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Harnessing *Chnoospora minima* (Hering) Papenfuss (Scytosiphonaceae, Ochrophyta) for pharmaceutical application: Antioxidant, antibacterial, tyrosinase and elastase inhibition properties

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Abstract

Marine algae are untapped alternative sources of bioactive substances with important biological activities that can be harness for pharmaceutical application. The proximate composition and some important biological properties of brown macroalga, *Chnoospora minima* (Hering) Papenfuss were studied. Results showed that proximate composition of *C. minima* contain high carbohydrate (33.09 ± 0.14%), protein (25.89 ± 0.01%) and ash (18.79 ± 0.02%) content. The seaweed contain a total phenolic content of 9.90 ± 0.08 mg gallic acid equivalents (GAE)/g. Antioxidant efficiency of *C. minima* were observed to have potent 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^+$) scavenging activity and good copper reduction capacity with IC_{50} value of 129 µg/mL and 28.59 µg/mL, respectively. *In vitro* evaluation of the tyrosinase and elastase inhibition properties showed that *C. minima* extract has potent enzyme inhibitory activities with half maximal inhibitory concentration (IC_{50}) values of 36.0 µg/mL and 56.0 µg/mL, respectively more effective than kojic acid and tocopherol. The algal extract showed effective antibacterial activities against *Staphylococcus aureus* minimum inhibitory concentration ((MIC) = 125 µg/mL), *Listeria monocytogenes* (MIC = 250 µg/mL), and *Aeromonas hydrophila* (MIC = 250 µg/mL). The study is the first documented report in the Philippines describing the noteworthy biological activities of *C. minima* that can be harnessed as source of novel bioactive compounds for human use.

Keywords: Algae, Bioactive compound, Biological activity, *Chnoospora minima,* Marine, Philippines, Seaweed, Tropical

1. Introduction

Seaweeds are composed of diverse species of marine organisms that provide us a number of important compounds such as sterols, polyphenols, flavonoids, tannins, alkaloids, proteins, fatty acids, carotenoids, and enzymes that can be use as alternative sources of active metabolites for drug development and nutraceutical [1]. To date, several marine algae have been documented to have antioxidant, antibacterial, antifungal, anticoagulant, anticancer, anti-diabetes, and anti-inflammatory activities [1-5]. In addition, some species are being consumed as fresh or dried vegetables that can be added in numerous dishes which provides valuable nutrients that can benefit humans. The wide array of biological activities and nutritional benefits that can be obtained from these organisms caught the attention of several researchers to study the chemical composition and search for novel compounds from these inexpensive and naturally occurring marine resources [6,7]. In the coastal areas of the Philippines, brown algae are the most common and frequently encountered vegetation despite the presence of several herbivorous predators. The ability of these seaweeds to proliferate and co-exist with its predator shows that the alga are capable of developing active metabolites to avoid these predators [6]. In addition, these bioactive metabolites are also synthesized as a protection against oxidizing substances and free radicals that affect the seaweeds because of exposure to unfavorable habitat conditions [6]. The bioactive properties of seaweeds are

associated with several bioactive metabolites such as phenolic compounds, phlorotannins, bioactive peptides, and lipids. The action of these metabolites was supported by findings from earlier studies regarding some species of seaweeds from the genus *Sargassum*, *Padina,* and *Turbinaria* found in the Philippines such as *Sargassum vulgare*, *Sargassum ilicifolium*, *Sargassum siliquosum*, *Padina australis*, and *Turbinaria decurrens* where potent biological activities are documented [2-5,8]. The genera *Chnoospora* is quite common in the Philippine coasts, however, the chemical composition and bioactive properties of this group of seaweeds have never been reported in the country [2-5,8].

