaweeds, such as *Sargassum siliquosum, Turbinaria decurrens,* and *Ascophyllum nodosum* on the relationship of algal extract concentration and their antioxidant activities (Arguelles and Sapin, 2020a; 2020c; Jiménez et al., 2010). From the analysis results, it may be presumed that polyphenols presented in the seaweed crude extract played a significant role for its metal ion chelating and free radical scavenging abilities.

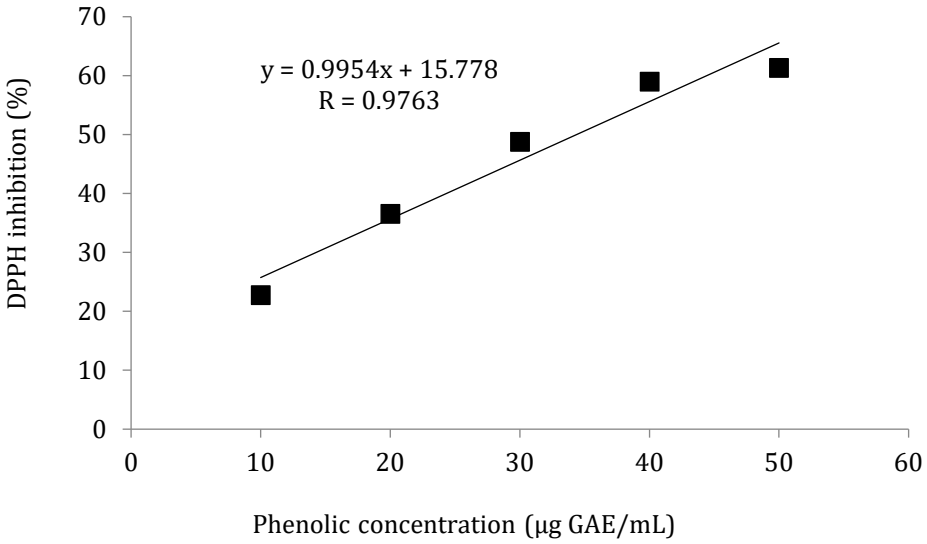

Figure 2. Correlation analysis between phenolic concentration and antioxidant activity via DPPH radical scavenging assay of *P. boryana*

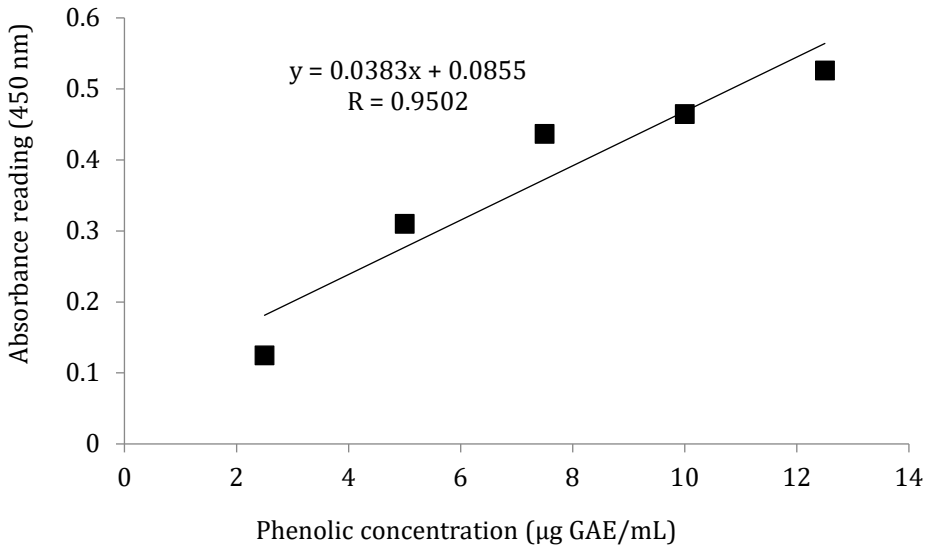

Figure 3. Correlation analysis between phenolic concentration and antioxidant activity via copper reduction antioxidant capacity (CUPRAC) assay of *P. boryana*

