Evaluation of Antioxidant Capacity, Tyrosinase Inhibition, and Antibacterial Activities of Brown Seaweed, *Sargassum ilicifolium* (Turner) C. Agardh 1820 for Cosmeceutical Application

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ABSTRACT

Seaweeds are a rich source of active compounds with excellent biological activities that are useful for formulation of cosmetic products. The investigation aims to study the biological and skin protection activities of *Sargassum ilicifolium* extract by evaluating its ability to scavenge free radicals, copper reducing capacity, tyrosinase inhibition and antibacterial properties. The results showed that *S. ilicifolium* extract has a total phenolic content of 4.86±0.07 mg GAE·g^{-1}. Analysis of the antioxidant capacity of *S. ilicifolium* extract exhibited potent DPPH (diphenyl-1, 2-picryl hydrazyl) radical scavenging activity and high capacity of reducing copper ions in a concentration-dependent manner, with IC_{50} values of 15.78 μg·mL^{-1} and 11.19 μg·mL^{-1}, respectively. Also, *S. ilicifolium* extract showed tyrosinase inhibition activity, with IC_{50} of 40.50 μg·mL^{-1}, more effective than that obtained for kojic acid with IC_{50} of 109.8 μg·mL^{-1}. In addition, potent antibacterial activity was exhibited by *S. ilicifolium* extract against common skin pathogenic bacteria such as Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, with minimum inhibitory concentration (MIC) values of 125 μg·mL^{-1}, 125 μg·mL^{-1}, and 250 μg·mL^{-1}, respectively. This study clearly demonstrates the effective biological and skin protecting properties of *Sargassum ilicifolium* as an alternative source of active ingredients useful for cosmeceutical application.

Keywords: Bioactive compounds, Cosmetic application, Marine, Phaeophyceae, Phenolic compounds

INTRODUCTION

Brown seaweeds are diverse group of marine algae that have several bioactive compounds with strong safety profiles for human use. These macroalgae are considered as natural raw materials that can be tapped for the development of important products for therapeutic and cosmetic applications with minimal side effects as compared to synthetic active compounds (Pereira, 2018). Brown seaweeds have bioactive compounds with potent antibacterial, antioxidant, anti-inflammatory, and tyrosinase inhibition activities that are beneficial as active ingredients for cosmetic products (Azam *et al*., 2017; Arguelles and Sapin, 2020). Notably, the phlorotannins and fucoxanthin of brown seaweed extracts are widely recognized as cosmeceutical agents (Pereira, 2018). Phlorotannins are polyketides made up of phloroglucinol (1,3,5-trihydroxybenzene) oligomers found almost exclusively in brown algae. This active substance is stored within cells of brown algae in vessels called physodes and have been reported to have potential health benefits such as antioxidant, antibacterial, anti-inflammatory, collagen boosting, and tyrosinase enzyme inhibition activities (Pereira, 2018; Pimentel *et al*., 2018). On the other

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hand, fucoxanthin is a carotenoid (orange-colored pigment) found in marine algae such as brown seaweeds (Phaeophyceae), diatoms (Bacillariophyta) and Chromophyta (Heterokontophyta or Ochrophyta) (Peng *et al*., 2011). It is an effective cosmetic ingredient with antioxidant and antibacterial activities as well as skin conditioning (providing moisture for the skin and activate skin cell renewal) properties (Peng *et al*., 2011; Pereira, 2018). These substances together with other active compounds such as fucoidans, alginates, fatty acids, and flavonoids derived from brown seaweeds are natural compounds that have greater market value as compared to artificial compounds being used in cosmetic application. Thus, strains of marine brown algae with potent skin protecting and hypo-pigmenting properties have been among the subjects of recent studies in marine natural products research.

Sargassum ilicifolium (Turner) C. Agardh is a fast-growing brown seaweed that is abundantly available along the coast of Catanuan, Quezon, Philippines. It is characterized by having discoid holdfasts with short and warty stems containing primary and secondary branches with elliptical-oblong leaves and globular gas vesicles (Trono, 1992). Structurally diverse active substances with reported pharmacological properties such as polysaccharides, terpenoids, sargaquinoic acids, plastoquinones, polyphenols, steroids, and glycerides are present in *S. ilicifolium* (Kordjazi *et al*., 2013; Namvar *et al*., 2014; Yende *et al*., 2018). Solvent extracts of *S. ilicifolium* using ethanol and ethyl acetate have been reported to possess immunomodulatory and antibacterial activities, respectively (Simpi *et al*., 2010; Jeyanthi *et al*., 2012). On the other hand, antioxidant, anticancer, analgesic, anti-inflammatory, and wound healing activities were also observed from crude extracts of this seaweed, showing the biotechnological potential of this alga (Kordjazi *et al*., 2013; Namvar *et al*., 2014; Premarathna *et al*., 2019). Due to diverse biological properties, research studies are being done on this species to uncover other potential health benefits relevant to the cosmetic industry (Yende *et al*., 2018).

