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Bioactive properties of *Sargassum siliquosum* J. Agardh (Fucales, Ochrophyta) and its potential as source of skin-lightening active ingredient for cosmetic application

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ABSTRACT

Seaweeds are notable in producing diverse kinds of polyphenolic compounds with direct relevance to cosmetic application. This investigation was done to assess the bioactive properties of a brown macroalga, *Sargassum siliquosum* J. Agardh. The alga has a total phenolic content of 30.34 ± 0.00 mg gallic acid equivalents (GAE) g^{-1}. Relative antioxidant efficiency showed that *S. siliquosum* exerted a potent diphenyl-1, 2-picrylhydrazyl scavenging activity and high ability of reducing copper ions in a dose-dependent manner with an IC_{50} value of 0.19 mg GAE ml^{-1} and 18.50 µg GAE ml^{-1}, respectively. Evaluation of antibacterial activities using microtiter plate dilution assay revealed that *S. siliquosum* showed a strong activity against bacterial skin pathogen, *Staphylococcus aureus* (minimum inhibitory concentration (MIC) = 125 µg ml^{-1} and minimum bactericidal concentration (MBC) = 250 µg ml^{-1}) and *Staphylococcus epidermidis* (MIC = 250 µg ml^{-1} and MBC = 500 µg ml^{-1}). *In vitro* study of tyrosinase inhibition showed that *S. siliquosum* has a greater inhibitory activity (IC_{50} of 65.0 µg GAE ml^{-1}) as compared to that of the known skin-lightening ingredient, kojic acid with an IC_{50} of 109.32 µg GAE ml^{-1}. This study is useful for improved utilization of *S. siliquosum* extract as a source of polyphenolic compounds important for the development of novel skin-lightening ingredient for the formulation of cosmetic products.

INTRODUCTION

Skin aging is a common and gradual degenerating physiological and biochemical process that affects the skin in several ways. During aging, skin becomes flimsy, thinner and gradually decreases its ability to maintain hydration and natural elasticity resulting in the formation of wrinkles (Pimentel *et al*., 2018; Wang *et al*., 2015). Biochemical processes such as enzymatic degradation (metalloproteinases) of elastin and collagen occur in the epidermal and dermal layers of the skin causing degeneration of the extracellular matrix. Besides, external factors such as ultraviolet radiation exposure results in the activation of tyrosinase and collagenases, resulting in melanin production, aging of skin, and formation of wrinkle (Wu *et al*., 2018). Thus, explorations on the use of naturally occurring source (such as seaweeds) of active ingredients in the formulation of personal care products that have beneficial effects in preventing skin aging are sought after in the pharmaceutical industry.

The value of the worldwide seaweed market in 2017 is about USD 11.48 billion and is estimated to attain USD 21.75 billion in 2025 with the Asia Pacific region to hold the largest global market share (Commercial Seaweed Market Size, 2019). The projected market growth of seaweed is at a rapid pace because of the health benefits associated with seaweed products, which motivates consumers to utilize these important resources. As reported by the Food and Agriculture Organization, a total of 23.8 million tons of seaweeds ($6.4 billion) are being harvested and

