CHEMICAL COMPOSITION AND *IN VITRO* ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *SARGASSUM VULGARE* C. AGARDH FROM LOBO, BATANGAS, PHILIPPINES

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ABSTRACT

Seaweeds are notable in producing diverse kinds of bioactive compounds with promising pharmacological properties. A study was conducted at the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños from July to November 2018 to evaluate the proximate composition and potential antioxidant and antibacterial properties of a brown macroalga, *Sargassum vulgare* C. Agardh. Determination of the total phenolic compounds using Folin-Ciocalteu reagent showed that the dried algal biomass have a total phenolic content of 10.13 ± 0.166 mg GAE/g. Relative antioxidant efficiency showed that *S. vulgare* exerted potent radical scavenging activity in a concentration dependent manner with EC_{50} value of 37.2 ± 0.015 µg GAE. The tested algal extract exhibited radical scavenging activity that is dose-dependent and positively correlated to its phenolic content. On the other hand, proximate composition of the dried macroalga showed that *S. vulgare* contains high carbohydrate, ash and crude fiber content of 34.18±0.32%, 27.09±0.00%, and 22.59±0.21 respectively. Methanolic extract of the macroalgal strain was subjected to microtiter plate dilution assay against a wide spectrum of pathogenic bacteria. *S. vulgare* showed pronounced activity against *Staphylococcus aureus* having MIC of 250 µg/ml. *Aeromonas hydrophila*, *Bacillus cereus* and Methicillin-resistant *S. aureus* were also moderately inhibited each having MIC of 500 µg/mL, 500 µg/mL, and 1000 µg/mL, respectively. Minimum bactericidal activity against *S. aureus* is higher than that of *Bacillus cereus* and *Aeromonas hydrophila*, having 500 µg/mL and 1000 µg/mL, respectively. On the other hand, Methicillin-resistant *S. aureus* exhibited MBC value of >1000µg/ml. This study showed the potential antioxidant and antibacterial activity of *S. vulgare*, which make it a suitable candidate for production of new bioactive compounds important for pharmacological and food industries.

Key words: brown macroalgae, phenolic content, proximate composition, radical scavenging activity, seaweed

INTRODUCTION

Macroalgae are noted as promising sources of biologically active metabolites as they are capable of synthesizing diverse types of secondary metabolic compounds marked by a broad extent of biological activities. They produce substances namely terpenoids, sterols, phenols, halogenated ketones, polysaccharides, water-soluble (B-complex and C) as well as fat-soluble (provitamin A and vitamins D, E and K), peptides, proteins, dietary fibers, minerals (such as magnesium, phosphorus, calcium, iodine, potassium, iron, and sodium), and polyunsaturated fatty acids and are described to possess antioxidant, antifungal, antiviral, and antibacterial activities (Arguelles et al. 2018; Cox et al. 2010; Chew et al. 2008). Recently, the occurrence of antibiotic resistant bacteria is a worldwide problem in health and medicine. To replace safer use of antibiotics, studies on the use of secondary metabolites from natural sources, including seaweeds have been increasing. Brown seaweeds such as

Sargassum spp. have been reported to have bioactive property antagonistic to a number of medically important bacteria Gram-negative and Gram- positive bacteria such *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pnemoniae*, *Clostridium perfringens*, *Staphylococcus aureus*, and the like (Oumaskour et al. 2012; Osman et al. 2010; Yoon et al. 2010). Thus, *Sargassum* has been regarded as candidate sustainable natural reserve resources with promising antibacterial biological activities associated to its secondary and primary metabolites (Yende et al. 2014).