Skin aging is a natural biological process present in all organisms that is highly stimulated by both intrinsic (physiological) and extrinsic (environmental) factors (Im *et al*., 2019). External factors such as exposure to UV radiation and air pollution cause skin aging and deterioration that leads to hyperpigmentation, formation of wrinkles, and skin thickening. Thus, it is important to develop cosmeceutical inhibitors that prevent the occurrence of skin aging (Im *et al*., 2019). Recently, phenolic compounds derived from natural resources such as seaweeds have become important active ingredients for topical creams used in the treatment of skin hyperpigmentation and other melanin-related skin disorders such as melanoma and cancer (Azam *et al*., 2017). The skin protecting effects such as whitening, antioxidant, anti-wrinkle, antibacterial, moisturizing, and anti-aging activities were reported from diverse species of seaweeds from different tropical countries (Jesumani *et al*., 2020). However, few documented studies have been conducted in the Philippines regarding the use of *S. ilicifolium* as a natural alternative source of tyrosinase inhibitor and skin care active ingredients for cosmetic application (Arguelles and Sapin, 2020).

The Philippines is a mega-diverse country with several species of seaweeds present in its marine waters with potential biological activities yet to be discovered (Arguelles *et al*. 2019). Despite having such diverse marine resources, only a few studies have reported on the antibacterial and antioxidant activities of these organisms. The current investigation is the first report in the Philippines exploring the use of *Sargassum ilicifolium* (Turner) C. Agardh as a natural source of active ingredients useful in cosmetic formulation. The study aims to evaluate *S. ilicifolium* as a natural alternative source of active ingredients useful for cosmetic application by determining the total phenolic content, antibacterial, antioxidant (using copper reduction antioxidant capacity (CUPRAC) and DPPH radical scavenging assays) and tyrosinase inhibitor activities of the seaweed extract. Also, correlation analysis of the antioxidant activities of the algal extract and the phenolic concentration is presented.

MATERIALS AND METHODS

Seaweed sampling and collection

Thalli of the macroalga *Sargassum ilicifolium* were collected from Catanauan (Lat. 13° 36' 20.88' N; Long. 122° 14" 18.24' E), Quezon, Philippines in November 2019. The seaweed was identified using relevant morphotaxonomic features according to Trono (1992) and AlgaeBase (Guiry and Guiry, 2020). The seaweed was washed several times with sterile tap water to remove associated algae and other solid debris and sand particles. The material was air-dried for six days before cutting it into small pieces. The seaweed was then powderized before subjecting it to extraction and biological assays (Arguelles *et al*., 2019).

Preparation of seaweed extract

The dried and coarsely powdered *Sargassum ilicifolium* biomass (1 g) was subjected to extraction using 30 mL acidified methanol (1 HCl: 80 CH_3OH: 10 H_2O) with stirring for 1 h using an ultrasonic bath (Gao *et al*., 2002). The liquid soluble active constituents of the seaweed extract in the flask were concentrated using a rotary evaporator (BUCHI™ RotavaporTM Scholar System) subject to reduced pressure at 40 ºC. The concentrated *S. ilicifolium* extract was kept in sterile screw-capped tubes at 4 ºC until it was used for the biological assays (Gao *et al*., 2002; Arguelles and Sapin, 2020). The extract yield of *S. ilicifolium* was calculated using the equation:

$$\text{Yield (\%)} = \frac{\text{Weight of the algal extract (g)}}{\text{Weight of the dried algal biomass (g)}} \times 100$$

Determination of total phenolic content (TPC)

The concentration of phenolic content present in *Sargassum ilicifolium* was analyzed using the Folin-Ciocalteu method (Nuñez-Selles *et al*., 2002). A volume (0.5 mL) of *S. ilicifolium* extract was mixed until homogenous using a vortex mixer with 10% sodium carbonate solution and Folin-Ciocalteau's reagent in equal volumes. The solution was set aside for 5 min and the volume was then adjusted by adding 5 mL of sterile distilled water. An absorbance reading of each sample was determined at 720 nm wavelength using an Ultraviolet-Visible spectrophotometer. The total phenolic content was set using standard gallic acid calibration curve with prepared range of concentrations of 20-100 $\mu g \cdot mL^{-1}$. The overall phenolic content of *S. ilicifolium* is expressed as milligrams of gallic acid equivalent (GAE) per gram of the seaweed sample.

