Philippine Journal of Science
151 (S1): 9-24, Marine Botany
ISSN 0031 - 7683
Date Received: 11 May 2021

Chemical Composition and Bioactive Properties of *Sargassum aquifolium* (Turner) C. Agardh and Its Potential for Pharmaceutical Application

Eldrin DLR. Arguelles[1]* and **Arsenia B. Sapin**[2]

[1]Philippine National Collection of Microorganisms (PNCM); [2]Food Laboratory
National Institute of Molecular Biology and Biotechnology (BIOTECH)
University of the Philippines Los Baños (UPLB), College 4031 Laguna, Philippines

Seaweeds are known sources of marine bioactive substances with diverse biological activities important in the synthesis of medically important novel drugs. The proximate and elemental compositions as well as bioactive properties of a brown macroalga, *Sargassum aquifolium* (Turner) C. Agardh, were studied. Results showed that proximate composition of *S. aquifolium* contain high carbohydrate (32.29 ± 0.17%) and ash (30.19 ± 0.14%) content. Elemental composition of the seaweed exhibited a decreasing order of Na > Ca > K > Mg > Mn > Fe > Zn > Cu > Pb > Cr > Cd. The seaweed had a total phenolic content (TPC) of 5.74 ± 0.04 mg GAE/g. Antioxidant activities of *S. aquifolium* were characterized by having potent $ABTS^+$ [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] scavenging activity and high copper reduction capacity with IC_{50} value of 107 µg/mL and 21.01 µg/mL respectively. Evaluation of tyrosinase and elastase inhibition activities showed that *S. aquifolium* extract has potent inhibition activities with IC_{50} of 39.00 µg/mL and IC_{50} of 231.00 µg/mL, respectively – more effective than kojic acid and tocopherol. In addition, *in vitro* assessment of α-glucosidase and α-amylase inhibition property showed that *S. aquifolium* extract has potent inhibitory activity as compared to acarbose (standard anti-diabetic drug) with IC_{50} of 15.60 and 59.0 µg/mL, respectively. Also, the *S. aquifolium* extract exhibited effective antimicrobial activities against bacterial pathogens such as penicillin acylase-producing *Bacillus cereus* (MIC = 125 µg/mL), *Staphylococcus saprophyticus* (MIC = 250 µg/mL), methicillin-resistant *Staphylococcus aureus* (MRSA) (MIC = 250 µg/mL), *Staphylococcus epidermidis* (MIC = 250 µg/mL), and *Pseudomonas aeruginosa* (MIC = 500 µg/mL). The current investigation is a pioneering study in the Philippines that shows the potential of *S. aquifolium* as novel source of bioactive compounds with important use for pharmaceutical applications.

Keywords: biological activity, chemical composition, marine, phenolic compounds, Philippines, seaweeds

INTRODUCTION

In recent years, several studies were conducted on seaweeds and their chemical composition for their potential health promotion properties as nutraceuticals and functional foods (Arguelles 2020; Magdugo *et al.* 2020). Seaweeds are natural renewable sources of biologically active phytochemicals such as phenolic compounds, vitamins, tocopherols, lipids, dietary fiber, carotenoids, sterols, flavonoids, and phycobilins. These compounds have a wide spectrum of bioactivities (*i.e.* antioxidant, antimicrobial, anticancer, and antidiabetic activities) applicable for pharmaceutical and industrial use (Cox *et*

*Corresponding Author: edarguelles@up.edu.ph

al. 2010; Arguelles *et al.* 2019). These biological activities are often associated to the adaptive response of seaweeds to harsh environmental conditions in the marine ecosystem like fluctuating oxygen concentration and temperature, grazing, allelopathy, and high light intensity. Such conditions can lead to the formation of strong oxidizing agents and other free radicals that are harmful to the organism. However, seaweeds are often rarely affected from any severe effects (such as photodynamic damage) during metabolism since their cells was able to develop protective substances with biological and antioxidative activities (Cox *et al.* 2010; Matsukawa *et al.* 1997).

Polyphenols are groups of bioactive compounds that are characterized by having a chemical structure with benzenic ring replaced by one or more hydroxyl (OH) groups. These active compounds are categorized to different groups of active metabolites such as flavonoids (*e.g.* flavones, flavonols, flavanones, and chalcones), tocopherols, lignins, phenolic acids, and tannins, which are common in marine seaweeds (Arguelles and Sapin 2020a, b, c). Phenolic compounds are essential substances needed for reproduction and growth of seaweeds and act as antibiotics, natural antioxidants, and natural protective agents against grazing marine organisms (Cox *et al.* 2010; Arguelles 2021b). The reported potent antioxidant property of macroalgae has led the application of these resources in human food diet. Commercially available substances from seaweeds such as fucoxanthin, agar, micronutrients, and alginates are already available in the market, which can be used as a supplement to generate functional foods and other important products (Magdugo *et al.* 2020). These compounds were proven to have chemo-preventive activities against mutagenesis and carcinogenesis, which is beneficial to human health. In cosmetic industry, polyphenols derived from seaweeds also earned popularity as an effective natural anti-wrinkling, anti-aging, and whitening agent with well-documented skin-protecting activity against ultraviolet (UV) radiation (Namjooyan *et al.* 2019).

Seaweeds are considered renewable natural resources found abundantly in the Philippine coastline, yet the commercial and pharmaceutical use of these organisms are largely untapped. In other countries, several studies had documented different types of biological activities in seaweeds showing the promising use of these algae as rich source of bioactive compounds (Kim *et al.* 2014; Chang and Teo 2016; Phasanasophon and Kim 2018; Zarate *et al.* 2020; Lee *et al.* 2020; Susano *et al.* 2021; Baek *et al.* 2021). However, reports on the bioactive properties of seaweeds in the Philippines are still limited (Canoy and Bitacura 2018; Arguelles 2020; Magdugo *et al.* 2020; Arguelles 2021b; Arguelles and Sapin 2020a, b, c). Thus, the current investigation was conducted to assess the TPC, antioxidant activities, anti-diabetic (using α-amylase and α-glucosidase inhibition assay), antibacterial, as well as tyrosinase and elastase inhibition activities of *S. aquifolium* crude extract. The antioxidant activity was done using two methods: copper reduction antioxidant capacity (CUPRAC) and $ABTS^{+}$ radical scavenging assays. Also, the relationship on the phenolic content of the algal extract and its antioxidant activities were established in this study.

MATERIALS AND METHODS

Seaweed Sampling and Collection

The brown seaweed, *S. aquifolium* was collected on 07 Mar 2021 during low tide condition in the coast of General Nakar (lat. 14° 47' 36.66" N; long. 121° 37' 25.01" E), Quezon, Philippines. The seaweed was washed several times using sterile distilled water. Using soft brush bristles, the seaweed was gently scrubbed to remove epiphytes, excess sand particles, and other necrotic parts of the algal sample. *S. aquifolium* was oven-dried at 60 °C for 12 h. After drying, the dried biomass of seaweed was pulverized before subjecting it for solvent extraction (Arguelles and Sapin 2020a). The macroalga was identified using morphological characteristics and identification keys of Trono (1997) and Algae Base (website: www.algaebase.org). Air-dried seaweed samples were mounted on herbarium sheets in triplicates to serve as herbarium specimens. These voucher specimens were deposited at the College of Agriculture Herbarium of the Museum of Natural History at the University of the Philippines Los Baños, College, Laguna, Philippines.

Preparation of Seaweed Extract

Dried and pulverized biomass of *S. aquifolium* (1 g) was subjected to extraction using 30 mL acidified methanol (1 HCl: 80 CH_3OH: 10 H_2O) in an ultrasonic bath for 30 min with continuous stirring for 1 h (Gao *et al.* 2002). The sample mixture was then centrifuged at 12,000 rpm for 20 min at a temperature of 20 °C. The algal extract was further concentrated using a rotary evaporator set at 40 °C under reduced pressure. The concentrated algal extract was kept under refrigerated condition (4 °C) to preserve its biological activity for use to different biological assays needed in the investigation (Arguelles *et al.* 2019).