C. minima Papenfuss is a brown seaweed belonging to the family Scytosiphonaceae commonly found in several coastal areas of tropical countries. The thallus of this seaweed is 20-25 cm tall, yellowish-brown in color, dichotomously branched and not entangled; branches are about 1 mm broad, subcylindrical with several tufts of colorless hairs; ultimate branchlets are short with acute apices [7,9]. This seaweed is reported to have potent biological properties and other pharmaceutical benefits such as anti-angiogenesis, anticoagulant, anti-inflammatory, and antiproliferative activities [7]. However, limited reports are available for its antioxidant, antimicrobial, anti-wrinkling, and whitening properties [7]. The Philippine marine coastal areas are known to have diverse species of seaweeds (including *C. minima*) with bioactive properties that are yet to be explored. However, only few studies were documented about the biological and therapeutic properties of these organisms [2-5,8]. And to date, no documented biological properties are reported for *C. minima* strains found in the country. Thus, the current study aims to document for the first time in the Philippines the bioactive properties of this seaweed with potential use as novel drugs for nutraceutical and pharmaceutical application. The study specifically aims to know the total phenolic content (TPC), antioxidant (using 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{+}$) and copper reduction antioxidant capacity (CUPRAC) assay, antibacterial as well as tyrosinase and elastase inhibition activities of *C. minima*. In addition, correlation analysis on the phenolic content of the seaweed extract and its antioxidant activity was established.

2. Materials and methods

2.1 Sample collection

The brown seaweed, *C. minima* was collected on 07 March 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 alga was identified based on key morphological characteristics using standard reference monograph material for seaweed identification and Algae Base [10,11]. The collected seaweed sample was cleaned using sterile distilled water several times to remove sand debris and other attached epiphytic organisms. *C. minima* was oven-dried at 60°C for 12 h. After drying, the dried seaweed biomass was pulverized before subjecting it for solvent extraction. 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 University of the Philippines (CAHUP) of the Museum of Natural History, University of the Philippines Los Baños, College, Laguna, Philippines.

2.2 Proximate composition analysis

The crude fat, moisture content, crude fiber, crude protein, and carbohydrate content were analyzed to know the proximate composition of *C. minima*. The proximate composition analysis of the algal sample was done in triplicates in all of the proximate parameters. The ash content of *C. minima* was analyzed by subjecting the algal sample to ignition at 450ºC for 6 h until an ash was produced [12]. On the other hand, moisture content of *C. minima* was analyzed by subjecting two grams of the algal biomass to complete dryness at 105ºC. The Weende method was used to know the crude fiber content of the seaweed sample. Briefly, 0.3 g of *C. minima* biomass was digested with 1.25% hydrochloric acid (HCl) followed by 1.25% sodium hydroxide (NaOH). The algal residue obtained was dried (at 105ºC for 3 h) and weighed [12]. The protein content of *C. minima* was analyzed using microkjeldahl protein analysis. *C. minima* biomass (1 g) was initially digested using 4 mL of concentrated sulfuric acid. The reaction mixture was then prepared for protein analysis using kjeltech apparatus (Foss Inc.) and the amount of nitrogen in crude protein was determined by calculation using the empirical factor 6.25. On the other hand, crude fat content of *C. minima* biomass was analyzed via the Soxhlet method using a Soxtec Total Fat Extractor (Foss Inc.). Initially, two grams of the dried seaweed biomass was placed in a thimble and subjected to lipid extraction using the solvent petroleum ether for about 16 h under 30-60ºC boiling range. The total carbohydrate content was calculated via difference method using the equation (1).

$$\%\, Carbohydrates = 100 - (\%\, Moisture\ +\ \%\, Protein + \%\, Crude\ Fat + \%\, Ash) \quad (1)$$

2.3 Seaweed extract preparation

The dried biomass of *C. minima* (1 g) was subjected to extraction using 30 mL acidified methanol (1 HCl: 80 methanol (CH_3OH): 10 hydrogen oxide (H_2O)) with stirring for 1 h in an ultrasonic bath. The mixture was then centrifuged at 12,000 rpm at a temperature of 20ºC for 20 min. The concentrated algal extract was further concentrated using a rotary evaporator set at 40°C under reduced pressure. The pooled *C. minima* extract was kept under refrigerated condition (4°C) to maintain its biological activity for use to the assays needed in the study [3,5,13].

2.4 Determination of total phenolic content (TPC)

The total phenolic content of *C. minima* was analyzed using Folin-Ciocalteau's reagent following the procedure of Arguelles and Sapin [2]. In this method, 0.5 mL of the diluted *C. minima* extract was added with equal volume of 10% sodium carbonate solution and Folin-Ciocalteau's reagent for 1 min. The reaction mixture was then placed at ambient room temperature for 5 min. The absorbance reading of the reaction mixture was noted using Ultraviolet-Visible (UV-Vis) spectrophotometer at a wavelength of 720 nm. The TPC of *C. minima* extract was expressed as milligrams of gallic acid equivalents (GAE) per g (mg GAE/g) of the algal sample [2,3].