DPPH radical scavenging assay

The capacity of *Sargassum ilicifolium* to scavenge free radicals was done following the protocol of Ribeiro *et al*. (2008) for 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with few modifications. Briefly, 100 µL crude extract of *S. ilicifolium* (prepared at five concentrations: 10.0, 20.0, 30.0, 40.0, and 50.0 $\mu g \cdot mL^{-1}$) was thoroughly mixed until homogenous using a vortex mixer with 5.0 mL of 0.1 mM DPPH methanolic solution. The reaction mixture was set aside for 20 min at ambient temperature. Ascorbic acid (prepared at five concentrations: 8.0, 16.0, 24.0, 32.0, and 40.0 $\mu g \cdot mL^{-1}$) was used as the positive control in this assay. Absorbance of each sample was measured at a wavelength of 517 nm using an Ultraviolet-Visible spectrophotometer. The antioxidant activity was estimated using the formula:

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

where $A_{control}$ = absorbance of the control (DPPH solution without seaweed extract) and A_{sample} = absorbance of the seaweed sample (DPPH solution plus seaweed extract). The effective concentrations (IC_{50}) for *S. ilicifolium* extract and the control were determined upon analysis of DPPH inhibition (Ribeiro *et al*., 2008).

Copper reduction antioxidant capacity (*CUPRAC*) *assay*

The CUPRAC assay was done using the protocol of Alpinar *et al*. (2009). Briefly, 1 mL each of 1 M ammonium acetate buffer (pH 7), 0.0075 M neocuproine, and 0.01 M copper (II) chloride ($CuCl_2$) solutions were mixed until homogenous in a test tube. Thereafter, 0.5 mL of *Sargassum ilicifolium* extract prepared at varying concentrations (2.5, 5.0, 7.5, 10.0, and 12.5 $\mu g \cdot mL^{-1}$) as well as standard solutions of ascorbic acid (5.0, 10.0, 15.0, 20.0, and 25.0 $\mu g \cdot mL^{-1}$) were added to the initial reaction mixture. Using sterile distilled water, total volume was adjusted up to 4.1 mL for each prepared concentration. The reaction mixtures were set aside at ambient temperature for 30 min and the absorbance readings were taken at 450 nm (Arguelles *et al*., 2017). The effective concentrations (IC_{50}) for *S. ilicifolium* extract and the control (ascorbic acid) were determined upon analysis of copper reduction antioxidant capacity (Arguelles *et al*., 2017).

Tyrosinase inhibition assay

The capacity of the *Sargassum ilicifolium* extract to cause inhibition of the tyrosinase enzyme was evaluated *in vitro* following the methods described by Hapsari *et al*. (2012) with slight modifications. In this assay, mushroom tyrosinase was used as the enzyme while L-DOPA served as the substrate for the reaction. Initially, a solution of mushroom tyrosinase (250 units$\cdot mL^{-1}$, Sigma Sigma T- 3824), 0.1 M potassium phosphate buffer (pH 6.5), and 5 mM DOPA (3,4-dihydroxy-L-phenylalanine, Sigma D-9628) was prepared. An aliquot of 40 µL DOPA was mixed with 40 µL of *S. ilicifolium* extract (prepared at five concentrations: 25.0, 50.0, 75.0, 100.0, and 125.0 $\mu g \cdot mL^{-1}$) or 40 µL buffer (in the case of the control) in a microtiter well-plate. The volume was adjusted to 160 µL using phosphate buffer and 40 µL of mushroom tyrosinase was added to complete the reaction mixture. The microtiter plate containing the reaction mixtures was incubated for 15 min at ambient room temperature. The absorbance reading was taken at 490 nm using a microtiter plate reader. Tyrosinase inhibition was calculated using the equation below:

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

where Ac = the absorbance reading of the control, Ab = the absorbance reading of the blank, and As = the absorbance reading of the sample (*S. ilicifolium* extract).