Proximate Composition Analysis

The moisture content of *S. aquifolium* was analyzed by subjecting 2 g of *S. aquifolium* biomass to complete dryness at 105 °C until a constant weight was obtained. On the other hand, ash content of *S. aquifolium* was analyzed

by subjecting the algal sample to ignition at 450 °C for 6 h until an ash was produced (AOAC 2011). Analysis of the crude fiber was done following the Weende method (AOAC 2011). Briefly, 0.3 g of *S. aquifolium* biomass was digested with 1.25% HCI followed by 1.25% NaOH. The algal residue obtained was dried (at 105 °C for 3 h) and weighed. The crude fat content of *S. aquifolium* biomass was determined using a Soxtec Total Fat Extractor (Foss Inc.) following the Soxhlet method. Briefly, two grams of the dried seaweed biomass was placed in a thimble and subjected to extraction using petroleum ether (as solvent) for 16 h within 30–60 °C boiling range. The crude protein content of the seaweed sample was determined using micro-Kjeldahl protein analysis. Initially, dried *S. aquifolium* biomass (1 g) was digested using 4 mL concentrated sulfuric acid. The resulting solution was then subjected to protein analysis using a Kjeltech™ apparatus (Foss Inc.), and the total amount of nitrogen in crude protein was determined by calculation using the empirical factor 6.25. The total carbohydrate content was obtained *via* the difference method following the equation below:

$$\%\ carbohydrates = 100 - (\%\ moisture + \%\ protein + \%\ crude\ fat + \%\ \text{ash}) \quad (1)$$

Elemental Composition Analysis

The seaweed sample was subjected to dry ashing procedure following the standard methods (AOAC 2011). Biomass of *S. aquifolium* (1 g) was placed into a porcelain crucible and dried in a muffle furnace set at 550 °C for 5 h. The procedure was repeated until the differences in the weight of the sample were less than 0.05% and a white or grayish residue was observed. The residue was then dissolved in 10 mL of concentrated HCl (1:1 ratio for residue and HCl) and was heated slowly to completely dissolve the sample. The digestion solution was briefly subjected to heat using a hot plate (set at 100 °C) to further dissolve the ash. The recovered residue was cooled and filtered using Whatman filter paper and placed into a clean volumetric flask. Similarly, the blank was treated using a digestion experiment in the same way as the seaweed sample. Detection and quantification of calcium, sodium, manganese, potassium, magnesium, zinc, chromium, iron, cadmium, copper, and lead using an atomic absorption spectrophotometer Perkin Elmer AAnalyst 400. The instrument detection limit values for each element were expressed in mg/L in flame AAS and were found to be 0.25 for Ca, 0.12 for Mg, 0.25 for Na, 0.14 for Mn, 0.25 for K, 0.12 Fe, 0.10 for Zn, 0.03 for Cu, 0.12 for Pb, 0.15 for Cr, and 0.02 for Cd.

Determination of TPC

The Folin-Ciocalteu assay was used to analyze the TPC. This assay was done following the method proposed by Nuñez-Selles *et al.* (2002). Initially, about 0.5 mL of *S. aquifolium* crude extract was mixed with 0.5 mL 10% sodium carbonate solution and 0.5 mL of Folin-Ciocalteau's reagent for 1 min. The mixture was set aside for 5 min at ambient temperature. Furthermore, the volume of the reaction mixture was adjusted using 5 mL sterile distilled water. Absorbance reading of all the sample mixtures was taken at 720 nm using a UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). The TPC is presented as µg of gallic acid equivalent (GAE) per g of the algal extract (calibration curve equation: $y = 0.006415x - 0.0140$, $R^2 = 0.99978$).

Antioxidant Activities

The antioxidant activities of *S. aquifolium* extract were assessed using $ABTS^+$ radical scavenging and CUPRAC assay. Two different antioxidant assays were used in this study to show the different mechanisms that take into account the antioxidant activities of *S. aquifolium* extract.

$ABTS^+$ Scavenging Assay

The $ABTS^+$ scavenging activity of the algal extract was assessed following the procedure of Re *et al.* (1999) with some modifications. In this assay, $ABTS^+$ is transformed to its radical cation by the addition of sodium persulfate (blue in color) and absorbs light at 734 nm. The $ABTS^+$ radical cation is very reactive to different antioxidants (such as phenolic compounds) and converts the $ABTS^+$ radical cation (blue in color) to its neutral form (colorless) (Arguelles and Sapin 2020a).

Briefly, 40 µL of *S. aquifolium* extract prepared in different concentrations (30.0–150.0 µg GAE/mL) and 40 µL of 90% methanol (for the control) were added with 3 mL of $ABTS^+$ radical mixture with an initial absorbance reading of 0.72 ± 0.05 at 734 nm. The reaction mixtures were thoroughly stirred and kept at ambient temperature for 5 min. Absorbance reading of each prepared reaction solution was noted at 734 nm (Re *et al.* 1999), and the $ABTS^+$ inhibition (%) was calculated using the following equation:

$$Inhibition\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \quad (2)$$

where $A_{control}$ is the absorbance reading of the control and A_{sample} is the absorbance reading of the sample (algal extract). Ascorbic acid was used as the positive control in the assay. The $ABTS^+$ inhibition activity (%) was plotted with different prepared concentrations of *S. aquifolium* extract. The seaweed extract concentration that showed 50% $ABTS^+$ radical scavenging activity was considered the IC_{50}.

CUPRAC Assay

The ability of *S. aquifolium* extract to reduce cupric ions (Cu^{2+}) was analyzed using the protocols done by Alpinar *et al.* (2009). In this assay, 1 mL each of 0.01 M $CuCl_2$ solution, 1 M ammonium acetate buffer (pH 7), and 0.0075 M neocuproine were mixed in sterile test tubes containing 0.5 mL of *S. aquifolium* extract (5.0, 10.0, 15.0, 20.0, and 25.0 µg GAE/mL) and ascorbic acid as the standard antioxidant (Arguelles *et al.* 2017). The total volume for each reaction mixture was adjusted to 4.1 mL using sterile distilled water and was kept at room temperature for 30 min. The absorbance reading against a reagent blank was noted at 450 nm for both the *S. aquifolium* extract and ascorbic acid concentrations (Arguelles *et al.* 2017; Arguelles 2021a).

Whitening Property *via* Tyrosinase Inhibition Assay

The tyrosinase inhibition activity of *S. aquifolium* extract was analyzed *in vitro* following the procedure of Hapsari *et al.* (2012) with slight modifications. Solutions of 5 mM DOPA (3,4-dihydroxy-L-phenylalanine, Sigma D-9628), mushroom tyrosinase (250 units/mL, Sigma T- 3824) and 0.1M potassium phosphate buffer (pH 6.5) were prepared. An aliquot of 40 µL DOPA was mixed with 40 µL of *S. aquifolium* extract (at varying concentration: 15.0, 30.0, 45.0, 60.0, and 75.0 µg GAE/mL) or 40 µL buffer (for the control) in a 96-well microtiter plate. The total volume of each reaction mixture was adjusted to 160 µL by adding 40 µL of phosphate buffer and mushroom tyrosinase. The microtiter plate containing the reaction mixtures was kept for 15 min at ambient room temperature. The absorbance reading was taken at 490 nm using a microtiter plate reader. Percent tyrosinase inhibition was calculated using the formula below:

$$Inhibition\ (\%) = \left(\frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}}\right) \times 100 \quad (3)$$

where $A_{control}$ is the absorbance reading of the control, A_{blank} is the absorbance reading of the blank, and A_{sample} is the absorbance reading of the sample (seaweed extract). Kojic acid was used as the positive control in the assay.

Anti-aging and Anti-wrinkling Property *via* Elastase Inhibition Assay

The elastase inhibition activity of *S. aquifolium* extract was done using the procedure of Moon *et al.* (2010). Initially, solutions of elastase from porcine pancreas (50 µg/mL, Sigma E-7885), N-succinyl-$(ALA)_3$-p-nitroanilide (25 mM, Sigma S-4760), and 0.2M TRIS-HCl buffer (pH 8.0) were prepared. An aliquot (40 µL) of the algal extract or 40 µL buffer (for the control) was thoroughly mixed with 40 µL N-succinyl-$(ALA)_3$-p-nitroanilide in sterile test tubes. The volume of the mixture was adjusted to 1 mL using phosphate buffer and 40 µL elastase was added last in the solution. The blank tube was the one without the enzyme solution. After 20 min, 2 mL of TRIS-HCl buffer were added in the reaction mixtures and the absorbance reading of each sample was measured at 410 nm. Elastase inhibition was determined using Equation 3, with A_{sample} as the absorbance reading of the sample (algal extract). Tocopherol was used as the positive control in the assay.

Antidiabetic Activities

The antidiabetic properties of *S. aquifolium* extract were assessed using α-glucosidase and α-amylase inhibition assay. Two different antidiabetic assays were used in this study to show the potential of *S. aquifolium* extract in suppressing key carbohydrate hydrolyzing enzymes in the digestive system such as α-glucosidase and α-amylase.

α-Glucosidase Inhibition Assay

The ability of *S. aquifolium* extract to inhibit α-glucosidase was assessed by spectrophotometric assay using *p*-nitrophenyl-α-glucopyranoside (*p*NPG) as a substrate, following the methods of Nair *et al.* (2013). The presence of α-glucosidases in algal extract converts *p*NPG (substrate) to *p*-nitrophenol (*p*NP) and is measured spectrophotometrically at 410 nm wavelength. Initially, a mixture containing 75 µL of α-glucosidase (2.5 U/mL), 100 µL of seaweed extract, or 100 µL of 0.1 M phosphate buffer pH 6.8 (for the control) were mixed in sterile test tubes. The volume of the sample mixture was adjusted to 500 µL by adding 30 µL of 10mM *p*-nitrophenyl-α-D-glucopyranoside (Sigma N1337) and 295 µL buffer before incubation. The reaction mixtures were then kept at 37 °C for 12 min, after which 3 mL of 50 mM NaOH were added to the mixture. Absorbance reading of each reaction mixture (sample) was noted at 410 nm. The percent α-glucosidase inhibition was determined using the equation below:

$$\alpha\text{-}Glucosidase\ inhibition\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \quad (4)$$

α-Amylase Inhibition Assay

The inhibitory properties of *S. aquifolium* extract towards α-amylase was evaluated *in vitro*, following the protocols of Phoboo (2015) with few modifications. Initially, solutions of 0.02M sodium-phosphate buffer (pH 6.9) with 0.006M NaCl, α-amylase from porcine pancreas (0.5 mg/mL, Sigma A3176), and 1% starch solution were prepared. Different concentrations of the algal extract (with different phenolic concentrations) were prepared by dilution with water. With 50 µL of the α-amylase solution, 25 µL of the algal extract or 25 µL buffer (for the control) were thoroughly mixed in sterile test tubes. The volume was adjusted up to 250 µL by adding 175 µL phosphate buffer.