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utilized yearly (Pimentel *et al*., 2018). The leading countries for seaweed production are China and Indonesia generating 54% and 27%, respectively, of the total bulk production. Other Asia Pacific countries such as the Philippines, Korea, Japan, and Malaysia followed the leading countries with a total seaweed production share of 7.4%, 4.3%, 1.85%, and 1.39%, respectively (Pimentel *et al*., 2018). Conventionally, seaweeds are being utilized as food, ingredient for animal feeds, for medicinal purposes, or an alternative source of fertilizer. However, recent scientific evidence proved that these valuable resources could also be used for cosmetic application (Pimentel *et al*., 2018). The global need for cosmetic products is constantly increasing since there is a huge demand by millions of consumers for cosmetics and their active ingredients. In fact, the global cosmetic market is believed to increase to USD 429.8 billion by 2022 displaying a compound annual growth rate of 4.3% for the duration of 2016–2022 (Cosmetics Market by Category, 2019). Nowadays, the cosmetic industry is developing innovative personal care products that satisfy the needs and expectations of consumers by finding cheap, sustainable, and natural raw materials possessing diverse bioactive compounds as a source of active ingredients (Wu *et al*., 2018). Seaweeds are an excellent naturally occurring resource that can satisfy these prerequisites. These organisms are important marine resources that are known to possess biologically active substances such as lipids, polysaccharides, proteins, pigments, and polyphenol showing significant antioxidative, antibacterial, antitumor, tyrosinase inhibition, antiviral, and skin-lightening activities (Bourguiba *et al*., 2017). The integration of seaweed-derived constituents in the formulation of cosmetics has long been studied as several research findings on their potential beneficial attributes in preventing skin aging have been reported. Dolorosa *et al*. (2019) showed that the methanolic extract of *Eucheuma cottonii* and *Sargassum plagyophyllum* has a potent tyrosinase inhibition activity with an IC_{50} of 2,631.648 and 1,769.336 µg ml^{-1}, respectively. Furthermore, biologically active substances with potential use in cosmetics such as terpenoids, saponins, steroids, alkaloids, flavonoids, and tannins were also observed from the extracts. Besides, Jiménez *et al*. (2010) reported the antioxidant, tyrosinase inhibition, and antibacterial (against *Micrococcus luteus* and *Staphylococcus aureus*) activities of brown seaweed *Ascophyllum nodosum*. The acetone extract of the macroalga was able to display an IC_{50} value of 0.1 mg ml^{-1} with 65.6% inhibition of tyrosinase. These findings give highlights on the possible use of several seaweed species as a source of substances with bioactive properties that can be used in the formulation of cosmetic products.

The Philippine coast has suitable environmental conditions and several coastal water with a diverse number of marine seaweeds that may have lead bioactive compounds with potential use in the cosmetic industry yet to be explored. Relatively, few studies are known about the anti-melanogenesis, antioxidant, and antibacterial potential of Philippine seaweeds, and to date, the scientific investigations of its biological activities for the formulation of cosmetics are still limited in the country (Arguelles *et al*., 2019). Previously, Corpuz *et al*. (2013) studied the beneficial effects of *Sargassum siliquosum* extract as a potential source of naturally occurring antioxidants. The results suggest that the seaweed extract has bioactive substances that can act as a free radical scavenger with antioxidant and anticancer properties. This study is the first report in the Philippines exploring the biological activities of a brown macroalga, *S. siliquosum* J. Agardh, with potential use for cosmetic application. The current study sought to do screening for antibacterial, tyrosinase inhibition, and antioxidative activities as well as know the total phenolic content of *S. siliquosum* (making use of gallic acid as a reference standard). Furthermore, the correlation among phenolic content and antioxidant activity of the seaweed was analyzed.

MATERIALS AND METHODS

Collection of seaweed

The marine brown macroalga *S. siliquosum* J. Agardh was freshly collected on 24 November 2019 from Catanauan (Lat. 13° 36′ 20.88″ N; Long. 122° 14″ 18.24′ E), Quezon, Philippines. The seaweed was identified based on morphotaxonomic features according to Trono (1992) and Algae Base (web site: www.algaebase.org) (Guiry and Guiry, 2017). The collected macroalga was immediately washed with seawater using soft brush bristles to remove sand particles, animal castings, and attached detritus. The necrotic parts of the seaweed were also removed. The cleaned seaweed was immediately transferred to the laboratory using sterilized polythene bags. The seaweed sample was then washed several times with sterile tap water to take away excess salt on the external portion of the alga. The algal sample was then placed on a clean tissue paper to wipe off the water residues. The sample was air-dried (for 6 days), cut into small portions, and pulverized into a fine-grained powder before extraction and/or isolation of bioactive components.