2.5 $ABTS^+$ (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

The ability of *C. minima* extracts to scavenge $ABTS^+$ free radical was evaluated following the procedure of Re et al. [14] with a few modifications. Initially, 40 µL of *C. minima* extract prepared in various phenolic concentrations (37.5-187.5 µg GAE/mL) and 40 µL of 90% methanol (for the control) were mixed with 3 mL of $ABTS^+$ free radical mixture (initial absorbance reading of 0.72 ± 0.05 at 734 nm). The reaction mixtures were thoroughly mixed and were placed in ambient room temperature for 5 min. The absorbance reading of each reaction mixture was noted at 734 nm. The percent inhibition of $ABTS^+$ was determined using the formula:

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

The $ABTS^+$ inhibition activity (%) was plotted together with different prepared concentrations of *C. minima* extract. The seaweed extract concentration that showed 50% $ABTS^+$ radical scavenging activity was considered as the half maximal inhibitory concentration (IC_{50}).

2.6 Copper reduction antioxidant capacity (CUPRAC) assay

The copper ion reducing capacity of *C. minima* extract was assessed via colorimetric method following the procedure of Arguelles [3]. Briefly, 1 mL each of 1 M ammonium acetate buffer Positive potential of the Hydrogen ions ((pH) 7), 0.01 M copper chloride ($CuCl_2$) solution, and 0.0075 M neocuproine were mixed in sterile test tubes containing 0.5 mL of *C. minima* extract (6,12,18,24 and 32 µg GAE/mL of phenolic concentration) and ascorbic acid as the standard antioxidant. The volume for each mixtures were adjusted to 4.1 mL using water and were kept at room temperature for 30 min. The absorbance reading against the reagent blank was noted at 450 nm wavelength for both the *C. minima* extract and ascorbic acid concentrations [15].

2.7 Tyrosinase inhibition assay

The whitening property of *C. minima* extract was evaluated via tyrosinase inhibition assay following the procedure of Arguelles and Sapin [2]. Initially, solutions of 5 M DOPA (3,4-dihydroxy-L-phenylalanine, Sigma D-9628), 0.1 M potassium phosphate buffer, pH 6.5, and mushroom tyrosinase (250 units/mL, Sigma T- 3824) were prepared. Aliquot (40 µL) of DOPA is mixed with 40 µL of *C. minima* extract (at varying concentration: 15, 30, 45, 60, and 75 µg/mL) or 40 µL buffer (for the control) in a 96-well microtiter plate. The total volume of each mixture in the microtiter plate was adjusted to 160 µL by adding 40 µL of phosphate buffer and mushroom tyrosinase. The plates were kept for 15 min at room temperature and the absorbance reading of the reaction mixtures were taken at 490 nm using a microtiter plate reader. Percent tyrosinase inhibition of *C. minima* extract was calculated using the formula below:

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

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

2.8 Elastase inhibition assay

The anti-aging and anti-wrinkling properties of *C. minima* extract was evaluated using the protocol of Moon et al. [16]. Briefly, solutions of 0.2M TRIS-HCl buffer, pH 8.0, elastase from porcine pancreas (50 ug/mL, Sigma E-7885), and N-succinyl-$(ALA)_3$-p-nitroanilide (25 mM, Sigma S-4760) were prepared. Aliquot (40 µL) of *C. minima* extract or 40 µL buffer (for the control) were mixed with 40 µL N-succinyl-$(ALA)_3$-p-nitroanilide in sterile test tubes. The volume of the reaction mixture was adjusted to 1mL by adding first phosphate buffer and followed by 40 µL elastase in the solution. In this assay, the blank was the reaction mixture 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. Tocopherol was used as the positive control in the assay. Percent elastase inhibition was calculated using the formula:

$$Elastase\ Inhibition\ (\%) = \left(\frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}}\right) \times 100 \quad (4)$$

where A_{blank} is the absorbance of the blank, $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample (*C. minima* extract).