The fact that the harsh environments in which seaweeds are exposed to in combination with exposure to high light intensity and elevated concentrations of oxygen can induce development of free radicals and several potent oxidizing agents. However, seaweeds are scarcely affected from any significant photodynamic impairment during metabolism because of the development of protective adaptive mechanisms and synthesis of compounds (Cox et al. 2010). Many research studies have showed that seaweeds contain various phytochemicals (such as polyphenols vitamins C, E and carotenoids) with antioxidant activity, which are accountable for their valuable health effects. *Sargassum* spp. contains fucoxanthin, fucoidan, as well as phlorotannin compounds that play a role as oxidizing agent. Phlorotannin is a polyphenolic compound that is only found in brown algae known to have other biological activities such as antioxidant and anti-proliferative, angiotensin-I-converting enzyme inhibition, inhibitors of matrix metalloproteinases, and UV radiation absorption ability (Soleimani et al. 2018). Fucoidan is reported to have pharmacological properties including anticoagulant, antithrombotic, anti-inflammatory, anticancer, and antioxidative properties (Soleimani, et al. 2018; Morya et al. 2012). These biological activities of fucoidan is linked with its chemical structure particularly the sulfate groups that is dependent to the fucose monomer of fucoidan (Soleimani et al. 2018). Fucoxanthin is considered as one of the main carotenoid in *Sargassum*. This pigment is responsible for the transfer of light energy to photosynthetic reaction centers (particularly the chlorophyll *a*) for photosynthesis. It has been reported that this bioactive pigment are characterized to have effective anti-obesity, anti-inflammatory, antidiabetic and antioxidant activities (Xia et al. 2013). Due to its natural origin, seaweed-derived antioxidants pose a greater potential in collation to other synthetic sources such as butylatedhydroxy toluene (BHT) and butylatedhydroxy anisole (BHA).

The Philippine coast has an extensive coastline with great biodiversity of marine macroalgae yet to be explored. Despite their diversity in forms and types, relatively few studies are known about the antioxidant and antibacterial potential of these algae and to date, scientific investigations of their biological activities is still limited in the country. This study is the first report in the Philippines exploring the biological activities of a brown macroalga, *Sargassum vulgare* C. Agardh. The present investigation sought to do proximate composition analysis, screen for antibacterial activity and determine the amount of total phenolic compounds in *Sargassum vulgare* (using gallic acid as the standard). The antioxidative activity was evaluated and the correlation among antioxidant activity and total phenolic content was established.

MATERIAL AND METHODS

Seaweed collection and preparation. Fresh marine brown macroalga *Sargassum vulgare* C. Agardh was collected on 20 July 2018 from Lobo (Lat. 13° 35’ 54.1’ N; Long. 121° 15” 33.2’ E), Batangas, Philippines. The macroalaga was characterized and identified based on morphotaxonomic features according to Algae Base (web site: www.algaebase.org) and Trono (1992). The collected seaweed sample was thoroughly cleaned with filtered seawater to remove associated sand debris, planktons and loosely attached microorganisms and was immediately transferred to the laboratory using sterilized polythene bags. The seaweed sample was then washed rigorously with sterile tap water to take away excess salt on the external surface of the alga. The water was drained off, and the seaweed laid out on a clean blotting paper. The seaweed was again thoroughly washed with sterilized distilled water to eliminate the remaining surface salt and to avoid pumping out the solvent during the extraction

process. The algal sample was air-dried (for six days), chopped into short pieces and pulverized into fine-grained powder in a mixer grinder.

Preparation of seaweed extract for antibacterial screening. The powdered seaweed biomass was soaked in methanol (1g seaweed powder: 30 mL methanol) and extracted in an ultrasonic bath for 30 minutes and stirred for 1 hour. The algal extract was centrifuged at 12,000 rpm at 20ºC for 20 minutes. The algal extract was concentrated using a rotary evaporator at 40 °C until a crude extract was obtained, and kept at 4 °C (Arguelles 2018).

Total phenolic content. The total phenolic compound present in the macroalgal extract was calculated using Folin-Ciocalteu method based on the process done by Nuñez Selles et al. (2002). Briefly, macroalgal extract was diluted with sterile distilled water and to 0.5 ml of the diluted sample extract, 0.5 ml of Folin-Ciocalteu's reagent and 0.5 ml of 10% Na_2CO_3 solution were mixed in a test tube. The reaction mixture was then set aside to stand for 5 minutes and 5 mL of distilled water were mixed to the solutions. The spectrophotometric readings were determined at 720 nm using a Shimadzu UV-1601 spectrophotometer with reagent plus water as blank sample (Arguelles 2018). Calibration curve was constructed using gallic acid as the standard; the total phenol was expressed as gallic acid equivalents (GAE).