Seaweed extract preparation

The powdered algal biomass (1 g) was soaked in 30 ml of acidified methanol (1 HCl:80 CH_3OH:10 H_2O), extracted for 30 minutes using an ultrasonic bath, and stirred for 1 hour. The seaweed extract was centrifuged at a speed of 12,000 rpm at 20°C for 20 minutes. The collected seaweed extract was dried out by means of a rotary evaporator subjected to reduced pressure (at 40°C) until a concentrated crude macroalgal extract was gathered. The extract was kept at 4°C to maintain its activity before using it in a different assay (Arguelles *et al*., 2019).

Determination of total phenolic content

The phenolic content of *S. siliquosum* was estimated using the Folin–Ciocalteu reagent as given by Nuñez Selles *et al*. (2002). A portion (0.5 ml) of the diluted seaweed extract was thoroughly mixed with an equal volume of Folin– Ciocalteu reagent and 10% sodium carbonate solution for 1 minute. The mixture was then set aside for 5 minutes, and its volume was adjusted by adding 5 ml of sterile distilled water. The optical density (OD) reading of the reaction mixture was taken at 720 nm using an Ultraviolet-Visible spectrophotometer. A calibration curve was constructed using gallic acid with a prepared range of concentrations from 20 to 100 µg ml^{-1}. The total phenolic concentration (TPC) of the seaweed extract was presented as mg gallic acid equivalents (GAE) g^{-1} of the macroalgal samples.

Diphenyl-1, 2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH scavenging assay was performed based on the methods of Ribeiro *et al*. (2008) with few alterations. A portion

(100 µl) of the algal extract was thoroughly mixed with 5 mM of DPPH solution. The solution was set aside at room temperature for 20 minutes, and the OD for each solution was noted at 517 nm by means of a spectrophotometer. Ascorbic acid (prepared in different concentrations: 0.08, 0.16, 0.24, 0.32, and 0.40 mg ml^{-1}) was used as the positive control in this study. The percentage inhibition of DPPH was estimated using the following equation:

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

where $A_{control}$ is the OD reading of the control, and A_{sample} is the OD reading of the algal sample. IC_{50} was calculated to denote the concentration (expressed in mg GAE ml^{-1}) of the macroalgal extract that can exhibit a 50% scavenging of the DPPH radical.

Copper reduction antioxidant capacity (CUPRAC) assay

The CUPRAC assay was done following the method described by Alpinar *et al*. (2009). Briefly, 1 ml each of 0.01 M $CuCl_2$, 0.0075 M neocuproine, and 1 M ammonium acetate buffer (pH 7) solutions were thoroughly mixed in a test tube. Subsequently, 0.5 ml of the prepared seaweed extract at varying concentrations (5, 10, 15, 20, and 25 µg GAE ml^{-1}) as well as ascorbic acid standard solutions was combined in the initial mixture. The total volume for each concentration was adjusted up to 4.1 ml by adding sterile distilled water. The mixtures were set aside at ambient temperature for 30 minutes, and the OD of each tested sample was measured at 450 nm.

Tests microorganisms

Type cultures of three pathogenic Gram-negative bacteria (*Pseudomonas aeruginosa* BIOTECH 1824, *Enterobacter aerogenes* BIOTECH 1145 and *Escherichia coli* BIOTECH 1825) and two Gram-positive bacteria (*Staphylococcus epidermidis* BIOTECH 10098 and *S. aureus* BIOTECH 1823) were acquired from the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB). The type cultures of pathogenic organisms were precultured using Luria–Bertani medium with shaking overnight at 37°C. The morphological and biochemical tests were checked continuously to ensure purity (Arguelles, 2018).