Previous studies reported that polyphenols from brown seaweeds, such as phlorotanins, are considered to be strong heavy metal chelators, which are thought to be accountable for the chelating ability of the sample extract (Chew et al., 2008). Also, earlier studies reported that macroalgae with polyphenolic antioxidants are capable of inhibiting the free-radical chain reaction by hydrogen atom donation to fatty acid radicals causing termination of the chain reactions (Karawita et al., 2005). The existence of polyphenolic compounds in seaweeds is a defense

mechanism to protect the algal thallus from UV radiation (Karawita et al., 2005).

3.5 Antibacterial activity

Seaweeds are commonly exposed to harsh environmental conditions, such as high light intensity and herbivory (Jayawardena et al., 2020). As a coping mechanism to these conditions, seaweeds are capable of forming diverse bioactive compounds with important pharmaceutical application. These compounds have potent biological activity that can be used in the development of novel drugs against medically important bacteria (Jayawardena et al., 2020). The antibacterial activity of *P. boryana* crude extract against eight pathogenic bacteria was evaluated *in vitro* using microtiter plate dilution assay. *P. boryana* exhibited inhibitory activities against *Micrococcus luteus* and *Staphylococcus epidermidis* both with MIC and MBC value of 125 and 250 µg/mL, respectively (Table 4). The antibacterial property of *P. boryana* crude extract against *M. luteus* is more potent than that observed by Sameeh et al. (2016) from ethanol extract of *P. boryana* with MIC value of 250 µg ml^{-1}. Also, *P. boryana* crude extract exhibited stronger inhibitory activity against *S. epidermidis,* in comparison to other species of seaweeds, such as *Sargassum siliquosum* and *Ishige okamurae* (Kim et al., 2018; Arguelles and Sapin, 2020a). *P. boryana* was able to inhibit *Aeromonas hydrophila* and *Staphylococcus aureus,* both of which showed MIC and MBC value of 250 µg/mL and 500 µg/mL, respectively. This antibacterial activity was similar to those observed from earlier studies wherein different seaweed species, such as *Caulerpa racemosa, Dictyopteris membranacea, Enteromorpha prolifera, Cladostephus verticillatus, Hypnea musciformis, Turbinaria ornata* and *Sargassum wightii*, exhibited antibacterial activities against *Aeromonas hydrophila* and *Staphylococcus aureus* (Alghazeer et al., 2013; Vijayabaskar and Shiyamala, 2011). On the other hand, no antibacterial activity was observed on *P. boryana* crude extract against *Pseudomonas aeruginosa, Bacillus cereus*, *Pseudomonas fluorescens* and *Klebsiella pneumoniae*. In contrast, Sameeh et al. (2016) reported *P. boryana,* when extracted with ethanol and acetone, exhibited antibacterial activity against *K. pneumoniae.* It is possible to assume that variation in antibacterial activities in *P. boryana* strains could be due to the difference in the synthesis of secondary metabolites with diverse antagonistic activities. In addition, such intraspecific variability may be due to seasonal variations, stage of active growth as well as sexual maturity of the seaweed (Srikong et al., 2017). Differences in the method of assay as well as extraction protocols and its effectiveness to recover inhibitory bioactive compounds in the macroalga could also lead to variation in susceptibilities of the target pathogenic bacteria (Srikong et al., 2017). The results of the study showed that Gram-negative bacteria are considered less susceptible to *P. boryana* crude extract than Gram-positive bacteria. These observations can be attributed to variation in the composition and structure of the bacterial cell wall, affecting permeability barriers of the cell. Gram-negative bacteria are known by having cell wall with thick murine layer and an outer membrane that serve as an added protection and inhibit active compounds to pass through the cell (Cox et al., 2010; Srikong et al., 2017). Also, previous studies indicated that the genus *Padina* possess structurally diverse bioactive compounds, such as alkaloids, saponin, flavanoids, tannins, steroids, and terpenoids (Jayawardena et al., 2020; Tenorio-Rodriguez et al., 2017). These compounds have strong antimicrobial and antioxidant activities that can be used to inhibit the growth of pathogenic bacteria.