Determination of effective concentration (IC_{50})

The effectiveness of antioxidant and tyrosinase inhibition activities was evaluated by comparing the IC_{50} values of the seaweed extract and the control standards. The lower the IC_{50} values, the higher the antioxidant and tyrosinase inhibition activities. For the DPPH assay, IC_{50} is defined as the concentration that inhibits DPPH radical by 50 %. On the other hand, IC_{50} for the CUPRAC assay is defined as the effective concentration that gives an absorbance reading of 0.5 at 450 nm. IC_{50} for tyrosinase inhibition assay is defined as the effective concentration that can inhibit tyrosinase by 50 %. Calculation of the IC_{50} values of *Sargassum ilicifolium* extract and the controls (ascorbic acid and kojic acid) was done by interpolation using its antioxidant and tyrosinase inhibition activities and their corresponding extract concentrations (Tables 1-3). The calculation for IC_{50} was done using the following formula:

$$Y_2 = \left(\frac{(X_2 - X_1) - (Y_3 - Y_1)}{X_3 - X_1}\right) + Y_1$$

where Y_1 = inhibition below 50 %; Y_2 = 50 %; Y_3 = inhibition above 50 %; X_1 = the concentration that gave inhibition below 50 %; X_2 = the concentration that gave 50 % inhibition and X_3 = the concentration that gave inhibition above 50 %.

Antibacterial assay

Three pathogenic Gram-negative bacteria (*Enterobacter aerogenes* BIOTECH 1145, *Pseudomonas aeruginosa* BIOTECH 1824 and *Escherichia coli* BIOTECH 1825) and four Gram-positive bacteria (Methicillin-resistant *Staphylococcus aureus* BIOTECH 10378, *Staphylococcus epidermidis* BIOTECH 10098, *Staphylococcus aureus* BIOTECH 1823, and *Bacillus cereus* BIOTECH 1509) were procured from the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños. The bacterial pathogens were pre-cultured using Luria–Bertani (LB) medium and incubated at 37 ºC with shaking for 24 h.

The antibacterial assay was done using the two-fold serial dilution technique following the procedures described by Arguelles *et al*. (2019). Briefly, 100 µL of test bacterial cultures (cell density of 1×10^6 cells·mL^{-1}) grown using Luria-Bertani medium were mixed with 100 µL of *Sargassum ilicifolium* extract at varying concentrations (1000 µg·mL^{-1}-7.8125 µg·mL^{-1}) in a 96-well microtiter plate. Acidified methanol was also included in the assay as the control.

The experimental microdilution plate was kept for 12 h in an incubator at 35 °C and observed for growth of bacteria (turbidity) in each well, after which minimum inhibitory concentrations (MIC) of *Sargassum ilicifolium* extract were noted. The minimum bactericidal concentration (MBC) of the seaweed extract was evaluated by plating a loopful of sample obtained from each MIC well that showed growth inhibition of bacteria into a new culture (tryptic soy agar) medium (Arguelles, 2018). These plates were kept at 35 °C in an incubator for 24 h. After incubation, the petri plates were checked for the presence of bacterial colony growth for each dilution subculture. No visible colony growth would mean that the algal extract was bactericidal at that particular dilution.

Statistical analyses

The extraction yield, antibacterial, antioxidant and tyrosinase inhibition activity data obtained in this study are presented as means±SD of three replicates. The correlation between antioxidant activities and the phenolic concentration was assessed by calculating Pearson's linear correlation coefficient (r). Data analysis was performed with Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Extraction yield of Sargassum ilicifolium

Extraction yield is a significant parameter being used to assess the effectiveness and economic value of a material or product (Nurjanah *et al*., 2017; Arguelles and Martinez-Goss, 2020). It is the percentage of the crude extract that can be utilized from the sample. The crude extract of *Sargassum ilicifolium* obtained from this study was characterized to have a brownish green color, which can be attributed to algal pigments such as fucoxanthin, chlorophyll, and carotenoids (Nurjanah *et al*., 2017). The yield of *S. ilicifolium* crude extract was 12.02± 0.042 %, which is higher than that obtained by Artemisia *et al*. (2019) from hot water extract of *S. ilicifolium* (2.89-3.49 %) and *S. turbinaroides* (6.00-7.36 %) at different extraction temperatures. Several factors are reported to have an effect on the extraction yield of a sample such as extraction method, temperature, natural condition of the sample, solvent used in the extraction, and particle size of the sample (Nurjanah *et al*., 2017; Arguelles and Martinez-Goss, 2020). Thus, optimization of the extraction condition is important for large-scale utilization of bioactive substances from *S. ilicifolium*.