Microdilution antibacterial assay

The minimum inhibitory concentration (MIC) of the macroalgal extract was analyzed by using a two-fold serial dilution technique according to the method done by Arguelles (2018), with few modifications. Using a 96-well microtiter plate, 100 µl of pathogenic bacterial cultures (cell density of 1×10^5 cells ml^{-1}) were placed to a 100 µl of seaweed extract prepared in varying dilutions (1,000–7.8125 µg ml^{-1}). The acidified methanol was also included as the negative control. The experimental plate was stored at 35°C in an incubator for 24 hours, after which MICs were noted. MIC is the minimum concentration of the test seaweed extract that can cause the inhibition of bacterial growth after an incubation time of 12 hours. On the other hand, MBC was analyzed by plating a loopful of sample from each MIC well that showed the inhibition of growth into newly prepared culture (tryptic soy agar) medium (Arguelles, 2018). The culture 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 bacterial growth would mean that the seaweed extract was bactericidal at that particular dilution. MBC value is the minimum extract concentration, at which no observable bacterial growth on agar subculture was noted (Arguelles *et al*., 2019).

Tyrosinase inhibition assay

The tyrosinase inhibitory activity of *S. siliquosum* extract was assayed in *in vitro* conditions by using a microplate reader following the procedures described by Hapsari *et al*., (2012) with few alterations. The solutions of 5 mM DOPA (3,4-dihydroxy-L-phenylalanine, Sigma D-9628), 0.1 M potassium phosphate buffer, pH 6.5, and mushroom tyrosinase (250 units ml^{-1}, Sigma T-3824) were prepared. To 40 µl of DOPA (3,4-dihydroxy-L-phenylalanine), 40 µl of seaweed extract and 40 µl of buffer (in the case of the control) were mixed in a microtiter well plate. Phosphate buffer was added to the solution to come up with a total volume of 160, and finally, 40 µl of tyrosinase was added. The blank used was the OD without the enzyme solution. After 15 minutes, the OD at 490 nm was noted using a microplate reader. Tyrosinase inhibition activity was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(Ac) - (As - Ab)}{(Ac)} \times 100$$

where A_c is the OD of the control, A_b is the OD of the blank, and A_s is the OD of the sample.

Statistical analyses

Each experiment run was done at three experimental replicates and the results are shown as means ± standard deviations (mean ± SD). The statistical tests for the linear correlation coefficient needed for correlation analysis were analyzed using MS Office Excel 2007.

RESULTS AND DISCUSSION

Total phenolic content (TPC)

The TPC detected in the seaweed extract was analyzed employing the Folin–Ciocalteu method and was presented in GAE (calibration curve equation: $y = 0.0682x - 0.0214$, $R^2 = 0.997$). The result indicated that the TPC in the analyzed *S. siliquosum* extract is 30.34 ± 0.00 mg GAE g^{-1}. The amount of phenolic substances obtained in this study is greater than that observed by Kim *et al*. (2005) from Korean seaweeds such as *Ecklonia cava, Ishige okamurae,* and *Sargassum siliquastrum* with TPC of 2.27, 0.63, and 0.29 mg GAE g^{-1}, respectively. Similarly, Arguelles *et al*. (2019) reported a lower total phenolic content (10.13 ± 0.166 mg GAE g^{-1}) for *Sargassum vulgare* from Lobo, Batangas. The total phenolic content of a sample is highly reliant on the polarity of extractant (solvent) utilized during the process of extraction. Normally, polyphenols have high solubility in polar solvents (acidified methanol), which results in recovering a high concentration of these desired compounds in the sample extracts (Arguelles *et al*., 2019).

Antioxidant activity

A versatile antioxidant system is important in search for a seaweed-based formulation of cosmetic products. When studying the potential antioxidant activity of seaweeds, the use of

only one assay is inadequate for determining some of the involved mechanisms of antioxidant activity. Therefore, two methods of antioxidant assays were used to verify the antioxidative potential of the seaweed extract used in this investigation. The antioxidant activity of *S. siliquosum* extract was evaluated using CUPRAC assay and DPPH radical scavenging activity assay.