Table 4. Antibacterial activities of *P. boryana* extract

Bacterial pathogen	Minimum inhibitory concentration (µg/mL)	Minimum bactericidal concentration (µg/mL)
Gram-positive bacteria		
Micrococcus luteus BIOTECH 1793	125	250
Staphylococcus epidermidis BIOTECH 10098	125	250
Staphylococcus aureus BIOTECH 1823	250	500
Bacillus cereus BIOTECH 1509	>1000	ND
Gram-negative bacteria		
Aeromonas hydrophila BIOTECH 10090	250	500
Pseudomonas aeruginosa BIOTECH 1824	>1000	ND
Pseudomonas fluorescens BIOTECH 1123	>1000	ND
Klebsiella pneumoniae BIOTECH 1754	>1000	ND

*ND = Not detected

To date, only few studies in the Philippines exploring the potential of seaweeds as a new source of bioactive compounds that can be used against pathogenic bacteria, are available (Arguelles and Sapin, 2020a; 2020c; Arguelles et al., 2019b). This investigation is the first report in the Philippines regarding antibacterial activities of *P. boryana* against *Micrococcus luteus, Staphylococcus epidermidis, Staphylococcus aureus,* and *Aeromonas hydrophila*. The results suggested that bioactive substances, such as polyphenols, were presented in the seaweed crude

extract, which caused inhibition of bacterial growth by disrupting the integrity of bacterial cell wall and cell membrane. Thus, additional investigation should be done to isolate, purify and identify these bioactive substances and to further elucidate the mode of action that causes these antimicrobial activities.

4. CONCLUSION

This investigation is a pioneering study in the Philippines that focuses on the biochemical composition and biological properties of *P. boryana* with potential use as novel source of bioactive substances important for pharmaceutical industry. *P. boryana* is capable of producing a high concentration of carbohydrates, proteins, and other essential microelements with direct relevance to pharmaceutical and food industries. Also, the algal crude extract shows a promising antioxidant activity, which is found to be more potent than the antioxidant activity of commercially available antioxidant tested in the study. The seaweed crude extract shows potent antibacterial activities against pathogenic bacteria that are beneficial in terms of preventive and therapeutic purposes against microbial infection. Comprehensive studies on the identification of the active compounds as well as elucidation of the reaction mechanism of these bioactive constituents of *P. boryana* crude extract should be done to further assess other biological activities *in vivo*.

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REFERENCES

Al-Enazi, N. F., Awad, A. S., Zain, M. E., and S. E. Alqasuomi. (2018). Antimicrobial, antioxidant and anticancer activities of *Laurencia catarinensis*, *Laurencia majuscula* and *Padina pavonica* extracts. *Saudi Pharmaceutical Journal,* 26(1), 44-52.

Alghazeer, R., Whida, F., Abduelrhman, E., Gammoudi, F., and Azwai, S. (2013). Screening of antibacterial activity in marine green, red and brown macroalgae from the western coast of Libya. *Natural Science*, 5(1), 7-14.

Alpinar, K., Özyurek, M., Kolak, U., Guclu, K., Aras, Ç., Altun, M., Celik, S. E., Berker, K. I., Bektasoglu, B., and Ampal, R. (2009). Antioxidant capacities of some food plants wildly grown in Ayvalik of Turkey. *Food Science Technology Research*, 15(1), 59-64.

Arguelles, E. D. L. R. (2018). Proximate analysis, antibacterial activity, total phenolic content and antioxidant capacity of a green microalga *Scenedesmus quadricauda* (Turpin) Brébisson. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences,* 20(1), 150-158.