This mixture was then added (at timed intervals) with 250 µL starch solution and was incubated for 20 min. After incubation, the mixture was halted by adding 400 µL of DNS color reagent also at timed intervals. On the other hand, the blank used in the assay consisted of 400 mL DNS reagent and 500 mL buffer. The test tubes containing the reaction mixture were subjected to a boiling water bath for about 5 min, cooled, and further diluted with 5 mL sterile distilled water. The absorbance reading of the sample mixtures and control were noted at a wavelength of 540 nm. The percent (%) inhibition was determined using the formula below:

$$\alpha\text{- Amylase inhibition } (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \qquad (5)$$

Antibacterial Activity

Five Gram-negative bacteria (*Serratia marcescens* BIOTECH 1748, *Aeromonas hydrophila* BIOTECH 10089, penicillin acylase-producing *Escherichia coli* BIOTECH 1634, *Pseudomonas aeruginosa* BIOTECH 1824, and *Pseudomonas fluorescens* BIOTECH 1123) and five Gram-positive bacteria (*Staphylococcus epidermidis* BIOTECH 10098, *Staphylococcus saprophyticus* BIOTECH 1802, methicillin-resistant *Staphylococcus aureus* BIOTECH 10378, *Listeria monocytogenes* BIOTECH 1958, and penicillin acylase-producing *Bacillus cereus* BIOTECH 1509) were tested against *S. aquifolium* crude extract using microtiter plate dilution assay. These reference pathogens were obtained from the PNCM of BIOTECH-UPLB. Initially, bacterial pathogens were pre-cultivated using Luria Bertani broth medium and incubated at 37 °C with shaking for 24 h. The purity and viability of each test organism was regularly monitored by conducting regular biochemical tests and morphological characterization (Arguelles 2018).

Microtiter plate dilution (two-fold serial dilution technique) assay was used to know the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. aquifolium* extract (Arguelles 2021a). Briefly, 100 µL of broth cultures of the bacterial pathogens (cell density of 1 x 10^6 cells/mL) were gently mixed with 100 µL of *S. aquifolium* extract set at different dilutions (1000 to 7.8125 µg/mL). The antibacterial assay was done in triplicates and was incubated for 12 h in an incubator set at 35 °C. After incubation, MICs of *S. aquifolium* extract against different bacterial pathogens were taken. On the other hand, MBC was analyzed using the method done by Arguelles (2018). Loopful of samples obtained from microtiter plate wells that exhibited no visible bacterial growth from the MIC assay were inoculated onto fresh tryptic soy agar plates. These plates were kept at 35 °C for 24 h and evaluated for bacterial growth. The absence of bacterial colony growth would mean that the algal extract was bactericidal at that specific dilution (Arguelles *et al.* 2019).

Statistical Analyses

The data obtained from the experiments are expressed as means ± standard deviations (mean ± SD) of three replicates. The statistical tests for the linear correlation coefficient important in correlation analysis were determined using Microsoft Office Excel 2016.

RESULTS AND DISCUSSION

Proximate Composition

Seaweeds were reported to have high amounts of polysaccharides, minerals, polyunsaturated fatty acids, vitamins, and dietary fibers that could be used as nutrition enhancers, preservatives, and sources of healthy food (El-Manawy *et al.* 2019). These substances play an important role in the color enhancement and nutritional content of human food (El-Manawy *et al.* 2019). *Sargassum aquifolium* contained a high concentration of carbohydrate, ash, protein, and crude fiber with percent composition of 32.29 ± 0.17, 30.19 ± 0.14, 16.89 ± 0.09, and 10.03 ± 0.15%, respectively (Table 1). This result conforms to previous studies showing that the proximate composition of several seaweed species considers carbohydrates as the most abundant biomolecule of the macroalgae constituting up to 90% of the total dry biomass (Radha 2018). The mineral content of the algal biomass was reflected by the amount of ash present in the seaweeds. Ash content of *S. aquifolium* was greater than those reported for *S. vulgare* (27.09 + 0.00%), *Gracilaria salicornia* (19.2 ± 0.72%), and *Gracilaria edulis* (7.83 ± 0.06%) but was lower than that observed for *Codium intricatum* (37.16 ± 0.21%) (Radha 2018; Arguelles *et al.* 2019; Arguelles 2020). Moisture is a quality factor that provides information in the preservation or shelf life of some products and significantly affects the stability of food material (Syad *et al.* 2013; Arguelles 2020; Arguelles and Martinez-Goss 2021). The moisture content of *S. aquifolium* was 6.74

Table 1. Proximate composition of *Sargassum aquifolium*.

Proximate composition	Percent composition (%)
Moisture content	6.74 ± 0.10
Ash content	30.19 ± 0.14
Crude protein	16.89 ± 0.09
Crude fat	3.86 ± 0.07
Crude fiber	10.03 ± 0.15
Carbohydrate	32.29 ± 0.17

± 0.10%, which suggests the stability of this seaweed for storage. Proteins play several important biological functions such as transport and storage (of biomolecules) as well as enzymatic catalysis that are crucial for the growth and proliferation of seaweeds (Radha 2018; Arguelles *et al.* 2018; Arguelles 2020). On the other hand, dietary fibers of seaweeds are known to exhibit important bioactive properties like anti-mutagenic, anti-tumor, antioxidant, and anti-coagulant activity and perform a critical role in lipid metabolism (Syad *et al.* 2013). The protein content of the *S. aquifolium* was greater than those reported for seaweeds collected from the Red Sea coast at Hurghada in Egypt such as *Caulerpa racemosa* (4.80 ± 0.4%), *Padina boergesenii* (5.90 ± 0.25%), and *Sargassum aquifolium* (5.40 ± 0.2%) (El-Manawy *et al.* 2019). The dietary fiber content of *S. aquifolium* was greater than those obtained by Syad *et al.* (2013) for *Sargassum wightii* (17.0 ± 1.19%) and *Gelidiella acerosa* (13.45 ± 1.076%) but was lower than those observed by El-Manawy *et al.* (2019) for *Padina boergesenii* (36.20 ± 2.70%), *Polycladia myrica* (34.7 ± 2.2%), and *Sargassum aquifolium* (33.1 ± 2.4%).

The current study documented that the proximate composition of *S. aquifolium* varies when compared to different seaweed species reported from earlier studies (Radha 2018; El-Manawy *et al.* 2019). A variation on the chemical composition of the macroalgae may be due to differences in season, geographic area, salinity, dissolved oxygen, and water temperature, by which these algae can be signaled to inhibit or stimulate the synthesis of diverse chemical components. Despite these differences in the chemical composition of seaweeds, reports on some of the pharmacologically important macroalgae show that several of these organisms still possess significantly high amounts of vitamins, protein, minerals, and other nutrients important for human nutrition (Syad *et al.* 2013; Radha 2018; El-Manawy *et al.* 2019).

Elemental Composition

Seaweeds are known as an alternative food source with an important role in providing global food security (Salehi *et al.* 2019). These organisms contain minerals that play a significant role in several metabolic processes in humans like cell transport and catalytic function of metalloenzymes (serving as cofactors). Even though minerals are important in the growth and development of seaweeds, these substances are not synthesized by these organisms but rather obtained by absorption from the marine environment. In addition, several ecological factors such as salinity, temperature, pH, and light intensity also affect the amount of minerals present in seaweeds (Salehi *et al.* 2019; Arguelles *et al.* 2019). The mineral content of seaweed biomass is usually reflected by the ash content of the alga. Ash content of seaweeds is considered as a benchmark of quality for the evaluation of the nutritional and bifunctional properties of the edible seaweeds (Reka *et al.* 2017; Arguelles 2020).

The average concentration of some important minerals in *Sargassum aquifolium* is presented in Table 2. The elemental distribution in *S. aquifolium* was observed to be in decreasing order of Na > Ca > K > Mg > Mn > Fe > Zn > Cu > Pb > Cr > Cd. *Sargassum aquifolium* has a significant concentration of minerals (such as calcium, sodium, potassium, and phosphorus), as with those reported for *C. intricatum*, *Ulva reticulata*, *Gracilaria edulis*, and *Sargassum naozhouense* (Peng *et al.* 2012; Reka *et al.* 2017; Arguelles 2020). Sodium (31,103 ± 0.61 ppm) was the most abundant microelement in the seaweed biomass, followed by calcium (29,291.10 ± 10.02 ppm), potassium (17,192 ± 1,087 ppm), and magnesium (16,773 ± 101 ppm). These microelements were greater than those obtained by Yangthong (2017) from *Sargassum binderi* with sodium, calcium, potassium, and magnesium content of 75, 323, 155, and 99 ppm, respectively. However, *S. aquifolium* showed a lower concentration of sodium and potassium as compared to that obtained for *S. naozhouense* (an edible brown seaweed) with an estimated concentration of 32,500 ppm and 41,700 ppm, respectively. Such variation in the concentration of microelements among several species of seaweed may be due to ecological factors (such as pH, salinity, temperature, and light intensity), differences in the geographical location, and age (during the time of collection) of the seaweed (Arguelles 2020). In addition, *S. aquifolium* is also composed of trace elements like copper, manganese, lead, zinc, iron, cadmium, and chromium. Manganese exhibited the highest concentration among the trace microelements (1005.17 ± 2.01 ppm), followed by iron (398.44 ± 1.94 ppm) and zinc (9.05 ± 0.31 ppm). The

Table 2. Concentrations of macro and micro-elements of *Sargassum aquifolium*.