DPPH radical scavenging activity

DPPH radical scavenging assay uses DPPH to check the ability of the antioxidative compounds (algal extract) acting as radical scavengers of proton. Substances possessing antioxidative activity will bring about the change of color from purple chromogen radical to the pale yellow hydrazine (Arguelles *et al*., 2017; Goh *et al*., 2010). This method has been used considerably as an initial method for screening of novel antioxidants because of its simplicity, reproducibility, and stability (Chen *et al*., 2008; Jiménez *et al*., 2010). *Sargassum siliquosum* exerted a potent radical scavenging activity against DPPH free radical showing inhibition percentage that is dose dependent (Table 1 and Fig. 1). The activity of the seaweed extract to scavenge DPPH increases when the tested algal extract concentration is also increased. Furthermore, *S. siliquosum* extract exhibited greater antioxidant activity than the positive control (ascorbic acid) with an IC_{50} of 0.19 mg GAE ml^{-1} and 0.23 mg GAE ml^{-1}, respectively.

The coefficient of correlation (R^2) among phenolic concentration and antioxidant activity of *S. siliquosum* using DPPH scavenging assay is shown in Figure 1. It is evident from this result that a good positive correlation ($R^2 = 0.93208$) exists between TPC and free radical scavenging activity for the acidified methanolic extract of *S. siliquosum*. This proves that the antioxidant properties of the seaweed extract are enhanced by phenolic compound present in the sample. The result of this investigation agrees with the earlier studies done by Arguelles *et al*. (2019) and Jiménez *et al*., (2010) on antioxidant properties (using DPPH radical scavenging assay) of brown seaweeds, *A. nodosum* and *S. vulgare*, where a direct relationship between phenolic concentration and antioxidant activity occurs. Also, IC_{50} (0.19 mg GAE ml^{-1}) obtained by *S. siliquosum* extract exhibited a greater antioxidant activity compared to *A. nodosum* (IC_{50} = 1.55 mg ml^{-1}) and *S. vulgare* (IC_{50} = 37.2 mg GAE ml^{-1}).

Copper reduction antioxidant capacity assay

The CUPRAC assay is a spectrophotometric method of determining the antioxidant activity of an extract by evaluating the capacity of the sample extract to reduce Cu (II) to Cu (I). Reducing power of varying concentration of *S. siliquosum* extract and ascorbic acid is shown in Table 2. *Sargassum siliquosum* extract showed a concentration-dependent ability of copper ion

Table 1. DPPH radical scavenging activity of phenolics from *S. siliquosum* extract.

Test sample	Phenolic concentration (mg GAE ml^{-1})					IC_{50}*
	0.1	0.2	0.3	0.4	0.5	
	DPPH inhibition (%)					
S. siliquosum	31.60 ± 0.99	51.51 ± 0.11	63.21 ± 1.20	71.03 ± 1.53	75.91 ± 0.11	0.19 mg GAE ml^{-1}
	Concentration (mg ml^{-1})					
	0.08	0.16	0.24	0.32	0.40	
	DPPH inhibition (%)					
Ascorbic acid	17.65 ± 0.05	34.21 ± 0.44	51.32 ± 0.05	68.58 ± 0.00	83.86 ± 0.00	0.23 mg ml^{-1}

*IC_{50} – is the effective concentration that can scavenge or inhibit DPPH free radical by 50%.