2.9 Antibacterial activity assay

The bacterial pathogens used in the antibacterial assay were obtained from the Philippine National Collection of Microorganisms, BIOTECH-UPLB. Four Gram-positive bacteria (*Staphylococcus aureus* BIOTECH 1823, *Staphylococcus saprophyticus* BIOTECH 1802, *Listeria monocytogenes* BIOTECH 1958, and *Bacillus cereus* BIOTECH 1635) and four Gram-negative bacteria (*Enterobacter aerogenes* BIOTECH 1145, *Aeromonas hydrophila* BIOTECH 10090, *Escherichia coli* BIOTECH 1634, and *Pseudomonas putida* BIOTECH 1506) were tested against *C. minima* crude extract using microtiter plate dilution assay. These bacterial pathogens were initially pre-cultivated using Luria Bertani (LB) broth medium and were incubated for 24 h at 37ºC with shaking. The purity and viability of each bacterial pathogens were monitored by conducting biochemical tests and morphological characterization regularly [4,5].

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *C. minima* extract was determined via two-fold serial dilution technique (microtiter plate dilution assay). Briefly, 100 µL of broth cultures of the bacterial test pathogens (cell density of 1x 10^6 cells/mL) were mixed with 100 µL of *C. minima* extract prepared at various dilutions (1000 µg/mL - 7.8125 µg/mL). Streptomycin was used in the assay as the control. The antibacterial assay was done in triplicates and were incubated for 12 h in an incubator set at 35°C. After incubation, MICs of *C. minima* extract against the bacterial pathogens were noted. On the other hand, MBC was determined using the protocol done by Arguelles et al. [8] Loopful of samples obtained from microtiter well plate that exhibited no visible bacterial growth from the MIC assay were inoculated onto a fresh rich culture medium (tryptic soy agar) plates. The plates were incubated at 35°C for 24 h and were evaluated for presence and absence of bacterial growth. Absence of bacterial growth (colony formation) would mean that the *C. minima* extract was bactericidal (completely killed) at that specific dilution [8,4,5].

2.10 Statistical analyses

The data obtained from the experimental assay are expressed as means ± standard deviations (mean ± SD) of three replicates [5,8]. The statistical tests used in correlation analysis to obtain the linear correlation coefficient were done using Microsoft Office Excel 2016.

3. Results and discussion

3.1 Proximate composition analysis

C. minima exhibited high concentrations of carbohydrate, ash, and protein (Table 1). The average carbohydrate content of the seaweed is 33.09 ± 0.14% which is greater than those observed for *Porphyra* sp. (20.59 ± 0.24%) and *C. minima* (3.87 ± 0.66%) from Matara district in Sri Lanka [17]. Carbohydrate is a key component for metabolism since it provides the energy needed for several important biological processes (such as respiration). The most common carbohydrates observed in brown macroalgae are cellulose, fucoidan, alginates, and laminaran [17,18]. In seaweeds, synthesis of carbohydrates are generally favored by light intensity and temperature while decreasing the lipids and protein content of the alga [18]. Protein and ash content of *C. minima*

were 25.89 ± 0.01% and 18.79 ± 0.02% (dry weight), respectively. The protein content are higher than those reported for *C. minima* (13.70. ± 0.2%), *Padina tetrastromatica* (11.39 ± 0.02%), and *Hormophysa triquetra* (15.34 ± 0.01%) but is comparable to that of *Porphyra* sp. (21.14 ± 0.04%) [17,18]. On the other hand, ash content was lower than those recorded for Philippine seaweeds such as *S. vulgare* (27.09 ± 0.00%) and *C. intricatum* (37.16 ± 0.21%) [4,8]. The amount of protein and ash in seaweeds varies not only among species of a certain genera but also between habitats and maturity level and are highly influenced by environmental conditions [4,8,18]. The lipid content of *C. minima* is greater than those seaweeds obtained from Gulf of Mannar such as *Padina tetrastromatica*, *Chnoospora minima*, *Sargassum wightii*, and *Hormophysa triquetra* with reported lipid content of 0.55 ± 0.002%, 0.45 ± 0.002%, 0.21 ± 0.001%, and 0.11 ± 0.001%, respectively [18]. The data obtained in this study for the lipid content (%) remained in the reported range (<10% on dry weight) from earlier studies for different seaweed species [4,8]. Although seaweeds contain low concentration of lipids, polyunsaturated fatty acid (PUFA) derived from this organism is considered more superior as compared to those found in vegetables in terms of its application to human diet [8]. *C. minima* exhibited a crude fiber and moisture content of 9.13 ± 0.11% and 6.23 ± 0.09%, respectively. The crude fiber content of *C. minima* is within the documented range of those earlier studies for brown seaweeds [8,17]. The dietary fiber derived from seaweeds have important biological properties such as anti-tumor and antiviral activities that have potential pharmaceutical application [10,19]. Variations in the proximate composition seaweeds (between species and strains) are possible if analyzed and collected at different season and geographical area. The results of the current study will serve as a baseline information that can be use in assessing the most suitable seasonal period for harvest of *C. minima* for large-scale production and sustainable use [4,8,20].