	Elemental parameter* (in ppm)										
	Ca	Mg	Na	Mn	K	Fe	Zn	Cu	Pb	Cr	Cd
Sargassum aquifolium	29,291.10 ± 10.02	16,773 ± 101	31,103 ± 0.61	1005.17 ± 2.01	17,192 ± 1,087	398.44 ± 1.94	9.05 ± 0.31	3.17 ± 0.01	2.61 ± 0.13	1.19 ± 0.02	0.99 ± 0.06

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

amount of manganese, iron, and zinc reported in this study was greater than those reported by Arguelles (2020) from *Codium intricatum* with concentrations of 32.40 ± 2.01, 290.53 ± 24.74, and 4.65 ± 0.25 ppm, respectively. The content levels of other heavy metals (Cu, Pb, Cr, and Cd) were comparable to previous reports on seaweeds (Peng *et al.* 2012; Reka *et al.* 2017; Salehi *et al.* 2019). These heavy metals are detrimental to other organisms if consumed in high concentrations. Table 2 shows that the presence of these microelements in *S. aquifolium* is below the limits of toxicity and confirms the potentiality of this seaweed as an alternative source of microelements for human use. Also, the current study documented important elements like sodium, calcium, potassium, iron, and magnesium in considerable concentration (in *S. aquifolium* biomass). Thus, showing the potential application of these elements as additives in diet formulation for improved quality of food needed in addressing nutrient deficiency and malnutrition in the country.

TPC

The Folin-Ciocalteu method was used to determine the TPC of *S. aquifolium* (expressed in mg GAE/g). Polyphenols are abundantly found in seaweeds and have been documented to contain diverse biological properties such as antioxidant, antibacterial, and tyrosinase inhibition properties (Arguelles and Sapin 2020b). These compounds could assist the seaweeds by providing important adaptive defense against oxidative stress and grazers such as marine herbivores. Phenolic compounds are considered key substances in several algal extracts that play a major part in the bioactive properties of these organisms (Arguelles and Sapin 2020a). Thus, several studies were conducted that focused on the quantification and identification of these bioactive compounds. In this study, *S. aquifolium* was observed to contain a TPC of 5.74 ± 0.04 mg GAE/g. The TPC of *S. aquifolium* was higher than those obtained from other brown seaweeds such as *Sargassum polycystum* (0.37 mg GAE/g), *Sargassum binderi* (0.267 mg GAE/g), *Turbinaria conoides* (0.09 mg GAE/g), *Turbinaria ornata* (1.07 mg GAE/g), and *Zonaria tournefortii* (0.78 mg GAE/g) (Boonchum *et al.* 2011; Chakraborty *et al.* 2013; Fu *et al.* 2015; Fellah *et al.* 2017). On the other hand, the TPC of *S. aquifolium* was lower than that obtained by Dang *et al.* (2018) for *S. aquifolium* and *Padina* sp. from Bateau Bay in Australia with phenolic contents of 67.78 and 124.65 mg GAE/g, respectively. Variation in the amount of phenolic compounds extracted on several seaweed species is dependent on factors such as the polarity of the solvent. In general, phenolic compounds are highly soluble to polar solvents such as acidified methanol, allowing high recovery of these important compounds in *S. aquifolium* extract (Arguelles 2021b). Other extrinsic factors (*e.g.* irradiation, sampling, salinity, and season) and intrinsic factors (*e.g.* stage of growth of the macroalgae) can also affect the amount of phenolic content in seaweeds. In this study, a single preliminary collection of brown seaweed *S. aquifolium* was done during the dry season. Thus, variations in the phenolic content of *S. aquifolium* are possible if collected and analyzed at different geographical areas and seasons.

Antioxidant Activities

The genus *Sargassum* is known to possess diverse kinds of compounds with potent antioxidant activities (Arguelles and Sapin 2020b). These antioxidants are important in food preservation and in preventing the oxidation of important biomolecules in the human body (Köksal *et al.* 2017). The antioxidant activities of *S. aquifolium* extract from General Nakar, Quezon were evaluated using $ABTS^+$ and CUPRAC assay. The $ABTS^+$ radical scavenging assay is an initial test for the assessment of the potential antioxidant activity of the seaweed extract in quenching free radicals. On the other hand, CUPRAC assay is an antioxidant activity test used to measure the metal chelating activity of the algal extract. It is an important assay since metals (*e.g.* Cu and Fe) have the potential to cause the formation of OH radicals (*via* Fenton reaction), which can result in the oxidation of important cellular structures of an organism (Köksal *et al.* 2017).

The $ABTS^+$ scavenging activity and IC_{50} value of *S. aquifolium* extract are presented in Table 3. The $ABTS^+$ scavenging activity of *S. aquifolium* extract exhibited the highest antioxidant activity of 66.10 ± 1.20% at 150 µg GAE/mL extract concentration. The analysis shows that the $ABTS^+$ scavenging activity of *S. aquifolium* extract increases with an increase in the phenolic concentration of the algal extract (30.0–150.0 µg GAE/mL). The computed IC_{50} value of the seaweed extract was 107.0 µg/mL, which is more potent than that obtained from standard antioxidant, ascorbic acid (IC_{50} = 161.0 µg/mL). This IC_{50} value is more effective than those obtained from other seaweeds such as *Gracilaria edulis* (0.56 ± 0.01 mg/mL), *Acetabularia acetabulum* (6.3 mg/mL), and *Halimeda tuna* (16.1 mg/mL) (Sivaramakrishnan *et al.* 2017; Gunathilaka *et al.* 2019). However, *S. aquifolium* extract is less potent as compared to *Turbinaria decurrens* extract with an IC_{50} value of 49.31 µg/ml (Arguelles and Sapin 2020a). The result of this study suggests that *S. aquifolium* extract may contain bioactive compounds such as phenolic compounds that act as effective antioxidants capable of inhibiting oxidation through free radical scavenging activity.

CUPRAC assay was utilized in this study to assess the capacity of *S. aquifolium* extract to inhibit oxidation *via* a metal chelation mechanism. The *Sargassum aquifolium* extract exhibited a dose-dependent copper

Table 3. $ABTS^+$ radical scavenging activity and IC_{50} value of phenolics from *Sargassum aquifolium* and ascorbic acid.

Sample	Extract concentration (µg GAE/mL)					IC_{50}*
	30.0	60.0	90.0	120.0	150.0	
	$ABTS^+$ inhibition (%)					
Sargassum aquifolium	16.74 ± 0.61	30.64 ± 0.20	43.48 ± 0.10	54.89 ± 0.20	66.10 ± 1.20	107 µg/mL
	Concentration (µg/mL)					
	37.5	75.0	112.5	150.0	187.5	
	$ABTS^+$ inhibition (%)					
Ascorbic acid**	12.24 ± 0.80	23.21 ± 0.00	36.08 ± 0.30	47.40 ± 0.40	55.98 ± 0.20	161 µg/mL

*IC_{50} is the effective concentration that inhibits the activity of $ABTS^+$ cation radical by 50%, the value of which is computed by interpolation.
**A reference antioxidant

Table 4. Copper reduction antioxidant capacity and IC_{50} value of phenolics from *Sargassum aquifolium* and ascorbic acid.

Sample	Extract concentration (µg GAE/mL)					IC_{50}*
	5.0	10.0	15.0	20.0	25.0	
	CUPRAC (absorbance at 450 nm)					
Sargassum aquifolium	0.138 ± 0.004	0.256 ± 0.006	0.372 ± 0.011	0.480 ± 0.006	0.579 ± 0.001	21.01 µg/mL
	Concentration (µg/mL)					
	10.0	20.0	30.0	40.0	50.0	
	CUPRAC (absorbance at 450 nm)					
Ascorbic acid**	0.114 ± 0.005	0.227 ± 0.000	0.334 ± 0.013	0.439± 0.013	0.534 ± 0.012	46.46 µg/mL

*IC_{50} is the effective concentration that gives CUPRAC value of 0.5 absorbance reading at 450 nm, the value of which is computed by interpolation.
**A reference antioxidant

ion reduction ability (Table 4). The maximum copper ion reduction ability (CUPRAC value = 0.579 ± 0.001) of the seaweed extract was observed at 25 µg GAE/mL concentration while for the standard (ascorbic acid), the highest reduction was observed at 50 µg/mL concentration with a corresponding absorbance of 0.534 ± 0.012. The observed trend in this antioxidant assay is analogous to those observed from the $ABTS^+$ antioxidant assay, in which 150 µg GAE/mL concentration exhibited the highest antioxidant activity. The computed IC_{50} value of *S. aquifolium* extract was 21.01 µg/mL, which is more potent than that obtained from ascorbic acid with an IC_{50} value of 46.46 µg/mL. This observation is relatively more potent than that obtained for *Turbinaria ornata* (IC_{50} value = 24.34 µg/mL) but is less effective to other brown seaweeds such as *Sargassum siliquosum* (IC_{50} value = 18.50 µg/mL), *Sargassum ilicifolium* (IC_{50} value = 11.19 µg/mL), and *Turbinaria decurrens* (IC_{50} value = 17.2 µg/mL) collected from the coastal area of Catanauan, Quezon, Philippines (Arguelles 2021b; Arguelles and Sapin 2020a, b, c). The study showed that *S. aquifolium* extract has strong copper reducing power activity, which can be attributed to bioactive compounds (such as polyphenols and phlorotannins) present in the seaweed. The existence of these bioactive compounds in *S. aquifolium* extract and its potent antioxidant activity proved the potential use of this seaweed as an alternative source of natural antioxidants applicable for human use.