Total phenolic content (TPC)

Phenolic compounds present in several species of seaweeds are recognized as natural sources of antioxidants for human use (Azam *et al*., 2017; Arguelles *et al*., 2019). In this investigation,

estimation of the TPC of the acidified methanolic *Sargassum ilicifolium* extract was done using Folin-Ciocalteu's phenol reagent and is expressed in terms of gallic acid equivalent (GAE). The total phenolic content of *S. ilicifolium* extract was 4.86±0.07 mg GAE·g^{-1}. The phenols present in *S. ilicifolium* are assumed to be substances responsible for the antioxidant, tyrosinase inhibitory and antibacterial activities of the extract. The TPC of the algal sample is greater than that obtained from other species of *Sargassum* such as *S. siliquastrum* (0.29 mg GAE·g^{-1}), *S. polycystum* (0.37 mg GAE·g^{-1}), and *S. binderi* (0.063 mg GAE·g^{-1}) (Kim *et al*., 2005; Boonchum *et al*., 2011; Fu *et al*., 2015). However, it is lower than the TPC obtained from other species of brown seaweeds such as *Turbinaria conoides* (105.97 mg GAE·g^{-1}), *Turbinaria ornata* (69.63 mg GAE·g^{-1}), *Sargassum siliquosum* (30.34 mg GAE·g^{-1}), and *Sargassum vulgare* (10.13±0.166 mg GAE·g^{-1}) (Chakraborty *et al*., 2013; Arguelles *et al*., 2019; Arguelles and Sapin, 2020). Variation in the total phenolic concentrations obtained from *S. ilicifolium* and other seaweed species can be attributed to environmental factors (such as seasonal variation, salinity, and irradiation) as well as the type of extraction solvent used. Generally, polar solvents like ethanol, methanol and acetone are effective extraction substances for phenolic compounds in algal samples (Oucif *et al*., 2017; Arguelles *et al*., 2018).

Antioxidant activity

The biological activities reported to be possessed by most of the commercially available cosmeceuticals are attributed to antioxidant activities of the active ingredients. Several synthetic antioxidants used in cosmetic products are available, but are known to pose toxicity concerns when used frequently at high concentration. Thus, harnessing naturally derived antioxidants from seaweeds with good antioxidant activity may be useful for skin care product formulation. In this study, two antioxidant activity assays (DPPH radical scavenging activity and copper reduction antioxidant capacity (CUPRAC)) were utilized to assess the antioxidant properties of *Sargassum ilicifolium* extract.

DPPH radical scavenging activity

Free radical inhibition using natural antioxidants has become a popular therapeutic treatment for skin aging because of its hypo-pigmenting, anti-wrinkle, moisturizing and antibacterial activities. DPPH free radical scavenging activity assay was used to assess the ability of *Sargassum ilicifolium* extract to do hydrogen donation and scavenge free radicals. The study showed that the algal extract exhibited potent free radical scavenging properties and can be used as a natural antioxidant for cosmetics. The antioxidant capacity of the *S. ilicifolium* extract increases in a concentration-dependent manner, with IC_{50} value of 15.78 µg·mL^{-1}, more potent than the positive control, ascorbic acid, with IC_{50} value of 23.38 µg·mL^{-1} (Table 1). This finding is similar to previous reports on different seaweed species such as *Ulva fasciata*, *Sargassum vulgare*, *S. swartzii*, *S. vachellianum*, and *Chaetomorpha antennina*, which also showed potent antioxidant free radical (DPPH) scavenging activity (Jose and Kurup, 2016; Arguelles *et al*., 2019; Jesumani *et al*., 2020). Also, these studies reported that seaweed extracts having higher phenolic content exhibited higher antioxidant activity, which is similar with the trends observed in the current study. *S. ilicifolium* exhibited an IC_{50} value showing it to be more effective than those reported by Sanger *et al*. (2019) from seaweeds obtained from Sulawesi, Indonesia, namely *Turbinaria decurrens* (10.01±0.53 mg·mL^{-1}), *G. salicornia* (12.81±0.93 mg·mL^{-1}), *Halymenia durvilae* (14.17±1.06 mg·mL^{-1}), *Sargassum olygocystum* (15.38±1.17 mg·mL^{-1}) and *H. macroloba* (18.54±1.25 mg·mL^{-1}), which indicates its promising activity as a potent antioxidant for use in skin care products. Antioxidant activity of brown seaweeds is linked to bioactive substances present in the extract such as phenolic compounds (phlorotannins and fucoidans), sulfated polysaccharides, bioactive peptides, pigments (carotenoids, fucoxanthin, and chlorophylls), lipids, vitamins, and terpenoids. These active substances are known to have free radical scavenging ability, metal-chelating ability, or peroxidation properties (Yende *et al*., 2018; Arguelles *et al*., 2019).