DPPH radical scavenging assay. The radical scavenging activity of the macroalgal extract on 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined following the protocol of Ribeiro et al. (2008) with some modifications. Briefly, 100 µl of aliquot of algal extract was mixed to 5.0 ml of 0.1 mM DPPH methanolic solution. The reaction mixture was completely homogenized using a vortex mixer and then cast aside for 20 minutes at ambient temperature. The spectrophotometric readings of the sample solution were determined utilizing a UV-VIS spectrophotometer at 517 nm. The percent inhibition (%) was estimated by using the mathematical formulae specified by Ribeiro et al. (2008).

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

where: A_{sample} = absorbance reading of the test sample (DPPH solution plus test sample) and $A_{control}$ = absorbance reading of the control (DPPH solution without sample). In this study, gallic acid (a synthetic antioxidant) was utilized as the positive control. The percentage scavenging activity of the macroalgal extract was analyzed and the concluding result was expressed as an EC_{50} value (the amount of the macroalgal extract exhibiting 50% scavenging of the DPPH radical expressed in µg/ml).

Proximate composition analysis. The procedure for the proximate chemical composition analysis of *S. vulgare* was determined by following the standard AOAC (2011) methods. Crude lipid of *S. vulgare* was obtained from the powderized macroalga using petroleum ether as the solvent in a Soxhlet extractor (Bhuiyan et al. 2016; Siddique et al. 2011). After the extraction process, the solvent was evaporated to dryness and the residue was further dried until an unvarying weight is obtained at 105°C. Carbohydrates or nitrogen free extract was obtained by deducting the sum of moisture, ash, crude fiber, crude protein, and crude fat from 100. On the other hand, protein content of *S. vulgare* was analyzed by Kjeldahl method. A conversion factor of 6.25 was used to estimate the total amount of nitrogen in the crude protein (g per 100 g edible portion). To measure the crude fiber, 2 g of powderized seaweed sample was boiled together with a 0.3 N H_2SO_4. The solution was then filtered and thoroughly washed using 200 ml of boiling water and NaOH (0.5 N), respectively, following the procedure done by Siddique et al. 2013 and Bhuiyan et al. 2016. The collected residue of the reaction mixture was further extracted and rinsed using acetone and boiling water. Lastly, the leftover material was further dried up at 105℃ for 3 h until unvarying weight is achieved. The moisture content of *S. vulgare* was analyzed by subjecting the macroalgal sample to dryness at 105℃ until unvarying weight is observed. Moisture content was calculated by getting the difference of the final mass of the algal

sample from the initial mass of the sample. Ash content of the seaweed sample was determined by calcinations in a muffle furnace at a temperature of 550℃ for 4 h.

Tests microorganisms. Type cultures of five pathogenic Gram-negative bacteria (*Pseudomonas aeruginosa* BIOTECH 1824, *Aeromonas hydrophila* BIOTECH 10089, *Salmonella typhimurium* BIOTECH 1826, *Escherichia coli* BIOTECH 1825, and *Enterobacter aerogenes* BIOTECH 1145) and four Gram-positive bacteria (Methicillin-Resistant *Staphylococcus aureus* BIOTECH 10378, *Listeria monocytogenes* BIOTECH 1958, *Staphylococcus aureus* BIOTECH 1823, and *Bacillus cereus* BIOTECH 1509) were acquired from the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), Philippines. The test organisms were pre-cultured using Luria Bertani (LB) medium overnight with shaking at 37 ºC. The morphological and biochemical tests were checked continuously to ensure purity (Arguelles 2018).