Table 1 Proximate composition of *Chnoospora minima*.

Proximate composition	Percent composition (%)
Moisture content	6.23 ± 0.09
Ash content	18.79 ± 0.02
Crude protein	25.89 ± 0.01
Crude fat	6.87 ± 0.04
Crude fiber	9.13 ± 0.11
Carbohydrate	33.09 ± 0.14

3.2 Total phenolic content (TPC)

Phenolic compounds are algal constituents that exhibit important bioactivities (such as antibacterial, antioxidant, and antidiabetic properties) that can be harness for pharmaceutical use. The TPC of *C. minima* was measured using the Folin-Ciocalteu reagent and is expressed in GAE per gram dry weight of the algal biomass. In this study, *C. minima* was observed to contain a total phenolic content of 9.90 ± 0.08 mg GAE/g. The TPC of *C. minima* is higher than those obtained from other brown seaweeds such as *Fucus serratus* (4.0 mg GAE/g), *Laminaria digitata* (2.93 mg GAE/g), and *Sargassum fusiforme* (6.0 mg GAE/g) [21,22]. However, it is lower than those obtained for *Saccharina latissima* (66.75 mg GAE/g) and *Undaria pinnatifida* (67.11 mg GAE/g) [23,24]. Generally, the amount of phenolic compounds in seaweeds is influenced by different ecological factors (e.g. seasonal variation, temperature, salinity, and light intensity), while their extraction and recovery analysis are highly affected by the chemical nature of the target phenolic compound, storage conditions, and presence or absence of interfering substances [6]. In this study, a polar solvent (acidified methanol) was used in the extraction to obtain the highest recovery of phenolic compounds in *C. minima* biomass since these compounds are highly soluble to this solvent.

3.3 Antioxidant activities

The antioxidant activities of *C. minima* extracts were assessed using $ABTS^+$ radical scavenging and CUPRAC assay. Two different antioxidant assays were used in this investigation to show the different mechanisms that take into account in the antioxidant activities of *C. minima* extract. Results showed that *C. minima* has potent antioxidant activity which is more effective than ascorbic acid (control). (Table 2). shows a dose-dependent scavenging activity of the seaweed extract against $ABTS^+$ free radicals. The computed IC_{50} of *C. minima* extract is 129 µg/mL which is more potent than ascorbic acid (control) with IC_{50} value of 161 µg/mL. Also, *C. minima* exhibited a more effective $ABTS^+$ radical scavenging activity than those obtained from aqueous extracts of *Amphiroa* sp*., Halimeda macroloba, Sargassum binderi,* and *Turbinaria conoides* with IC_{50} of 8.026 mg/mL, 14.397 mg/mL, 15.164 mg/mL, and 5.290 mg/mL, respectively [24]. In addition, *C. minima* extract also exhibited copper ion reduction ability (Table 3). The seaweed extract exhibited a concentration-dependent antioxidant

activity and potent copper reduction activity (IC_{50} of 28.59 µg/mL) more efficient than ascorbic acid (IC_{50} = 46.46 µg/mL). The copper reduction antioxidant capacity of *C. minima* is comparable to those observed from other brown seaweeds such as *Sargassum ilicifolium* (IC_{50} value of 11.19 µg/mL) and *Sargassum siliquosum* (IC_{50} of 18.50 µg/mL) [2,3]. The result observed in these antioxidant assays shows that *C. minima* is capable of inhibiting oxidation via free radical scavenging and metal chelation mechanisms. This potent activity maybe attributed to polyphenols that are present in the algal extract. Several seaweed-derived phenolic compounds (such as phlorotannins and phloroglucinol) are potent antioxidants that are capable of metal chelation as well as termination of autoxidation of free radicals by donating hydrogen atom from phenolic hydroxyl (OH) groups found in the chemical compound [24].