Copper reduction antioxidant capacity (*CUPRAC*)

Antioxidants have been proposed as an effective active ingredient of anti-aging and skin care products because of their ability to impede oxidative damage to skin caused by free radicals. CUPRAC assay is a technique used in assessing the antioxidant capability of an extract by evaluating the ability to reduce Cu (II) to Cu (I) and is useful in determining hydrophilic and liphophilic antioxidants (Arguelles, 2018). *Sargassum ilicifolium* extract exhibited copper ion reduction ability in a concentration-dependent manner. Table 2 shows the highest absorbance reading of 0.570 of *S. ilicifolium* extract at 12.5 µg·mL^{-1} concentration, in contrast with the standard, ascorbic acid, which had an absorbance of 0.542 at 25.0 µg·mL^{-1} concentration. The observed trend in this antioxidant assay is similar to that obtained from the DPPH assay, in which 50.0 µg·mL^{-1} concentration showed the highest DPPH inhibition of 74.06 %.

Table 1. DPPH free radical scavenging activity and IC_{50} value of phenolics from *Sargassum ilicifolium* and ascorbic acid.

Sample	**Extract concentration (µg•mL^{-1})**					**IC_{50} (µg•mL^{-1})**
Sargassum ilicifolium	10.0	20.0	30.0	40.0	50.0	
	DPPH inhibition (%)					
	39.67±0.118	57.57±0.178	65.86±0.000	70.50±0.180	74.06±0.120	15.78
Ascorbic Acid	**Concentration (µg•mL^{-1})**					
	8.0	16.0	24.0	32.0	40.0	
	DPPH inhibition (%)					
	17.65±0.005	34.21±0.440	51.32±0.050	68.58±0.000	83.86±0.000	23.38

Table 2. Copper reduction antioxidant capacity (CUPRAC) and IC_{50} value of phenolics from *Sargassum ilicifolium* and ascorbic acid.

Sample	**Extract concentration (µg•mL^{-1})**					**IC_{50} (µg•mL^{-1})**
Sargassum ilicifolium	2.5	5.0	7.5	10.0	12.5	
	CUPRAC (Absorbance at 450 nm)					
	0.123±0.001	0.239±0.004	0.343±0.000	0.437±0.000	0.570±0.001	11.19
Ascorbic Acid	**Concentration (µg•mL^{-1})**					
	5.0	10.0	15.0	20.0	25.0	
	CUPRAC (Absorbance at 450 nm)					
	0.112±0.002	0.213±0.007	0.328±0.004	0.429±0.012	0.542±0.011	23.15

Sargassum ilicifolium extract exhibited more potent antioxidant activity in relation to ascorbic acid with IC_{50} values of 11.19 μg·mL^{-1} and IC_{50} = 23.15 μg·mL^{-1}, respectively. This finding is similar to those obtained by Grina *et al*. (2020) from Moroccan seaweeds such as *Cystoseira humilis* (IC_{50} of 12.98 μg trolox equivalent mg^{-1}), *Gelidium sesquipedale* (IC_{50} of 8.25 μg trolox equivalent mg^{-1}) and *Bifurcaria bifurcata* (IC_{50} of 12.92 μg trolox equivalent mg^{-1}), which exhibited high antioxidant capacity as compared to α-tocopherol and butylated hydroxytoluene (synthetic antioxidants). This study reveals that *S. ilicifolium* extract contains phenolic compounds with antioxidant properties ascribed to its potent copper reducing ability.

Correlation analysis between antioxidant activity and phenolic content

Correlation analysis between antioxidant capacity and phenolic concentration of *Sargassum ilicifolium* extract using DPPH scavenging and copper reduction antioxidant capacity (CUPRAC) assay is presented in Table 3. Results of the analysis showed a positive correlation between antioxidant (CUPRAC and DPPH assay) capacity and the TPC of the seaweed extract with r = 0.94366 and r = 0.99861, respectively. This indicates that phenolic compounds found in *S. ilicifolium* contribute to the antioxidant properties of the seaweed extract. This observation is supported by earlier studies in other seaweeds that also reported a positive correlation between phenolic concentration and antioxidant activity (Sanger *et al*., 2019; Arguelles and Sapin, 2020; Jesumani *et al*., 2020). Phenolic compounds are abundantly present in several *Sargassum* species, and in this investigation the antioxidant properties of *S. ilicifolium* extract are strongly linked to these compounds. Thus, identification and structure elucidation of these substances are needed to better understand the reaction mechanism responsible for the antioxidant properties and their potential for cosmetic application (Arguelles *et al*., 2019).