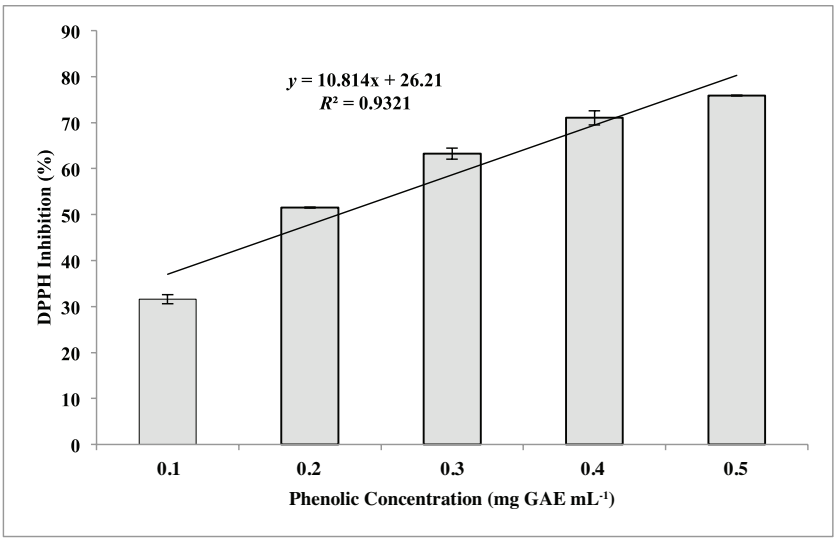

Figure 1. Simple regression correlation between phenolic content and antioxidant activity via DPPH radical scavenging assay of *S. siliquosum* extract.

reduction. In this method, a higher absorbance reading denotes a greater antioxidant capacity. The highest reducing activity was noted at 25 µg GAE ml^{-1} of prepared phenolic concentration as compared to other lower concentration. The trend observed in this assay is similar to those obtained from the DPPH assay, in which 0.5 mg GAE ml^{-1} exhibited the strongest antioxidant activity. The study observed that there is an increase in the antioxidant activity when the seaweed extract concentration was also increased. Furthermore, the investigation reveals that *S. siliquosum* extract contains bioactive metabolites of good antioxidant potential attributed from its copper reducing ability. It was reported that brown seaweeds possess bioactive compounds (mainly fucoxanthin and phlorotannins) with reduction potential such as antioxidative properties (Chakraborty *et al*., 2013). These compounds when present in high concentration exhibit a strong antioxidant property better than the commonly used antioxidant (carotenes, ascorbic acid, and α-tocopherol) in the food and pharmaceutical industry.

The positive correlation (R^2) between phenolic concentration and antioxidant activity of *S. siliquosum* using CUPRAC assay (R^2 = 1.00) shows that phenolic substances serve a vital part in the antioxidant capacity of the macroalga (Fig. 2). Besides, *S. siliquosum* extract exhibited a greater antioxidant activity than the positive control (ascorbic acid) with an IC_{50} value of 18.50 and 46.30 µg GAE mg ml^{-1}, respectively. The potent antioxidant activity of *S. siliquosum* extract against ascorbic acid proves its potential as a natural source of active ingredients used in the development of cosmetic products.

Tyrosinase inhibition assay

The tyrosinase inhibitory effect of *S. siliquosum* extract was determined spectrometrically using mushroom tyrosinase (Table 3). The results of the assay demonstrated that *S. siliquosum* extract is considered more potent in inhibiting tyrosinase enzyme with an IC_{50} value of 65 µg GAE ml^{-1} than kojic acid (positive control) with an IC_{50} value of 109.8 µg GAE ml^{-1}. Such result proves that the seaweed extract contains anti-melanogenic substances that are of potential use as skin-lightening active ingredient. Furthermore, comparing the activity of the acidified methanolic extract of *S.*