The correlation analysis and its coefficients between different extract concentrations and antioxidant activities of *Sargassum aquifolium* are presented in Table 5. It is observed that the phenolic content of the algal extract exhibited a strong positive correlation with the antioxidant activity using $ABTS^+$ and CUPRAC assay with correlation coefficients (R) of 0.99887 and 0.99936, respectively. Such results would mean that the potent antioxidant properties of the algal extract might be attributed to polyphenolic substances present in *S. aquifolium*. The correlation obtained in this study is similar to those observed from earlier studies done for other seaweed species such as *Turbinaria ornata*, *Turbinaria decurrens*,

Table 5. Correlation between phenolic content and antioxidant activities of *Sargassum aquifolium* extract.

Antioxidant assay	Regression equation	Correlation coefficient (R)
$ABTS^{+}$ radical scavenging assay	y = 0.4099x + 5.479	0.99887
CUPRAC assay	y = 0.0221x + 0.0332	0.99936

Sargassum siliquosum, and *Sargassum ilicifolium* on the relationship of phenolic content in the algal extract and its corresponding antioxidant activities (Arguelles 2021b; Arguelles and Sapin 2020a, b, c). The significant correlation between antioxidant activity and algal extract concentration suggests that phenolic compounds in *S. aquifolium* extracts can cause oxidation inhibition *via* free radical scavenging and metal chelation mechanisms. Thus, it is recommended that isolation and identification of phenolic compounds and other important bioactive substances should be done to further study the potential biotechnological applications of this seaweed.

Tyrosinase Inhibition Activity

Tyrosinase is an important enzyme liable for the biosynthesis of melanin – the key pigment in charge of skin color and browning of foods. Recently, several synthetic and naturally derived compounds have been widely used to inhibit tyrosinase, which was also tapped as a novel skin whitening agent in the cosmetic industry (Arguelles 2021b). The potential of *Sargassum aquifolium* was assessed as an effective tyrosinase inhibitor *in vitro* using mushroom tyrosinase (Table 6). *S aquifolium* extract exhibited potent tyrosinase inhibition property with an IC_{50} value of 39.0 µg/mL. It is considered more effective as compared to kojic acid (standard whitening substance) with a computed IC_{50} value of 101.0 µg/mL. The activity of the *S. aquifolium* extract is also more effective than other previously reported seaweeds with tyrosinase inhibition properties such as *Turbinaria ornata* (IC_{50} = 67.50 µg/mL), *Eucheuma cottonii* (IC_{50} = 234.44 µg/mL), *Sargassum ilicifolium* (IC_{50} = 40.50 µg/mL), and *Sargassum siliquosum* (IC_{50} = 65.0 µg/mL) (Chang and Teo 2016; Arguelles and Sapin 2020b, c; Arguelles 2021b). Tyrosinase inhibitors are popularly used in food applications as anti-browning compounds and in the cosmetic industry as depigmentation agents. Polyphenols can mimic the substrate of tyrosinase by causing competitive inhibition of the enzyme (Baek *et al.* 2021). Other bioactive compounds such as fucoidan (common in several species of brown seaweeds) can react with copper (found in the active site of tyrosinase) through sulfide atoms causing inhibition of tyrosinase in skin cells (Namjooyan *et al.* 2019; Arguelles and Sapin 2020c). In addition, non-phenolic compounds may also exhibit tyrosinase inhibition activity like carotenoids (*e.g.* fucoxanthin and astaxanthin), which was previously reported to have anti-tyrosinase activities (Chang and Teo 2016; Baek *et al.* 2021). Thus, additional studies are needed to further investigate the identity of the active compounds present in *S. aquifolium* extract that exhibit potent tyrosinase inhibition properties.

Elastase Inhibition Activity

Wrinkling is a result of direct exposure of epidermal cells and other connective tissues to harsh conditions that lead to dryness, fragility, sagging, and thinning of the skin (Shanura Fernando *et al.* 2018). Elastin and collagenous fibers are two of the most important components of the connective tissues that preserve the glossy and smooth characteristics of human skin. These substances are

Table 6. Tyrosinase inhibition activity and IC_{50} value of phenolics from *Sargassum aquifolium* and kojic acid.

Sample	Extract concentration (µg GAE/mL)					IC_{50}*
	15.0	30.0	45.0	60.0	75.0	
	Tyrosinase inhibition (%)					
Sargassum aquifolium	29.83 ± 2.92	41.93 ± 1.76	55.86 ± 1.43	69.31 ± 1.94	78.10 ± 1.65	39.0 µg/mL
	Concentration (µg/mL)					
	50.0	100.0	150.0	200.0	250.0	
	Tyrosinase inhibition (%)					
Kojic acid**	32.30 ± 1.02	49.75 ± 0.24	65.64 ± 2.38	72.86 ± 0.37	76.41 ± 0.43	101.0 µg/mL

*IC_{50} is the effective inhibitory concentration that inhibits tyrosinase activity by 50%, the value of which is computed by interpolation.
**A reference tyrosinase inhibitor and known whitening agent

vulnerable to degradation *via* the action of elastase and collagenases, which can result in changes in the structural features of the skin dermis causing the formation of wrinkles and skin aging (Shanura Fernando *et al.* 2018; Phasanasophon and Kim 2018). Thus, the evaluation of elastase and collagenase inhibition activity of seaweed extract is an initial step in assessing the potential anti-wrinkling activity of the alga (Shanura Fernando *et al.* 2018; Phasanasophon and Kim 2018). In this study, the ability of *S. aquifolium* to inhibit elastase was done *in vitro* (Table 7). *Sargassum aquifolium* extract exhibited a dose-dependent elastase inhibition activity. This would mean that the elastase inhibition activity of *S. aquifolium* extract increases with an increase in the phenolic concentration of the algal extract (100.0–300.0 µg GAE/mL). The IC_{50} value of *S. aquifolium* extract was 231.01 µg/mL, which is more potent than that obtained from tocopherol (wherein 50% inhibition of elastase was not achieved at 2500 µg/mL concentration). The IC_{50} value of *S. aquifolium* extract is considered more potent than that obtained for *Asparagopsis armata* (IC_{50} value = 2.50–3.387 mg/mL), *Lobophora variegata* (IC_{50} value = > 250.0 µg/mL), and *Dictyota* sp. (IC_{50} value = > 250.0 µg/mL) but is less effective to other brown seaweeds such as *Agarum cribrosum* (IC_{50} value = 16.13 µg/mL) and *Carpomitra costata* (IC_{50} value = 4.0 µg/mL) (Phasanasophon and Kim 2018; Zarate *et al.* 2020; Lee *et al.* 2020; Susano *et al.* 2021).

Earlier studies in the Philippines showed that several species of *Codium*, *Sargassum*, and *Turbinaria* exhibited antioxidant and anti-melanogenic activity. However, anti-aging and anti-wrinkling property such as elastase inhibition activity was not documented in the Philippines (Arguelles 2021b, 2020; Arguelles and Sapin 2020a, b, c). The current study is the first report in the Philippines showing the elastase inhibition activity of *Sargassum aquifolium* (Turner) C. Agardh as a promising source of naturally derived anti-aging and anti-wrinkling active ingredient useful for cosmetic application. It is recommended that additional studies on *S. aquifolium* extract should be done to identify the active compounds present in the seaweed to fully understand the mechanisms involved in elastase inhibition. Such information is crucial for mass production and large-scale utilization for cosmetic application of this important seaweed.