Table 2 $ABTS^+$ radical scavenging activity and IC_{50} value of phenolics from *Chnoospora minima* and ascorbic acid.

Sample	Phenolic concentration (µg GAE/mL)					IC_{50}* (µg/mL)
	37.5	75	112.5	150	187.5	
	$ABTS^+$ Inhibition (%)					
Chnoospora minima	17.86 ± 0.99	33.26 ± 0.70	45.92 ± 0.10	55.34 ± 0.30	60.13 ± 0.30	129
	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

*IC_{50} is the effective concentration that inhibits the activity of $ABTS^+$ (2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) cation radical by 50%. Computed by interpolation.
**A reference antioxidant.

Table 3 Copper reduction antioxidant capacity (CUPRAC) and IC_{50} value of phenolics from *Chnoospora minima* and ascorbic acid.

Sample	Phenolic concentration (µg GAE/mL)					IC_{50}* (µg/mL)
	6	12	18	24	30	
	CUPRAC value (Absorbance Reading at 450 nm)					
Chnoospora minima	0.128 ± 0.012	0.220 ± 0.011	0.327 ± 0.011	0.434 ± 0.013	0.520 ± 0.006	28.59
	Concentration (µg/mL)					
	10	20	30	40	50	
	CUPRAC value (Absorbance Reading 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

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

The correlation analysis between phenolic concentration of *C. minima* extracts and antioxidant activities using $ABTS^+$ free radical scavenging and CUPRAC assays is shown in (Table 4). Results showed a positive correlation among antioxidant activities ($ABTS^+$ radical scavenging and CUPRAC assays) and the phenolic concentrations of the seaweed extract with correlation coefficient of R=0.98158 and R=0.99930, respectively. Such result suggests that polyphenols play a significant role in the potent free radical scavenging and metal ion chelating activities exhibited by *C. minima* extract. In addition, the results support the findings that the acidified methanolic extract of *C. minima* can serve as a natural source of phenolic compounds that can be use for pharmaceutical application. The fadings of this analysis is similar to those previously documented studies about seaweeds showing positive correlations among antioxidant activities and phenolic contents such as *Laminaria digitata, Sargassum ilicifolium, Fucus serratus, Codium fragile, Sargassum vulgare,* and *Gracilaria gracilis* [3,8,21].

Table 4 Correlation between phenolic content and antioxidant activities of *Chnoospora minima* extract.

Antioxidant Assay	Regression Equation	Correlation Coefficient (R)
$ABTS^+$ Radical Scavenging Assay	y = 0.2843x + 10.516	0.98158
Copper Reduction Antioxidant Capacity (CUPRAC) Assay	y = 0.0166x + 0.0264	0.99930

3.4 Tyrosinase inhibition activities

Tyrosinase is a rate-limiting enzyme responsible for skin melanogenesis as well as browning of foods and vegetables. Thus, studies concerning discovery and synthesis of effective tyrosinase inhibitors that can be use in cosmetics and food processing for skin whitening and melanin hyperpigmentation are increasingly important [20,25]. The ability of *C. minima* extract to inhibit tyrosinase was evaluated *in vitro* using mushroom tyrosinase (Table 5). Results showed a concentration-dependent inhibition activity of the seaweed extract against tyrosinase. The computed IC_{50} of *C. minima* extract is 36 µg/mL which is more potent than the control (kojic acid) with computed IC_{50} value of 101 µg/mL. The result of this assay shows that the algal extract may contain bioactive substances with anti-melanogenic activities which can be use as an alternative source of whitening active ingredient. In addition, *C. minima* extract is more potent than those obtained from extracts of other brown algae from previous studies such as *Ecklonia stolinofera, Eucheuma cottonii, Turbinaria conoides, Sargassum plagyophyllum,* and *Ascophyllum nodosum,* with IC_{50} values of 0.345 mg/mL, 234.44 µg/mL, 188.5 µg/mL, 4.97 mg/mL, and 0.1 mg/mL respectively [26-30]. However, it is less potent than that observed for *Sargassum siliquosum* collected in Catanauan, Quezon in the Philippines with computed IC_{50} value of 18.50 µg/mL [2].