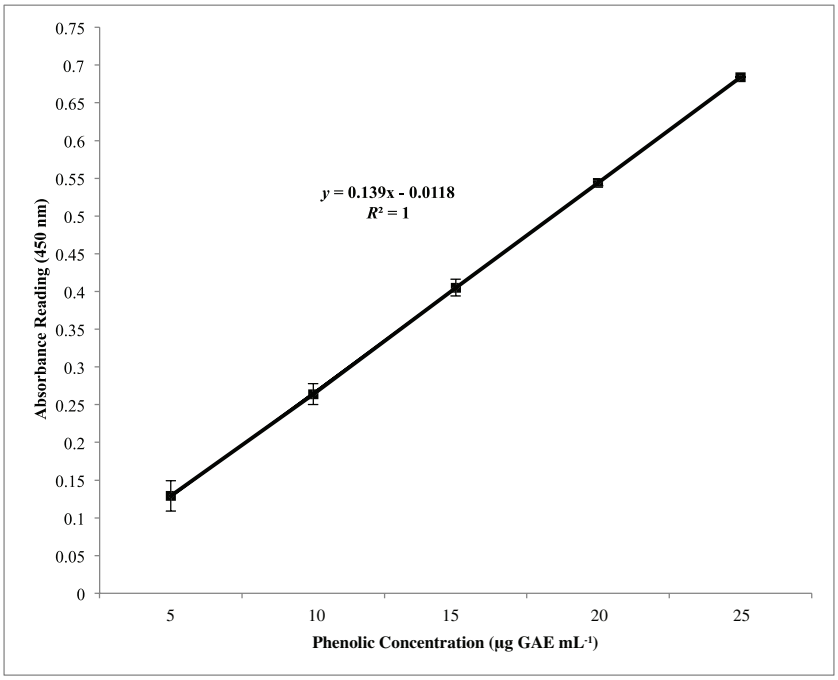

Figure 2. Simple regression correlation between phenolic content and antioxidant activity via CUPRAC assay of *S. siliquosum* extract.

Table 2. Copper reduction antioxidant capacity of phenolics from *S. siliquosum* extract.

Test sample	Concentration (µg GAE ml^{-1})					IC_{50}*
	5.0	10.0	15.0	20.0	25.0	
	CUPRAC (Absorbance at 450 nm)					
S. siliquosum	0.129 ± 0.020	0.264 ± 0.014	0.405 ± 0.011	0.544 ± 0.004	0.684 ± 0.000	18.50 µg GAE ml^{-1}
	Concentration (µg ml^{-1})					
	10.0	20.0	30.0	40.0	50.0	
	CUPRAC (Absorbance at 450 nm)					
Ascorbic acid	0.112 ± 0.002	0.213 ± 0.007	0.328 ± 0.004	0.429± 0.012	0.542 ± 0.011	46.30 µg ml^{-1}

*IC_{50} is the effective concentration that gives CUPRAC value of 0.5 absorbance reading at 450 nm. Computed by interpolation.

siliquosum against other seaweed extracts, such as the brown algae *Ecklonia stolonifera*, *A. nodosum*, and *Turbinaria conoides*, showed a greater tyrosinase inhibition activity with an IC_{50} value of 0.345 mg mL^{-1}, 0.1 mg ml^{-1}, and 188.85 µg ml^{-1}, respectively (Jimenéz *et al*., 2010; Kang *et al*., 2004; Sari *et al*., 2019).

The inhibitory activity exhibited by the algal extract in this assay can be ascribed to the hydroxyl groups present in phenolic substances in *S. siliquosum* extract that can lead to the formation of hydrogen bonds to the active site of the target enzyme (tyrosinase) causing stearic hindrances and changes in the enzyme conformation which results to lower activity. Furthermore, the presence of other known tyrosinase inhibitors such as fucoidan, fucoxanthin, terpenoids, and other polyphenols may also serve a crucial role in the inhibition of tyrosinase using the seaweed extract. These compounds have antiaging effects and are known to have a competitive enzymatic inhibitory effect against tyrosinase. Furthermore, these substances cause the suppression of the polymerization of metabolic intermediates needed for melanin synthesis (Jimenéz *et al*., 2010; Kim *et al*., 2008; Namjooyan *et al*., 2019).