Arguelles, E. D. L. R. (2020). Evaluation of nutritional composition and in vitro antioxidant and antibacterial activities of *Codium intricatum* Okamura from Ilocos Norte (Philippines). *Jordan Journal of Biological Sciences*, 13(3), 375-382.

Arguelles, E. D. L. R. (2021). Biochemical composition and bioactive properties of *Chlorella minutissima* (Chm1) as a potential source of chemical compounds for nutritional feed supplement and disease control in aquaculture. *Current Applied Science and Technology*, 21(1), 65-77.

Arguelles, E. D. L. R., and Sapin, A. B. (2020a). Bioactive properties of *Sargassum siliquosum* J. Agardh (Fucales, Ochrophyta) and its potential as source of skin-lightening active ingredient for cosmetic application. *Journal of Applied Pharmaceutical Science,* 10(7), 51-58.

Arguelles, E. D. L. R., and Sapin, A. B. (2020b). Bioprospecting of *Turbinaria ornata* (Fucales, Phaeophyceae) for cosmetic application: antioxidant, tyrosinase inhibition, and antibacterial activities. *Journal of the International Society for Southeast Asian Agricultural Sciences*, 26(2), 30-41.

Arguelles, E. D. L. R., and Sapin, A. B. (2020c). *In vitro* antioxidant, alpha-glucosidase inhibition and antibacterial properties of *Turbinaria decurrens* Bory (Sargassaceae, Ochrophyta). *Asia-Pacific Journal of Science and Technology*, 25(3), 1-9.

Arguelles, E. D. L. R., Laurena, A. C., Martinez-Goss, M. R., and Monsalud, R. G. (2017). Antibacterial activity, total phenolic content, and antioxidant capacity of a green microalga *Desmodesmus* sp. (U-AU2) from Los Baños, Laguna (Philippines). *Journal of Nature Studies,* 16(2), 1-13.

Arguelles, E. D. L. R., Laurena, A. C., Monsalud R. G., and Martinez-Goss, M. R. (2018). Fatty acid profile and fuel-derived physico-chemical properties of biodiesel obtained from an indigenous green microalga, *Desmodesmus* sp. (I-AU1), as a potential source of renewable lipid and high-quality biodiesel. *Journal of Applied Phycology*, 30, 411-419.

Arguelles, E. D. L. R., Laurena, A. C., Monsalud, R. G., and Martinez-Goss, M. R. (2019a). High lipid and protein-producing epilithic microalga, *Desmodesmus* sp. (U-AU2): a promising alternative feedstock for biodiesel and animal feed production. *Philippine Journal of Crop Science*, 44(2), 13-23.

Arguelles, E. D. L. R., Monsalud, R. G., and Sapin, A. B. (2019b). Chemical composition and *In vitro* antioxidant and antibacterial activities of *Sargassum vulgare* C. Agardh from Lobo, Batangas, Philippines. *Journal of the International Society for Southeast Asian Agricultural Sciences*, 25(1), 112-122.

Balasundari, V. K., Jeyashakila, R., Velayathum, P., Masilan, K., and Reshma, R. (2017). Proximate and mineral composition of brown seaweed from Gulf of Mannar. *International Journal of Fisheries and Aquatic Studies*, 5(5), 106-112.

Boonchum, W., Peerapornpisal, Y., Kanjanapothi, D., Pekkoh, J., Pumas, C., Jamjai, U., Amornlerdpison, D., Noiraksar, T., and Vacharapiyasophon, P. (2011). Antioxidant activity of some seaweed from the Gulf of Thailand. *International Journal of Agriculture and Biology,* 13(1), 95-99.

Chew, Y. L., Lim, Y. Y., Omar, M., and Khoo, K. S. (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT-Food Science and Technology*, 41(6), 1067-1072.

Chia, Y. Y., Kanthimathi, M. S., Rajarajeswaran, J., Khoo, K. S., and Cheng, H. M. (2015). Antioxidant, antiproliferative, genotoxic and cytoprotective effects of the methanolic extract of *Padina tetrastromatica* on human breast adenocarcinoma and embryonic fibroblast cell lines. *Frontiers in Life Science,* 8(4), 411-418.