Antidiabetic Activities

Diabetes mellitus is a disease characterized by having impaired insulin activity and/or insulin secretion resulting in hyperglycemia (Kim *et al.* 2014; Sim *et al.* 2019). Treatment of this disease is usually done *via* prevention of hyperglycemia, which is made possible by suppressing key carbohydrate hydrolyzing enzymes in the digestive system such as α-glucosidase and α-amylase. In this study, an assessment of the potential antidiabetic properties of *S. aquifolium* was done *in vitro via* α-amylase and α-glucosidase inhibition assay. The α-glucosidase and α-amylase inhibitory activities of *S. aquifolium* extract are presented in Tables 8 and 9. The algal extract exhibited a concentration-dependent reduction in α-glucosidase and α-amylase inhibition. *S. aquifolium* extract exhibited highest inhibition of α-glucosidase (72.04 ± 1.28%) and α-amylase (85.65 ± 0.87%) at extract concentration of 20 µg GAE/mL and 120 µg GAE/mL, respectively. The *S. aquifolium* extract has potent α-amylase (IC_{50} of 59.0 µg/mL) and α-glucosidase (IC_{50} of 15.60 µg/mL) inhibition activities – more effective as compared to that of acarbose (standard antidiabetic drug). The IC_{50} value of *S. aquifolium* extract against α-glucosidase is more effective than those obtained for *Ascophyllum nodosum* and *Fucus vesiculosus* collected from the coast of L'Isle Verte in Quebec, wherein the seaweeds exhibited α-glucosidase inhibition with an

Table 7. Elastase inhibition activity and IC_{50} value of phenolics from *Sargassum aquifolium* and tocopherol.

Sample	Extract concentration (µg GAE/mL)					IC_{50}*
	100.0	150.0	200.0	250.0	300.0	
	Elastase inhibition (%)					
Sargassum aquifolium	6.89 ± 1.09	18.37± 1.66	28.68 ± 2.23	60.10 ± 0.52	72.02 ± 1.09	231.0 µg/mL
	Concentration (µg/mL)					
	500	1000	1500	2000	2500	
	Elastase inhibition (%)					
Tocopherol**	16.58 ± 0.19	19.35 ± 0.06	26.08 ± 1.13	31.03 ± 0.95	38.22 ± 0.37	> 2500 µg/mL***

*IC_{50} is the effective concentration that inhibits elastase activity by 50%, the value of which is computed by interpolation.
**A reference elastase inhibitor and known anti-wrinkling agent
***IC_{50} was not determined because 50% inhibition was not achieved at 2500 µg/mL concentration.

Table 8. α-Glucosidase inhibition and IC_{50} of phenolics from *S. aquifolium* in comparison to acarbose.

Sample	Extract concentration (µg GAE/mL)					IC_{50}*
	4.0	8.0	12.0	16.0	20.0	
	α-Glucosidase inhibition (%)					
Sargassum aquifolium	1.63 ± 0.54	8.07 ± 0.20	22.60 ± 0.49	52.96 ± 1.43	72.04 ± 1.28	15.6 µg/mL
	Concentration (µg/mL)					
	2,000.0	4,000.0	6,000.0	8,000.0	10,000.0	
	α-glucosidase inhibition (%)					
Acarbose**	17.96 ± 1.36	31.69 ± 1.22	45.32 ± 1.90	57.26 ± 0.49	62.35 ± 0.49	6771 µg/mL

* IC_{50} is the effective concentration that inhibits α-glucosidase activity by 50%.
**A reference α-glucosidase inhibitor and anti-diabetic drug

Table 9. α-Amylase inhibition and IC_{50} of phenolics from *S. aquifolium* in comparison to acarbose.

Sample	Extract concentration (µg GAE/mL)					IC_{50}*
	40.0	60.0	80.0	100.0	120.0	
	α-Amylase inhibition (%)					
Sargassum aquifolium	23.36 ± 0.43	51.26 ± 0.35	66.16 ± 2.69	80.53 ± 0.30	85.65 ± 0.87	59 µg/mL
	Concentration (µg/mL)					
	60.0	120.0	180.0	240.0	300.0	
	α-Amylase inhibition (%)					
Acarbose**	35.41 ± 0.30	55.86 ± 0.78	67.11 ± 0.65	74.80 ± 0.43	80.90 ± 0.74	103 µg/mL

*IC_{50} is the effective concentration that inhibits α-amylase activity by 50%.
**A reference α-amylase inhibitor and anti-diabetic drug

IC_{50} value of 0.047 and 0.049 mg/mL, respectively (Kim *et al.* 2014). However, it is less potent than that observed by Arguelles and Sapin (2020a) for *Turbinaria decurrens*, wherein the seaweed exhibited an IC_{50} value of 11.0 µg/mL. On the other hand, the IC_{50} value of the *S. aquifolium* extract against α-amylase is more potent than those of *Sargassum wightii*, *Undaria pinnatifida*, and *Turbinaria ornata* with IC_{50} values of 378.3, 0.190, and 250 µg/mL, respectively (Unnikrishnan *et al.* 2014, 2015; Sim *et al.* 2019). This result is contrary to that observed by Gabbia *et al.* (2017) from the combined algal extracts of *F. vesiculosus* and *A. nodosum*, wherein inhibition of α-amylase had an IC_{50} value of 1.49 µg/mL. The potent activity of *S. aquifolium* extract against carbohydrate hydrolyzing enzymes shows the promising application of this seaweed as an alternative source of antidiabetic drug useful in the regulation and control of hyperglycemia.

The mechanisms involved in α-amylase and α-glucosidase inhibition differ among the type of bioactive substances involved in the inhibition (Kim *et al.* 2014). Phenolic substances are reported to have potent inhibition activities against α-amylase and α-glucosidase. These compounds are capable of intercepting the hydrogen ion (*via* hydrogen scavenging mechanism) being released from the active site of α-glucosidase (Kim *et al.* 2014). On the other hand, α-amylase is usually inhibited *via* complex formation (α-amylase and active substance), thus limiting the activity of the hydrolytic enzyme as well as slowing down the glucose diffusion from the active site of α-amylase (such as water-soluble dietary fibers), which causes a delay in the digestion of carbohydrates and glucose absorption (Kim *et al.* 2014). This study presented the use of bioactive compounds from seaweeds as an efficient enzyme inhibitor of the key starch-digesting enzymes. Results observed in this study support the promising use of *S. aquifolium* as a dietary supplement for the treatment of diabetes. Additional studies are needed on the identification and structure elucidation of the active compounds (in the algal extract) to be able to further understand the mode of action involved in the antidiabetic properties of the seaweed. In addition, analyzing the antidiabetic effects of *S. aquifolium* extracts in diabetic rats may provide valuable information on the hypoglycemic effects of the algal extract.

Antibacterial Activity

Brown seaweeds possess diverse compounds such as

alkaloids, polyphenols, terpenes, and carotenoids that possess biological activities with direct relevance to the treatment of diseases caused by bacterial infections (Arguelles *et al.* 2019). The antibacterial activity of brown seaweed *Sargassum aquifolium* was done *in vitro* using microtiter plate dilution assay against ten medically important bacterial pathogens. *Sargassum aquifolium* exhibited potent antibacterial activity against penicillin acylase-producing *Bacillus cereus* with MIC and MBC values of 125 and 250 µg/mL, respectively. This result is more effective than those observed by Ghania *et al.* (2019) and Ambreen *et al.* (2012) from algal extract of *Sargassum vulgare* (MIC = 3.75 mg/mL), *Cladostephus hirsutus* (MIC = 1.87 mg/mL), *Rissoella verruculosa* (MIC = 7.50 mg/mL), *Caulerpa racemosa* (MIC = 0.3 mg/mL), *Caulerpa sertularioides* (MIC = 0.20 mg/mL), and *Kappaphycus alvarezii* (MIC = 1.20 mg/mL). In addition, the *S. aquifolium* extract was able to inhibit the growth of MRSA*, Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* – each with MIC and MBC values of 250 and 500 µg/mL, respectively (Table 10). The antibacterial activity observed against *S. aureus* is more potent than that observed by Kosanić *et al.* (2019) with acetone extracts of *Dictyota dichotoma* (MIC = 1.25 mg/mL), *Padina pavonica* (MIC = 1.25 mg/mL), and *Sargassum vulgare* (MIC = 2.50 mg/mL) from the Adriatic Sea. On the other hand, Al-Zahrani *et al.* (2014) reported greater antibacterial activity of *Ulva* sp. extract against *S. epidermidis* (MIC = 4.0 µg/mL) and *S. saprophyticus* (MIC = 16.0 µg/mL). *Sargassum aquifolium* extract also exhibited antibacterial activity against *Pseudomonas aeruginosa* showing MIC and MBC values of 500 and 1000 µg/mL, respectively. A similar observation was also reported by Chong *et al.* (2011), wherein marine seaweeds such as *Padina australis* (MIC = 0.26 mg/mL) and *Sargassum polycystum* (MIC = 0.73 mg/mL) showed antibacterial activity against the pathogen. No antibacterial activity was observed from *S. aquifolium* extract against *Aeromonas hydrophila*, *Listeria monocytogenes*, *Serratia marcescens*, *Pseudomonas fluorescens*, and penicillin acylase-producing *Escherichia coli*. In general, *S. aquifolium* extract is more potent against Gram-positive bacteria than that of Gram-negative bacteria. The findings of this study are similar to those observed by Arguelles (2021, 2020a) and Cox *et al.* (2010), wherein Gram-negative bacteria are less susceptible to seaweed extracts as compared to Gram-positive bacteria. This is possible since Gram-negative bacteria are known to have a multilayered structure of cell wall that serves as an additional barrier of protection against active compounds that will enter the bacterial cell (Cox *et al.* 2010; Arguelles 2020b).