Antibacterial activity

In the Philippines, there are few studies on the use of *Sargassum* for its pharmaceutical properties with great potential for medicinal and cosmetic application, and thus, this area was studied in this investigation. The results of the analysis on the antibacterial properties of the acidified methanolic extract of *S. siliquosum* against common bacterial pathogens are shown in Table 4. Of the five pathogenic bacteria tested, two test organisms were inhibited by the seaweed extract. *Sargassum siliquosum* showed a pronounced activity against common skin pathogen such as *S. aureus* and *S. epidermidis* having MIC of 125 and 250 µg ml^{-1}, respectively. However, no inhibitory activity was observed against *E. aerogenes*, *E. coli,* and *P. aeruginosa*. Minimum bactericidal concentration against *S. aureus* is greater than that of *S. epidermidis*, having 250 and 500 µg ml^{-1}, respectively. These antibacterial activities exhibited by *S. siliquosum* are in agreement with those earlier antimicrobial studies of *Sargassum* (Arguelles *et al*., 2019; Osman *et al*., 2010; Rani *et al*., 2016).

The genus *Sargassum* is known in producing bioactive metabolites such as terpenoids, polyphenols, polysaccharides, and steroids (Arguelles *et al*., 2019). Therefore, *Sargassum* species are regarded as candidate marine organism with good bioactivities that can be used as an alternative raw material of active ingredients for the formulation of skincare products. Besides, this study shows that the algal extract is more potent for its antagonistic activity toward Gram-positive bacteria as compared to that of the Gram-negative bacteria. Such observation can be attributed to the complex cell wall composition of Gram-negative bacteria in contrast to that of the Gram-positive bacterial cells. In general, Gram-negative bacteria are characterized by a multilayered cell wall structure affecting the penetration of active compounds within the bacterial cells giving added protection for the bacteria (Arguelles *et al*., 2019; Amaro *et al*., 2011). Variations in the antibacterial activities between species and strains of *Sargassum* can be attributed to factors such as the kind of solvent used for extraction that can result in differences in the type of recovered bioactive substances and varying methods of

Table 3. Tyrosinase inhibition activity of phenolics from *S. siliquosum* extract.

Test sample	Concentration (µg GAE ml^{-1})					IC_{50}*
	25.0	50.0	75.0	100.0	125.0	
	Tyrosinase inhibition (%)					
S. siliquosum	25.48 ± 0.12	38.94 ± 0.84	57.34 ± 1.25	67.14 ± 0.53	73.33 ± 0.77	65.0 µg GAE ml^{-1}
	Concentration (µg ml^{-1})					
	50.0	100.0	150.0	200.0	250.0	
	Tyrosinase inhibition (%)					
Kojic acid	30.79 ± 1.09	47.22 ± 0.25	61.66 ± 0.27	69.81 ± 0.04	77.01 ± 0.25	109.8 µg ml^{-1}

*IC_{50} is the effective concentration that can inhibit tyrosinase by 50%.

Table 4. Antibacterial activities of *S. siliquosum* extract.

Bacterial pathogen	Minimum inhibitory concentration (µg ml^{-1})	Minimum bactericidal concentration (µg ml^{-1})
Gram-positive bacteria		
S. aureus BIOTECH 1823	125.00	250.00
S. epidermidis BIOTECH 10098	250.00	500.00
Gram-negative bacteria		
P. aeruginosa BIOTECH 1824	>1,000.00	ND
E. coli BIOTECH 1825	>1,000.00	ND
E. aerogenes BIOTECH 1145	>1,000.00	ND

*ND = Not Determined.

antimicrobial assay (Al-Judaibi, 2014). Thus, additional studies must be done on the optimization, purification, and identification of these bioactive substances for cosmetic and pharmaceutical application.

CONCLUSIONS

Sargassum siliquosum is capable of producing polyphenolic compounds with direct relevance to cosmetic application. The brown macroalga was able to exhibit good tyrosinase inhibition, antioxidant, and antibacterial properties that can be utilized for large-scale production. Additional studies focusing on the identification and purification of phenolic substances using high-performance liquid chromatography must be done to better understand the mechanisms of action of the active substances found in the seaweed extract. The results of this study are beneficial for the effective use of *S. siliquosum* extract as a source of polyphenolic compounds important for the development of novel skin-lightening ingredient for pharmaceutical and cosmetic industry.

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CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest.

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