Micro-dilution antibacterial assay. Minimum inhibitory concentration (MIC) of the macroalgal extract was determined by employing two-fold serial dilution technique following the procedures done by Arguelles 2018. Using a 96-well microtiter plate, 100 µl of bacterial cultures (approximately 1x 10^5 cells/ml) were added to 100 µl of macroalgal extract test samples prepared in different dilutions starting from 1000 µg/ml down to 7.8125 µg/ml. Methanol was also included as the negative control. The seeded plate was incubated overnight at 35°C in an ambient air incubator, after which the minimum inhibitory concentration was recorded. MIC is described as the lowest amount of the test algal extract that totally inhibited bacterial growth after a 12-h incubation period. After the incubation time, microtiter plates were checked for the absence or presence of bacterial growth. The minimum concentration of the algal extract at which there is no observable growth of bacteria was considered as the MIC for the extract–microbe combination under consideration. As for the controls, the MIC of tetracycline against each bacterial species was similarly determined. The experiments done in this study were conducted in triplicate and the microdilution trays were incubated at 35ºC for 12 h (Arguelles et al. 2017).

The minimum bactericidal activity (MBC) was determined by plating a loopful of bacterial sample from each MIC assay wells that showed growth inhibition into freshly prepared tryptic soy agar plates (Arguelles 2018). The petri plates were stored at 35°C for 24 hours and were checked for visible colony growth or lack of growth for each dilution subculturing. No growth indicated that the algal extract was bactericidal at that dilution. On the other hand, colony growth indicated that the macroalgal extract was bacteriostatic at that dilution. The lowest concentration that did not exhibit visible growth on agar subculture after the incubation period was regarded as MBC value (Arguelles 2018).

Statistical analyses. The experimental data are showed as means ± standard deviations (mean ± SD) of three parallel experimental measurements. The statistical test for the correlation analysis and linear correlation coefficient were evaluated using MS Office Excel 2007.

RESULTS AND DISCUSSION

Proximate composition analysis. The nutritional value of seaweeds is usually evaluated using its biochemical composition like carbohydrates, protein, ash content, lipids as well as crude fiber. The proximate composition based on dry weight of *Sargassum vulgare* C. Agardh was shown in Table 1. It was found that carbohydrate (34.18%) is the major component in dried seaweed. Carbohydrates constitute 30 – 60% of the total dry weight of brown seaweed, which is mainly composed of cellulose, fucoidan, alginates and laminaran (Vijay et al. 2017). The result of this study showed higher carbohydrate content as compared to other reported *Sargassum* species such as *Sargassum tennerimum* and *Sargassum wightii* with maximum carbohydrate content of 23.54% and 23.50%,

respectively (Manivannan et al. 2009; Vijay et al., 2017).

Table 1. Proximate composition of *Sargassum vulgare* C. Agardh.

Proximate composition	Percent composition (%)
Moisture Content	7.89±0.15
Ash Content	27.09±0.00
Crude Protein	7.69±0.23
Crude Fat	0.56±0.10
Crude Fiber	22.59±0.21
Carbohydrate	34.18±0.32

Dietary fibers from seaweeds contained some vital nutrients and substances that gained significant interests for nutraceuticals and functional foods for human utilization and consumption such as polysaccharides showing anticoagulant, antitumor, anti-herpetitic and antiviral biological activity (Ahmad et al. 2012). In this study, dried *S. vulgare* showed a relatively high crude fiber content of 22.59%. This result falls within the range of earlier published studies, which reported that dried seaweed is comprised of about 10% to 62% total fibers (Serrano et al. 2015; Ahmad et al. 2012). The protein content of the dried seaweed sample is 7.69%, which is within the range of those reported for Sargassaceae (Casas-Valdez et al. 2006). Variation in protein content of species within the genus *Sargassum* can be due to difference in seasonal period and geographic area (Ahmad et al. 2012). A study made by Manoonphatyaporn et al. (2014) showed difference in the chemical composition of brown macroalga *S. aquifolium* and *S. oligocystum* in three seasonal periods. *S. oligocystum* have significant ($p \leq 0.05$) high protein and vitamin C contents as compared to *S. aquifolium* in all seasons with observed highest protein content in rainy monsoon season while highest vitamin C content in hot dry season. On the other hand, other biochemical components such lipid, ash, carbohydrate, and crude fiber contents were not significantly different in each of the *Sargassum* species during seasons (Manoonphatyaporn et al. 2014). A single preliminary collection of brown seaweed *S. vulgare* was done in the current study during the dry season. Variations in the chemical composition of *S. vulgare* are possible if collected and analyzed at different season and geographical area. The present study provides baseline information to take into account the most suitable seasonal period for harvest of *S. vulgare* for sustainable use and management.