The Philippine coast provides suitable conditions for the growth and proliferation of diverse species of seaweeds that contain lead bioactive substances with promising use in the pharmaceutical industry. To date, several species of seaweeds (*Sargassum ilicifolium*, *Sargassum vulgare*, *Sargassum*, *Sargassum siliquosum*, *Codium intricatum*, *Turbinaria ornata*, and *Turbinaria decurrens*) have been reported in the country to have

Table 10. Antibacterial activities of *Sargassum aquifolium* extract.

Bacterial pathogen	MIC (µg/mL)	MBC (µg/mL)
Gram-positive bacteria		
Penicillin acylase-producing *Bacillus cereus* BIOTECH 1509	125.00	250.00
Methicillin-resistant *Staphylococcus aureus* BIOTECH 10378	250.00	500.00
Staphylococcus epidermidis BIOTECH 10098	250.00	500.00
Staphylococcus saprophyticus BIOTECH 1802	250.00	500.00
Listeria monocytogenes BIOTECH 1958	> 1000.00	ND
Gram-negative bacteria		
Pseudomonas aeruginosa BIOTECH 1824	500.00	1000.00
Pseudomonas fluorescens BIOTECH 1123	> 1000.00	ND
Penicillin acylase-producing *Escherichia coli* BIOTECH 1634	> 1000.00	ND
Serratia marcescens BIOTECH 1748	> 1000.00	ND
Aeromonas hydrophila BIOTECH 10089	> 1000.00	ND

*ND – none detected

antibacterial activities against a number of medically important bacterial pathogens (Arguelles *et al.* 2019; Arguelles and Sapin 2020a, b, c; Arguelles 2020, 2021b). This study is the first report in the Philippines regarding antibacterial activities of *Sargassum aquifolium* against *Staphylococcus saprophyticus*, penicillin acylase-producing *Bacillus cereus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and MRSA. This finding shows that Philippine seaweeds contain bioactive substances that demand further studies to know the structure and mode of action of the active compounds in the algal extract.

CONCLUSION

Sargassum aquifolium contains a high concentration of polyphenols and exhibited potent antioxidant, antibacterial, α-glucosidase, α-amylase, elastase, and tyrosinase inhibition activities with direct and relevant application to disease treatment. The results of this study show the high-value bioproducts that can be obtained from *S. aquifolium*. Thus, it is recommended to further work on the purification, characterization, and structure elucidation of bioactive substances present in the seaweed extract to understand the mechanisms involved in their bioactivities. Also, additional studies that will focus on the potential of *S. aquifolium* extract to other biological properties such as quorum sensing inhibition, anti-cancer, antifungal, and UV screen protection properties are recommended in future studies to further expand our knowledge regarding this seaweed. The findings of this study are useful in improving the proper utilization of Philippine seaweeds (such as *Sargassum aquifolium*) as an alternative source of bioactive substances crucial for the development of drugs for use in the pharmaceutical and cosmetic industries.

ACKNOWLEDGMENTS

The authors acknowledge the technical and financial support of the PNCM and the Food Laboratory of BIOTECH-UPLB. Also, the technical assistance of Mr. Emille Joseph A. Iranista, as well as the constructive comments given by the external reviewers for the refinement of the manuscript, is acknowledged with sincere gratitude.

REFERENCES

[AOAC] Association of Official Analytical Chemists. 2011. Official Methods of Analysis, 18th ed. Gaithersburg, MD. 2590p.

ALPINAR K, ÖZYUREK M, KOLAK U, GUCLU K, ARAS Ç, ALTUN M, CELIK SE, BERKER KI, BEKTASOGLU B, AMPAL R. 2009. Antioxidant capacities of some food plants wildly grown in Ayvalik of Turkey. Food Sci Technol Res 15 (1): 59–64.

AL-ZAHRANI A, AL-HAJ N, OMER H, AL-JUDAIBI A. 2014. Impact of extracts of marine macroalgae on multidrug-resistant bacteria. J Microbiol Res 4(6A): 18–24.

AMBREEN A, HIRA K, RUQQIA A, SULTANA V. 2012. Evaluation of biochemical component and antimicrobial activity of some seaweeds occurring at Karachi coast. Pak J Bot 44(5): 1799–1803.

ARGUELLES EDLR. 2021a. 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. Curr Appl Sci Technol 21(1): 65–77.

ARGUELLES EDLR. 2021b. Evaluation of antioxidant capacity, tyrosinase inhibition, and antibacterial activities of brown seaweed, *Sargassum ilicifolium* (Turner) C. Agardh 1820 for cosmeceutical application. J Fisher Environ 45(1): 64–77.

ARGUELLES EDLR, MARTINEZ-GOSS MR. 2021. Lipid accumulation and profiling in microalgae *Chlorolobion* sp. (BIOTECH 4031) and *Chlorella* sp. (BIOTECH 4026) during nitrogen starvation for biodiesel production. J Appl Phycol. 33: 1–11.

ARGUELLES EDLR. 2020. Evaluation of nutritional composition and *in vitro* antioxidant and antibacterial activities of *Codium intricatum* Okamura from Ilocos Norte (Philippines). Jordan J Biol Sci 13(3): 375–382.

ARGUELLES EDLR, SAPIN AB. 2020a. *In vitro* antioxidant, alpha-glucosidase inhibition, and antibacterial properties of *Turbinaria decurrens* Bory (Sargassaceae, Ochrophyta). Asia-Pacific J Sci Technol 25(3).

ARGUELLES EDLR, SAPIN AB. 2020b. Bioactive properties of *Sargassum siliquosum* J. Agardh (Fucales, Phaeophyta) and its potential as source of skin-lightening active ingredient for cosmetic application. J Appl Pharm Sci 10(7): 51–58.

ARGUELLES EDLR, SAPIN AB. 2020c. Bioprospecting of *Turbinaria ornata* (Fucales, Phaeophyceae) for cosmetic application: antioxidant, tyrosinase inhibition and antibacterial activities. J ISSAAS 26(2): 30–41.

ARGUELLES EDLR, MONSALUD RG, SAPIN AB. 2019. Chemical composition and *in vitro* antioxidant and antibacterial activities of *Sargassum vulgare* C.

Agardh from Lobo, Batangas, Philippines. J ISSAAS 25(1): 112–122.

ARGUELLES EDLR. 2018. Proximate analysis, antibacterial activity, total phenolic content and antioxidant capacity of a green microalga *Scenedesmus quadricauda* (Turpin) Brébisson. Asian J Microbiol Biotechnol Environ Sci 20(1): 150–158.

ARGUELLES EDLR, LAURENA AC, MONSALUD RG, MARTINEZ-GOSS MR. 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. J Appl Phycol 30: 411–419.

ARGUELLES EDLR, LAURENA AC, MARTINEZ-GOSS MR, MONSALUD RG. 2017. Antibacterial activity, total phenolic content, and antioxidant capacity of a green microalga *Desmodesmus* sp. (U-AU2) from Los Baños, Laguna (Philippines). J Nat Stud 16(2): 1–13.

BAEK SH, CAO L, JEONG SJ, KIM H-R, NAM TK, LEE SG. 2021. The comparison of total phenolics, total antioxidant, and anti-tyrosinase activities of Korean *Sargassum* species. J Food Qual (Article ID 6640789). https://doi.org/10.1155/2021/6640789

BOONCHUM W, PEERAPORNPISAL Y, KANJANAPOTHI D, PEKKOH J, PUMAS C, JAMJAI U, AMORNLERDPISON D, NOIRAKSAR T, VACHARAPIYASOPHON P. 2011. Antioxidant activity of some seaweed from the Gulf of Thailand. Int J Agric Biol 13: 95–99.

CANOY JL, BITACURA JG. 2018. Cytotoxicity and antiangiogenic activity of *Turbinaria ornata* Agardh and *Padina australis* Hauck ethanolic extracts. Anal Cell Pathol (Amst). p. 1–8. https://doi.org/10.1155/2018/3709491

CHAKRABORTY K, PRAVEEN NK, VIJAYAN KK, RAO GS. 2013. Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to *Turbinaria* spp. (Phaeophyta, Sargassaceae) collected from Gulf of Mannar. Asian Pac J Trop Biomed 3: 8–16.

CHANG VS, TEO SS. 2016. Evaluation of heavy metal, antioxidant and anti-tyrosinase activities of red seaweed (*Eucheuma cottonii*). Int Food Res J 23(6): 2370–2373.

CHONG CW, HII SL, WONG CL. 2011. Antibacterial activity of *Sargassum polycystum* C. Agardh and *Padina australis* Hauck (Phaeophyceae). Afr J Biotechnol 10: 14125–14131.

COX S, ABU-GHANNAM N, GUPTA S. 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int Food Res J 17: 205–220.

DANG TT, BOWYER MC, VAN ALTENA IA, SCARLETT CJ. 2018. Comparison of chemical profile and antioxidant properties of the brown algae. Int J Food Sci Tech 53: 174–181.

EL-MANAWY IM, NASSAR MZ, FAHMY NM, RASHEDY SH. 2019. Evaluation of proximate composition, antioxidant, and antimicrobial activities of some seaweeds from the Red Sea coast, Egypt. Egypt J Aquat Biol Fish 23(1): 317–329.

FELLAH F, LOUAILECHE H, DEHBI-ZEBBOUDJ A, TOUATI N. 2017. Seasonal variations in the phenolic compound content and antioxidant activities of three selected species of seaweeds from Tiskerth islet, Bejaia, Algeria. J Mater Environ Sci 8: 4451–4456.