Table 5 Tyrosinase inhibition activity and IC_{50} value of phenolics from *Chnoospora minima* and kojic acid.

Sample	Phenolic concentration (µg GAE/mL)					IC_{50}* (µg/mL)
	15	30	45	60	75	
	Tyrosinase inhibition (%)					
Chnoospora minima	26.79 ± 1.68	41.23 ± 0.77	66.41 ± 1.70	79.46 ± 1.01	87.46 ± 0.35	36
	Concentration (µg/mL)					
	50	100	150	200	250	
	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

* IC_{50} is the effective concentration that inhibits tyrosinase activity by 50%. Computed by interpolation.
**Reference tyrosinase inhibitor and whitening agent.

The potent tyrosinase inhibition property exhibited by *C. minima* may be attributed to phenolic compounds (such as Dieckol, fucoxanthin, and 7-phloroeckol) that are present in the algal extract. These compounds are capable of causing stearic hindrances and conformational changes in the active site of tyrosinase via hydrogen bond formation causing impaired activity of the enzyme [2]. In addition, other known active compounds such as fucoidans and other bioactive peptides may also be present in *C. minima* extract which can act as tyrosinase inhibitors with anti-aging (elastase and hyaluronidase inhibition effects) activities as well as competitive enzyme inhibition properties causing suppression of melanogenesis [2,20,26].

3.5 Elastase inhibition activities

Skin aging is a process caused by several alterations of the dermal connective tissue that results to a notable decrease in the amount of elastin and collagen causing loss of flexibility and strength of the dermal tissue [20,31]. This process is highly influenced by elastase and collagenase that breakdowns elastin and collagen in the extra-cellular matrix. The anti-aging activity of *C. minima* extract was assessed *in vitro* via elastase inhibition assay (Table 6). Results showed that *C. minima* extract exhibited highest inhibition activity at 75 µg GAE/mL with percent inhibition of 78.76 ± 0.04%. The computed IC_{50} of *C. minima* extract is 56 µg/mL which is more potent than tocopherol (control) with IC_{50} value of >2500 µg/mL since 50% elastase inhibition was not achieved at 2500 µg/mL concentration. The elastase inhibition activity of *C. minima* is comparable to that observed from other brown seaweeds such as *Fucus spiralis* and *Agarum cribrosum* with IC_{50} value of 3.0 µg/mL and 16.13 µg/mL, respectively [32,33]. The result of this assay shows that the seaweed extract may contain substances with anti-aging activities that can be use as an active ingredient for pharmaceutical and cosmetic application.

Table 6 Elastase inhibition activity and IC_{50} value of phenolics from *Chnoospora minima* and tocopherol.

Sample	Phenolic concentration (μg GAE/mL)					IC_{50}*
	15	30	45	60	75	
	Elastase inhibition (%)					
Chnoospora minima	11.41 ± 0.92	29.44 ± 0.09	35.56 ± 2.97	55.35 ± 1.31	78.76 ± 0.04	56 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 by 50%. Computed by interpolation.
**Reference elastase inhibitor and whitening agent.
***IC_{50} was not determined because 50% inhibition was not achieved at 2500 μg/mL concentration.

The strong elastase inhibitory property of *C. minima* may be attributed to high concentration of phenolic compounds (e.g., phlorotannins) which are reported to have potent antioxidant and anti-aging properties. These compounds are also known for its collagenase and hyaluronidase inhibition activity showing its potential in cosmeceutical application [32,33]. To the best of our knowledge, this investigation is a pioneering study in the Philippines showing the potential of *C. minima* as an alternative source of naturally derived active ingredients with anti-wrinkling and anti-aging properties. Thus, additional experiments that will target the isolation and identification of these compounds as well as other cell-based assays are needed for large-scale utilization of this seaweed.