Ash content of the powdered macroalga is 27.09%, which is considerably high. High amount of ash is linked with appreciable level of diverse mineral amount of mineral elements in the sample (Serrano et al. 2015; Matanjun et al. 2008). The total lipid content (0.56%) in the algal biomass was found relatively low and is considered as a minor proximate component. The result of this investigation fell within the ranges described previously by other studies (Gómez-Ordóñez et al. 2010; Casas-Valdez et al. 2006). Results of the total nutritional profile of *S. vulgare* recommends that the macroalga is a suitable candidate as alternative source of mineral and nutrition supplements as well as of food with high carbohydrate and crude fiber content. This study could also be considered as baseline information for further advanced study on nutritional value and as prospective resources for the development of seaweed-based products for enhanced animal and human nutrition. However, biological evaluation making use of animal and human feeding research is prescribed to prove the nutritional significance and benefits of *S. vulgare*.

Total phenolics and antioxidant activity. The total phenolic content present in the macroalgal extract was evaluated and is expressed as mg of GAE/g of the dried algal biomass (Table 2). The total phenolic content in the analyzed macroalgal biomass is 10.13 ± 0.166 mg GAE/g. Similar phenolic concentration was observed by Somanah et al (2012) from shallow water seaweeds such as the phaeophyceae (*Sargassum binderi, S. latifollium, S. duplicatum, Padina gymnospora, P. tetrastromatica,* and *Turbinaria ornata*), the rhodophyceae (*Gracilaria millardetti, Jania adhaerens,*

Haliptilon subulata) and the chlorophyceae (*Codium lucasii, C. intricatum, Ulva reticulata, U. lactuca* and *Chaetomorpha crassa* of Mauritius with total phenolic contents ranging from 4.00 to 264.38 mg gallic acid equivalent (GAE) 100 g^{-1} as well as Ganesan et al. (2008) likewise reported methanol extracts of Indian seaweeds which vary from 1.5 mg GAE/g to 4.1 mg GAE/g.

Table 2. Phenolic content and DPPH free radical scavenging activity of methanolic extract of *Sargassum vulgare.*

Seaweed	Total Phenolic Content* (mg GAE/g)	EC_{50} (µg GAE)
Sargassum vulgare C. Agardh	10.13 ±0.166	37.2 ±0.015

*Average of triplicate sample

DPPH radical scavenging activity. DPPH free radical scavenging activity assay is a preliminary test to assess the capacity of the algal extract to give hydrogen or to scavenge free radicals. The assay has been used extensively because of its susceptibility for the detection and screening of bioactive metabolites even at small amounts as well as high throughput screening. Antioxidant activity of marine algae is associated to the existence of bioactive substances which may come from vitamins and vitamin precursors (ascorbic acid, α-tocopherol, thiamine, niacin, and β-carotene), pigments (carotenoids and chlorophylls), phenolics (such as hydroquinones and polyphenolics), phospholipids (such as phosphatidylcholine), peptides, terpenoids, and other antioxidative substances, which are capable of scavenging free radicals produced during scavenging of oxygen-containing compounds, metal-chelating ability, or peroxidation (Cox et al. 2010; Yende et al. 2014). Anthocyanins and polyphenols are capable of donating hydrogen to scavenge DPPH reducing it to diphenylpicrylhydrazine. The present study showed that *S. vulgare* extract act as antioxidant since it possesses hydrogen-donating properties. Also, antioxidative activity of the seaweed extract intensifies in a concentration-dependent manner (Figure 1) with EC_{50} value of 37.2 ± 0.015 µg GAE (Table 2).