Cox, S., Abu-Ghannam, N., and Gupta, S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal,* 17, 205-220.

Fu, C. W. F., Ho, C. W., Yong, W. T. L., Abas, F., and Tan, C. P. (2015). Effects of phenolic antioxidants extraction from four selected seaweeds obtained from Sabah. *PeerJ PrePrints*, 3, 1-15.

Gao, L., Wang, S., Oomah, B. D., and Mazza, G. (2002). Wheat quality: antioxidant activity of wheat millstreams. In *Wheat Quality Elucidation* (Ng, P., and Wrigley, C. W., eds.) pp. 219-233. St. Paul, Minnesota: AACC International.

Goecke, F., Escobar, M., and Collantes, G. (2012). Chemical composition of *Padina fernandeziana* (Phaeophyceae, Dictyotales) from Juan Fernandez Archipelago, Chile. *Revista Latinoamericana de Biotecnologia Ambiental y Algal*, 3(2), 95-104.

Guiry, M. D., and Guiry, G. M. (2020). *AlgaeBase. World-wide electronic publication*. Galway: National University of Ireland. [Online URL : http://www.algaebase.org] accessed on May 9, 2020.

Horwitz, W., and Latimer, G. W. (2011). *Official Methods of Analysis of AOAC International*, 18th, Gaithersburg, MD: AOAC International.

Jayawardena, T. U., Wang, L., Sanjeewa, K. K. A., Kang, S. I., Lee, J. S., and Jeon, Y. J. (2020). Antioxidant potential of sulfated polysaccharides from *Padina boryana*; protective effect against oxidative stress in *In vitro* and *In vivo* zebrafish model. *Marine Drugs*, 18(4), 212.

Jiménez, J. T., O'Connell, S., Lyons, H., Bradley, B., and Hall, M. (2010). Antioxidant, antimicrobial, and tyrosinase inhibition activities of acetone extract of *Ascophyllum nodosum. Chemical Papers,* 64(4), 434-442.

Karawita, R., Siriwardhana, N., Lee, K. W., Heo, M. S., Yeo, I. K., and Lee, Y. D. (2005). Reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition properties of different solvent fractions from *Hizikia fusiformis*. *European Food Research and Technology*, 220(3-4), 363-371.

Kim, B., Kim, M. S., Park, S. K., Ko, S. C., Eom, S. H., Jung, W. K., and Kim, Y. M. (2018). Antibacterial effect of *Ishige okamurae* extract against cutaneous bacterial pathogens and its synergistic antibacterial effect against *Pseudomonas aeruginosa*. *Fisheries and Aquatic Sciences,* 21, 18.

Machu, L., Misurcova, L., Vavra Ambrozova, J., Orsavova, J., Mlcek, J., Sochor, J., and Jurikova, T. (2015). Phenolic content and antioxidant capacity in algal food products. *Molecules,* 20(1), 1118-1133.

Marinho-Soriano, E., Fonseca, P. C., Carneiro, M. A. A., and Moreira, W. S. C. (2006). Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresource Technology*, 97(18), 2402-2406.

Matanjun, P., Mohamed, S., Mustapha, N. M., and Muhammad, K. (2008). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. *Journal of Applied Phycology*, 21(1), 75-80.

Mwalugha, M. H., Wakibia, J. G., Kenji, G. M., and Mwasaru, M. A. (2015). Chemical composition of common seaweeds from the Kenya coast. *Journal of Food Research,* 4(6), 28-38.

Nagayama, K., Iwamura, Y., Shibata, T., Hirayama, I., and Nakamura, T. (2002). Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. *Journal of Antimicrobial Chemotherapy,* 50(6), 889-893.

Novaczek, I., and Athy, A. (2001). *Sea vegetable recipes for the Pacific Islands*, Suva, Fiji Islands: University of the South Pacific, pp. 1-36.