Tyrosinase inhibition activity

Tyrosinase is a multi-copper oxidative enzyme important in melanin synthesis via L-tyrosine hydroxylation converting it to 3,4-dihydroxyphenylalanine (DOPA) as well as the transformation of DOPA to dopaquinone. Excessive production of this enzyme will lead to hyperpigmentation in skin that can cause skin disorders such as lentigo and melisma (Im *et al*., 2019). Phenolic compounds are considered as one of the largest sources of natural active substances that have anti-tyrosinase activity. Many phenolic compounds have been documented to exhibit tyrosinase inhibition properties by attacking the enzyme's active site, leading to an unchangeable substrate-like interaction causing inactivation of tyrosinase. Also, other forms of flavonoids are capable of copper ion chelation in tyrosinase, causing inhibitory activity against the target enzyme (Im *et al*., 2019).

The ability of *Sargassum ilicifolium* extract to inhibit tyrosinase was analyzed *in vitro* using mushroom tyrosinase, an enzyme responsible for L-DOPA formation (Table 4). The highest tyrosinase inhibition activity observed for *S. ilicifolium* extract was 89.78 % at 125 μg·mL^{-1} concentration. *Sargassum ilicifolium* extract

Table 3. Correlation between phenolic content and antioxidant activities of *Sargassum ilicifolium* extract.

Antioxidant Assay	Regression of Extract Concentration (x) on Antioxidant Activity (y)	Correlation Coefficient (r)	p value*
DPPH radical scavenging assay	y = 0.8171x + 37.019	0.94366	0.015916
Copper reduction antioxidant capacity (CUPRAC) assay	y = 0.0218x + 0.0148	0.99861	0.000062

Note: *Correlation is significant at p<0.05

Table 4. Tyrosinase inhibition activity and IC_{50} value of *Sargassum ilicifolium* extract and kojic acid.

Sample	Extract concentration (μg·mL^{-1})					IC_{50} (μg·mL^{-1})
Sargassum ilicifolium	25.0	50.0	75.0	100.0	125.0	
	Tyrosinase inhibition (%)					
	32.71±0.232	60.72±0.260	80.69±0.650	85.87±0.177	89.78±0.203	40.50
Kojic Acid	Concentration (μg·mL^{-1})					
	50.0	100.0	150.0	200.0	250.0	
	Tyrosinase inhibition (%)					
	30.79±0.110	47.22±0.250	61.66±0.270	69.81±0.040	77.01±0.250	109.80

exhibited potent anti-tyrosinase activity with IC_{50} value of 40.50 μg·mL^{-1} in comparison to that obtained for kojic acid (positive control) with IC_{50} of 109.8 μg·mL^{-1}. This result suggests that *S. ilicifolium* extract is more effective than kojic acid and that the seaweed extract has anti-melanogenic activities. The IC_{50} of *S. ilicifolium* obtained in the current study is more potent than those previously documented seaweeds such as *Cystoseira humilis* (IC_{50} = 84.1 μg·mL^{-1}), *Ascophyllum nodosum* (IC_{50} = 0.1 mg·mL^{-1}), *Eucheuma cottonii* (IC_{50} = 234.33 μg·mL^{-1}) and *Grateloupia lancifolia* (IC_{50} = 256 μg·mL^{-1}) (Jiménez *et al*., 2010; Yen and Kim, 2012; Chang and Teo, 2016; Grina *et al*., 2020). Based on these results, *S. ilicifolium* could be considered as a natural active ingredient in the formulation of skin whitening products because of its ability to inhibit tyrosinase as well as its potent antioxidant activity (Jesumani *et al*., 2020). Variation in the anti-tyrosinase properties of seaweed extracts is caused by environmental and physiological factors such as habitat, harvesting condition and algal species (Arguelles and Sapin, 2020). Tyrosinase inhibitors derived from seaweeds are natural sources of active ingredients for cosmetics and treatment of skin disorders. Dieckol and 7-phloroeckol (from *Ecklonia cava*) as well as fucoxanthin (from *Laminaria japonica*) were reported to have higher inhibitory activity than some of the commercially available tyrosinase inhibitors (like kojic acid and arbutin) and exhibited suppressing activity against melanogenesis in UV-B irradiated mice (Grina *et al*., 2020).