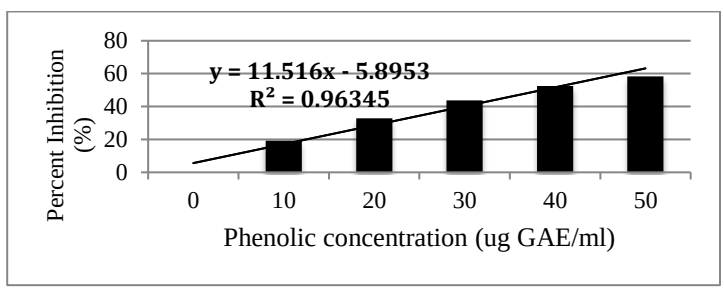

Fig. 1. Correlation between total phenolic content and total antioxidant activity (DPPH radical scavenging assay) of *S. vulgare*.

The antioxidative potential of several brown macroalgal species (such as *Fucus* spp. and *Sargassum* spp.) from Danish coast were reported to possess scavenging ability on DPPH with EC_{50} ranging from 85.7-1466.7 µg/ml (Farvin and Jacobsen 2013). This result is considerably less effective as compared to that of *S. vulgare* in the current study (EC_{50} = 37.2 ±0.015 µg GAE), which demonstrates the great potential of the alga assayed in this study as alternative source of naturally-derived antioxidants. The reported antioxidative potential of *S. vulgare* could be used as a replacement for commercially available artificial antioxidants (such as butylated hydroxyanisole (BHA), propyl gallate, butylated hydrotoluene (BHT) and tert-butylhydroquinone), which have been studied to have tumorigenic and carcinogenic effects at high doses (Chia et al. 2015). The capability of *S. vulgare* extracts to scavenge free radicals may have potential food industry application for

lengthening the shelf life of processed food products during storage and distribution (Cox et al. 2010).

Correlation study between total phenolic content and antioxidant activity. In this study, strong correlation between DPPH assay and total phenolic content (R^2=0.963) indicated that bioactive compounds such as polyphenols present in *S. vulgare* extract are involved in antioxidant activity by scavenging DPPH (Figure 1). Previous studies reported that a positive correlation exists among phenolic compounds and antioxidant activity in seaweeds (Wang et al. 2009; Matanjun et al. 2008). Many brown macroalgae species contain polyphenolics and in this investigation the antioxidative activity of *S. vulgare* extracts could be ascribed to these important bioactive compounds. Further identification of phenolic components is necessary to get more appropriate understanding regarding correlation between phenolics and antioxidant activity (Arguelles et al. 2017).

Screening for antibacterial activity. *Sargassum* produces various bioactive metabolites such as polysaccharides, polyphenols, terpenoids, steroids, glycerides, plastoquinones and the like (Soleimani et al. 2018; Yende et al. 2014). Thus, *Sargassum* has been regarded as a promising natural resource with captivating bioactivities coupled to its synthesized biochemical metabolites (Soleimani et al. 2018; Yende et al. 2014). The results of the inhibitory activity of the methanolic extract of the macroalga *S. vulgare* against pathogenic bacteria are summarized in Table 3.

Table 3. Antibacterial activity of *Sargassum vulgare* extract.

Test organism	Minimum inhibitory concentration (µg/ml)	Minimum bactericidal concentration (µg/ml)
Gram-positive bacteria		
Staphylococcus aureus BIOTECH 1823	250.00	500.00
Bacillus cereus BIOTECH 1509	500.00	1000.00
Methicillin-Resistant *Staphylococcus aureus* BIOTECH 10378	1000.00	>1000.00
Listeria monocytogenes BIOTECH 1958	>1000.00	ND
Gram-negative bacteria		
Aeromonas hydrophila BIOTECH 10089	500.00	1000.00
Pseudomonas aeruginosa BIOTECH 1824	>1000.00	ND
Escherichia coli BIOTECH 1825	>1000.00	ND
Enterobacter aerogenes BIOTECH 1145	>1000.00	ND
Salmonella typhimurium BIOTECH 1826	>1000.00	ND