Nuñez Selles, A., Castro, H. T. V., Aguero, J. A., Gonzalez, J. G., Naddeo, F., De Simone, F., and Rastrelli, L. (2002). Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. *Journal of Agricultural Food Chemistry,* 50(4), 762-766.

Pangestuti, R., and Kim, S. K. (2011). Biological activities and health benefit effects of natural pigments derived from marine algae. *Journal of Functional Foods.* 3(4), 255-266.

Ribeiro, S. M. R., Barbosa, L. C. A., Queiroz, J. H., Knödler, M., and Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. *Food Chemistry*, 110(3), 620-626.

Robledo, D., and Pelegrin, Y. F. (1997). Chemical and mineral composition of six potentially edible seaweed species of Yucatán. *Botanica Marina,* 40, 301-306.

Salosso, Y., Aisiah, S., Toruan, L. N. L., and Pasaribu, W. (2020). Nutrient content, active compound, and antibacterial activity of *Padina australis* against *Aeromonas hydropilla. Pharmacognosy Journal*, 12(4), 771-776.

Sameeh, M. Y., Mohamed, A. A., and Elazzazy, A. M. (2016). Polyphenolic contents and antimicrobial activity of different extracts of *Padina boryana* Thivy and *Enteromorpha* sp marine algae. *Journal of Applied Pharmaceutical Science*, 6(9), 87-92.

Shanmuganathan, B., and Devi, K. P. (2016). Evaluation of the nutritional profile and antioxidant and anti-cholinesterase activities of *Padina gymnospora* (Phaeophyceae). *European Journal of Phycology*, 51(4), 482-490.

Sivaramakrishnan, T., Swain, S., Saravanan, K., Kiruba, S. R., Roy, S. D., Biswas, L., and Shalini, B. (2017). *In vitro* antioxidant and free radical scavenging activity and chemometric approach to reveal their variability in green macroalgae from South Andaman Coast of India. *Turkish Journal of Fisheries and Aquatic Sciences,* 17(3), 639-648.

Srikong, W., Bovornreungroj, N., Mittraparparthorn, P., and Bovornreungroj, P. (2017). Antibacterial and antioxidant activities of differential solvent extractions from the green seaweed *Ulva intestinalis. ScienceAsia,* 43(2), 88-95.

Tenorio-Rodriguez, P. A., Murillo-Alvarez, J. I., Campa-Cordova, A. I., and Angulo, C. (2017). Antioxidant screening and phenolic content of ethanol extracts of selected Baja California Peninsula macroalgae. *Journal of Food Science and Technology,* 54(2), 422-429.

Trono, G. C. Jt. (1997). *Field Guide and Atlas of the Seaweed Resources of the Philippines*, 1st, Makati City, Manila: Bookmark Inc., pp. 27-303.

Vijayabaskar, P., and Shiyamala, V. (2011). Antibacterial activities of brown marine algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar biosphere reserve. *Advances in Biological Research,* 5(2), 99-102.

Wang, T., Jónsdóttir, R., and Ólafsdóttir, G. (2009). Total phenolic compounds, radical scavenging and metal

chelation of extracts from Icelandic seaweeds. *Food Chemistry*, 116(1), 240-248.

Wong, K. H., and Cheung, P. C. K. (2000). Nutritional evaluation of some subtropical red and green seaweeds Part I: proximate composition, amino acid profiles and some physico-chemical properties. *Food Chemistry*, 71(4), 475-482.

Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C., and Attia, H. (2011). Chemical composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia. *Food Chemistry*, 128(4), 895-901.

Yuan, Y., Zhang, J., Fan, J., Clark, J., Shen, P., Li, Y., and Zhang, C. (2018). Microwave assisted extraction of phenolic compounds from four economic brown macroalgae species and evaluation of their antioxidant activities and inhibitory effects on α-amylase, α-glucosidase, pancreatic lipase and tyrosinase. *Food Research International,* 113, 288-297.