Antibacterial activity

Cosmetic products have active ingredients possessing antibacterial activities that help maintain and preserve normal skin microflora such as *Staphylococcus aureus* on human skin surfaces (Jesumani *et al*., 2020). These microorganisms are not harmful normally, but can cause severe infections when the skin is suddenly wounded. Also, *Staphylococcus aureus* is capable of producing toxins on the skin epidermis that results in formation of blisters and pimples (Jesumani *et al*., 2020). Skin diseases such as atopic dermatitis can be exacerbated by *S. aureus*, which can increase the severity of the disease by causing inflammation and systemic infections. In addition, other pathogenic bacteria such as *Escherichia coli*, *Staphylococcus epidermidis* and *S. saprophyticus* are causative agents for soft tissue and skin infections (Arguelles, 2018; Jesumani *et al*., 2020).

Antibacterial activities of *Sargassum ilicifolium* against bacterial skin pathogens were evaluated using microtiter plate dilution assay. The results of the antibacterial assay of the seaweed extract are presented in Table 5. Of the seven bacterial strains tested, three bacterial test organisms were inhibited by the algal extract. *Sargassum ilicifolium* exhibited good antibacterial activities against *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), and *Staphylococcus epidermidis* having MIC of 125 μg·mL^{-1}, 125 μg·mL^{-1}, and 250 μg·mL^{-1}, respectively. The antibacterial

Table 5. Antibacterial activities of *Sargassum ilicifolium* extract.

Test organism	Minimum inhibitory concentration ($\mu g \cdot mL^{-1}$)	Minimum bactericidal concentration ($\mu g \cdot mL^{-1}$)
Gram-positive bacteria		
Staphylococcus aureus	125.00	250.00
BIOTECH 1823		
Methicillin-resistant		
Staphylococcus aureus	125.00	250.00
BIOTECH 10378		
Staphylococcus epidermidis	250.00	500.00
BIOTECH 10098		
Bacillus cereus	>1000.00	ND
BIOTECH 1509		
Gram-negative bacteria		
Escherichia coli	>1000.00	ND
BIOTECH 1825		
Pseudomonas aeruginosa	>1000.00	ND
BIOTECH 1824		
Enterobacter aerogenes	>1000.00	ND
BIOTECH 1145		

Note: *ND – None detected

activity of *S. ilicifolium* extract against three strains of *Staphylococcus* obtained in this study is more potent than those reported from other species of brown seaweeds such as *Sargassum polycystum*, *Padina australis* and *S. oligocystum*, with MIC values of 0.156 $mg \cdot mL^{-1}$, 0.417 $mg \cdot mL^{-1}$, and 3.175 $mg \cdot mL^{-1}$, respectively (Chiao-Wei *et al*., 2011; Tajbakhsh *et al*., 2011). No inhibitory activity was detected against *Escherichia coli*, *E. aerogenes*, *B. cereus*, and *P. aeruginosa*. Minimum bactericidal concentration (MBC) of *S. ilicifolium* extract against Methicillin-resistant *S. aureus* and *S. aureus* was 250 $\mu g \cdot mL^{-1}$. It is more potent than *S. epidermidis*, with MBC value of 500 $\mu g \cdot mL^{-1}$. The results of the assay showed that Gram-positive bacteria are often more receptive to the inhibitory effects of the algal extract, which is similar to previous studies showing the advantage of Gram-negative bacteria in terms their cell wall composition (Tajbakhsh *et al*., 2011; Jesumani *et al*., 2020). Generally, Gram-negative bacteria possess a thick peptidoglycan layer and an outer membrane that serve as an effective barrier that prevents the entry of antibiotics and other bioactive substances into the cell (Arguelles and Sapin, 2020; Jesumani *et al*., 2020). Variation in antibacterial activity of *S. ilicifolium* extract and of other seaweed extracts from earlier studies can be attributed to variation in the type of solvent used as well as method of extraction done in the study (Arguelles *et al*., 2019).

This is a pioneering study in the Philippines documenting the antibacterial properties of *Sargassum ilicifolium* in opposition to bacterial pathogens: *Staphylococcus aureus*, *S. epidermidis* and Methicillin-resistant *S. aureus*. The antibacterial properties of this seaweed clearly demonstrate the existence of bioactive substances in the extract with promising biological activity, and it can be used as an alternative source of natural active ingredients for cosmetic product formulation.

CONCLUSIONS

The present study suggests that *Sargassum ilicifolium* extract has promising biological properties such as antioxidant activity, tyrosinase inhibition property, and antibacterial activity (against bacterial skin pathogens) useful for formulation of cosmetic products. The results obtained in this investigation are based on *in vitro* assays and preliminary screening. Thus, *in vivo* experimental studies and skin trials are needed to further assess the effectiveness of the active compounds found in the seaweed extract. Also, isolation and identification of the active compounds are recommended for future studies in order to better comprehend the fundamental mechanisms involved in the skin protection properties of this seaweed.

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