*ND = Not Determined

S. vulgare showed pronounced activity against *Staphylococcus aureus* having MIC of 250 µg/ml. *Aeromonas hydrophila*, *Bacillus cereus* and Methicillin-resistant *S. aureus* were also moderately inhibited each having MIC of 500 µg/mL, 500 µg/mL, and 1000 µg/mL, respectively. No activity was observed against *Listeria monocytogenes, Eschrichia coli, Salmonella typhimurium* and *Pseudomonas aeruginosa*. Minimum bactericidal activity against *S. aureus* (500 µg/mL) is higher

than that of *Bacillus cereus* and *Aeromonas hydrophila* having 1000 µg/mL. On the other hand, MBC value against Methicillin-resistant *S. aureus* is >1000 µg/ml. These experimental findings are similar with previous studies (Osman et al. 2010; Oumaskour et al. 2012; Ali et al. 2016). However, other studies such as Ibtissam et al. (2009) and El Shayaf et al. (2016) reported that methanolic extract of *S. vulgare* did not exhibit antibacterial activity against *S. aureus*. Notable variations in the results of the current study and the findings of other investigations can be attributed to several factors such as intraspecific differences in the kind of secondary metabolites produced by the macroalga, often associated to seasonality variations (Osman et al. 2010; Omar et al. 2012). Also, differences in capability of the method of extraction to recuperate the bioactive substances as well as variations in the method of antimicrobial assay would cause differences in sensitivities of the tested bacterial strains (Gonzalez et al. 2001; Osman et al., 2010). Lastly, differences in the stage of active metabolic growth and sexual maturity of the macroalgae can also cause variations in its biological activity (Al-Judaibi 2014). This study also reports that the methanolic extract of the alga is more potent for its activity towards Gram-positive bacteria, especially *S. aureus* and *B. cereus* than Gram-negative bacteria. Differences in terms of antibacterial activity of the extract may be due to the considerably intricate structure of the Gram-negative bacterial cell wall. Generally, Gram-negative bacteria are characterized by a complex, multilayered cell wall structure affecting the penetration of active compounds within the bacterial cells causing added protection for the organism against antibiotics (Arguelles 2018). The cell membrane of Gram-negative bacteria is associated with degradative enzymes in the periplasmic space, which has the capability of degrading unfamiliar molecules introduced from the external environment of the cell (Kim and Lee 2008). Bioactive compounds from seaweeds acts against bacterial pathogen by altering the permeability of bacterial cell and loss of important internal macromolecules or by the intrusion with the membrane structure and function resulting to cellular instability and loss of integrity that leads to cell death (Arguelles 2018).

To the best of our knowledge, this paper is the first report in the Philippines about antibacterial activity of *S. vulgare* strain against Methicillin-resistant *Staphylococcus aureus, Bacillus cereus, Staphylococcus aureus,* and *Aeromonas hydrophila*. The general antibacterial activity assessed from the present study demonstrates the existence of biologically active compounds in the seaweed extract of which displayed promising antimicrobial activity effective against medically important Gram-positive and Gram-negative bacteria. Thus, further study should be conducted to purify and identify these bioactive substances. *Sargassum* is a rich resource of the Philippine archipelago, hence the availability of this alga for industrial use.

CONCLUSION

Sargassum vulgare C. Agardh represents a potential source of polyphenols and other bioactive compounds for the production of natural antibiotics and antioxidants. Further studies are needed to know the chemical structure and determine the nature of bioactive compounds responsible of the activity of the algal extract that showed promising antibacterial activity. Not only the existence of useful compound which cause this macroalga engrossing but also its diversity and the feasibility of not only collecting them but also of optimizing its growth at varying environmental conditions, resulting to enhanced production of important bioactive compounds for food and pharmaceutical industry.

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