FU CWF, HO CW, YONG WTL, ABAS F, TAN CP. 2015. Effects of phenolic antioxidants extraction from four selected seaweeds obtained from Sabah. PeerJ PrePrints 3: e1249v1.

GABBIA D; DALL'ACQUA S, DE MARTIN S, DI GANGI IM, BOGIALLI S, CAPUTI V, ALBERTONI L., MARSILIO I, PACCAGNELLA N, CARRARA M, GIRON MC, DE MARTIN S. 2017. The Phytocomplex from *Fucus vesiculosus* and *Ascophyllum nodosum* controls postprandial plasma glucose levels: an *in vitro* and *in vivo* study in a mouse model of NASH. Mar Drugs 15(2): 41.

GAO L, WANG S, OOMAH BD, MAZZA G. 2002. Wheat quality: antioxidant activity of wheat millstreams. In: Wheat Quality Elucidation. Ng P, Wrigley CW eds. St. Paul, MN: AACC International. p. 219–233.

GHANIA A, NABILA BB, LARBI B, ELISABETH M, PHILIPPE G, MARIEM B, KHADIDJA KK, WACILA BR, FAWZIA AB. 2019. Antimicrobial and antiparasitic activities of three algae from the northwest coast of Algeria. Nat Prod Res 33(5): 742–745.

GUNATHILAKA TL, SAMARAKOON KW, RANASINGHE P, PEIRIS LDC. 2019. *In vitro* antioxidant, hypoglycemic activity, and identification of bioactive compounds in phenol-rich extract from the marine red algae *Gracilaria edulis* (Gmelin) Silva. Molecules 24(20): 3708. doi:10.3390/molecules24203708.

HAPSARI R, ELYA B, AMIN J. 2012. Formulation and evaluation of antioxidant and tyrosinase inhibitory effect from gel containing the 70% ethanolic *Pleurotus*

ostreatus extract. Int J Med Aromat Plants 2(1): 135–140.

KIM K-T, RIOUX L-E, TURGEON SL. 2014. Alpha-amylase and alpha-glucosidase inhibition is differentially modulated by fucoidan obtained from *Fucus vesiculosus* and *Ascophyllum nodosum*. Phytochemistry 98: 27–33.

KOSANIĆ M, RANKOVIĆ B, STANOJKOVIĆ T. 2019. Brown macroalgae from the Adriatic Sea as a promising source of bioactive nutrients. J Food Meas Charact 13(1): 330–338.

KÖKSAL E, TOHMA H, KILIÇ Ö, ALAN Y, ARAS A, GÜLÇIN I, BURSAL E. 2017. Assessment of antimicrobial and antioxidant activities of *Nepeta trachonitica*: analysis of its phenolic compounds using HPLC-MS/MS. Sci Pharm 85(24): 1–14. doi:10.3390/scipharm85020024

LEE KW, HEO SH, LEE J, PARK SI, KIM M, SHIN MS. 2020. Antimicrobial, antioxidative, elastase and tyrosinase inhibitory effect of supercritical and hydrothermal *Asparagopsis armata* extract. Int J Adv Cult Technol 8(3): 231–240.

MATSUKAWA R, DUBINSKY Z, KISHIMOTO E, MASAK K, MASUDA Y, TAKEUCHI T, CHIHARA M, YAMAMOTO Y, NIKI E, KARUBE I. 1997. A comparison of screening methods for antioxidant activity in seaweeds. J Appl Phycol 9: 29–35.

MAGDUGO RP, TERME N, LANG M, PLIEGO-CORTÉS H, MARTY C, HURTADO AQ, BEDOUX G, BOURGOUGNON N. 2020. An analysis of the nutritional and health values of *Caulerpa racemosa* (Forsskål) and *Ulva lactuca* (Linnaeus) – two Chlorophyta collected from the Philippines. Molecules 25(12): 2901. https://doi.org/10.3390/molecules2512290

MOON JY, YIM EY, SONG G, LEE NH, YUN CG. 2010. Screening of elastase and tyrosinase inhibitory activity from Jeju Island plants. EurAsian J Biosci 4: 41–53.

NAIR SS, KAVREKAR V, MISHRA A. 2013. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. Eur J Exp Biol 3(1): 128–132.

NAMJOOYAN F, FARASAT M, ALISHAHI M, JAHANGIRI A, MOUSAVI H. 2019. The anti-melanogenesis activities of some selected brown macroalgae from northern coasts of the Persian Gulf. Braz Arch Biol Technol 62. http://dx.doi.org/10.1590/1678-4324-2019180198

NUÑEZ-SELLES A, CASTRO HTV, AGUERO JA, GONZALEZ JG, NADDEO F, DE SIMONE F, PASTRELLI 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. J Agric Food Chem 50: 762–766.

PENG Y, XIE E, ZHENG K, FREDIMOSES M, YANG X, ZHOU X, WANG Y, YANG B, LIN X, LIU J, LIU Y. 2012. Nutritional and chemical composition and antiviral activity of cultivated seaweed *Sargassum naozhouense* Tseng et Lu. Mar Drugs 11(1): 20–32. doi: 10.3390/md11010020.

PHASANASOPHON K, KIM SM. 2018. Antioxidant and cosmeceutical activities of *Agarum cribrosum* phlorotannin extracted by ultrasound treatment. Nat Prod Commun 13(5): 565–570.

PHOBOO S. 2015. *In vitro* assays of anti-diabetic and anti-hypertensive potential of some traditional edible plants of Qatar. J Med Active Plants 4: 22–29.

RADHA P. 2018. Proximate analysis and mineral composition of seaweeds of Manamelkudi Coast, Pudukkottai District, India. Int J Curr Microbiol App Sci 7(8): 3121–3128.

RE R, PELLEGRINE N, PROTEGGENTE A, PANNALA A, YANG M, RICE-EVANS C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26: 1231–1237.

REKA P, THAHIRA BANU A, SEETHALAKSHMI M. 2017. Elemental composition of selected edible seaweeds using SEM-energy dispersive spectroscopic analysis. Int Food Res J 24(2): 600–606.

SALEHI B, SHARIFI-RAD J, SECA AML, PINTO DCGA, MICHALAK I, TRINCONE A, MISHRA AP, NIGAM M, ZAM W, MARTINS N. 2019. Current trends on seaweeds: looking at chemical composition, phytopharmacology, and cosmetic applications. Molecules 24: 4182. doi:10.3390/molecules24224182

SHANURA FERNANDO IP, ASANKA SANJEEWA KK, SAMARAKOON KW, KIM H-S, GUNASEKARA UKDSS, PARK Y-J, ABEYTUNGA DTU, LEE WW, JEON Y-J. 2018. The potential of fucoidans from *Chnoospora minima* and *Sargassum polycystum* in cosmetics: antioxidant, anti-inflammatory, skin-whitening, and antiwrinkle activities. J Appl Phycol 30: 3223–3232. https://doi.org/10.1007/s10811-018-1415-4

SIM S-Y, SHIN Y-E, KIM H-K. 2019. Fucoidan from *Undaria pinnatifida* has anti-diabetic effects by stimulation of glucose uptake and reduction of basal lipolysis in 3T3-L1 Adipocytes. Nutr Res 65: 54–62.

SIVARAMAKRISHNAN T, SWAIN S, SARAVANAN

K, KIRUBA SR, ROY SD, BISWAS L, 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 J Fish Aquat Sci 17: 639–648.

SUSANO P, SILVA J, ALVES C, MARTINS A, GASPAR H, PINTEUS S, MOUGA T, GOETTERT MI, PETROVSKI Z, BRANCO LB, PEDROSA R. 2021. Unravelling the dermatological potential of the brown seaweed *Carpomitra costata*. Mar Drugs 19(3): 135. https://doi.org/10.3390/md19030135

SYAD AN, SHUNMUGIAH KP, KASI PD. 2013. Seaweeds as nutritional supplements: Analysis of nutritional profile, physicochemical properties and proximate composition of *G. acerosa* and *S. wightii*. Biomed Prev Nutr 3: 139–144.

TRONO GC. 1997. Field guide and atlas of the seaweed resources of the Philippines. Manila: Bookmark Inc. 303p.

UNNIKRISHNAN P, SUTHINDHIRAN K, JAYASRI MA. 2015. Antidiabetic potential of marine algae by inhibiting key metabolic enzymes. Front Life Sci 8: 148–159.

UNNIKRISHNAN PS, SUTHINDHIRAN K, JAYASRI MA. 2014. Inhibitory potential of *Turbinaria ornata* against key metabolic enzymes linked to diabetes. BioMed Res Int 1–10.

YANGTHONG M. 2017. Chemical and mineral compositions of *Sargassum* spp. from Bo Mao Beach, Chumphon Province, Thailand. J Fisher Environ 41(3): 1–5.

ZARATE R, PORTILLO E, TEIXIDÓ S, PINHEIRO DE CALVO MAA, NUNES N, FERRAZ S, SECA AML, ROSA GP, BARRETO MC. 2020. Pharmacological and cosmeceutical potential of seaweed beach-casts of Macaronesia. Appl Sci 10: 5831. doi:10.3390/app10175831