3.6 Antibacterial activities

Seaweeds contains lead bioactive substances with notable antibacterial activities that can be harnessed for medical use. In this study, antibacterial activities of *C. minima* extract against some medically important pathogenic bacteria was evaluated *in vitro* using microtiter plate dilution assay. *C. minima* exhibited antibacterial activities against *Staphylococcus aureus, Listeria monocytogenes*, and *Aeromonas hydrophila* with MIC ranging from 125-250 μg/mL (Table 7). In addition, the minimum bactericidal concentration (MBC) of *C. minima* is considered more potent in *Staphylococcus aureus* (250 μg/mL) than those observed for *Listeria monocytogenes* (500 μg/mL), and *Aeromonas hydrophila* (500 μg/mL). The antibacterial property of *C. minima* extract against *S. aureus* is more potent than those observed from methanol extracts of *Padina boergeseni*, *Padina antillarum*, and *Ulva flexuosa* from the Persian Gulf with MIC value of 15, 7.5, and 3.75 mg/mL, respectively [34]. Also, *C. minima* extract exhibited a stronger antibacterial activity against *L. monocytogenes* as compared to other seaweed species obtained from the coast of Vona in Turkey such as *Enteromorpha linza, Ulva rigida, Cystoseira barbata, Corallina officinalis, Ceramium ciliatum*, and *Padina pavonica* with MIC value of >2.2, >10, >2.5, >2.5, >2.5, and >1.25 mg/mL, respectively [35]. On the other hand, streptomycin exhibited antibacterial activities against all bacterial pathogens with MIC and MBC ranging from 250-1000 μg/mL. The antibacterial activity of *C. minima* can be attributed to bioactive substances present in the crude algal extract such as phenolic compounds which are reported to have potent antioxidant and antibacterial properties. These phenolic compounds upon exposure to bacterial cells can cause alteration in the cell membrane permeability which results to loss of cellular stability and cell death [8,36,37].

To the best of our knowledge, this paper is the first report about antibacterial activity of *C. minima* against *L. monocytogenes* and *Aeromonas hydrophila*. Thus, showing the great potential of this seaweed as novel and cheap sources of antibiotics for pharmaceutical application. In addition, the current study shows that *C. minima* extract are more effective towards Gram-positive bacteria, especially *L. monocytogenes* and *S. aureus* than Gram-negative bacteria. Such observation in the antibacterial activities can be attributed to differences in structure and function of bacterial cell wall. Gram-negative bacteria have complex and multilayered structure of cell wall which serve as an additional barrier that inhibits the penetration of antibiotics within the bacterial cells. [8,38].

Table 7 Antibacterial activities of *Chnoospora minima* extract and streptomycin (control).

Bacterial Pathogen	*C. minima* extract		Streptomycin	
	Minimum inhibitory concentration (µg/mL)	Minimum bactericidal concentration (µg/mL)	Minimum inhibitory concentration (µg/mL)	Minimum bactericidal concentration (µg/mL)
Gram-positive bacteria				
Staphylococcus aureus BIOTECH 1823	125.00	250.00	250.00	500.00
Listeria monocytogenes BIOTECH 1958	250.00	500.00	500.00	1000.00
Staphylococcus saprophyticus BIOTECH 1802	ND	ND*	250.00	500.00
Bacillus cereus BIOTECH 1635	ND	ND	500.00	1000.00
Gram-negative bacteria				
Aeromonas hydrophila BIOTECH 10090	250.00	500.00	500.00	1000.00
Pseudomonas putida BIOTECH 1506	ND	ND	500.00	1000.00
Escherichia coli BIOTECH 1634	ND	ND	500.00	1000.00
Enterobacter aerogenes BIOTECH 1145	ND	ND	500.00	1000.00

*ND = No antibacterial activity was observed.
ND=No antibacterial activity was detected.

4. Conclusion

The study showed that *C. minima* (Hering) Papenfuss contain high amounts of carbohydrate, protein, ash, and phenolic compounds. In addition, the seaweed extract exhibited promising bioactivities such as antibacterial and antioxidant activities as well as elastase and tyrosinase inhibition properties that can be harness for pharmaceutical application. This study is considered as a preliminary investigation in the potential biotechnological use of *C. minima*. Thus, additional studies are recommended to identify the active compounds and further understand the reaction mechanisms involved in the active substances present in the algal extract. Also, *in vivo* experimental trials are also needed to support the effectivity and safety of *C. minima* extract for treatment of human diseases.

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6. References

[1] Gunathilaka TL, Samarakoon KW, Ranasinghe P, Peiris LDC. *In vitro* antioxidant, hypoglycemic activity, and identification of bioactive compounds in phenol-rich extract from the marine red algae *Gracilaria edulis* (Gmelin) Silva. Molecules. 2019;24(20):3708.

[2] Arguelles EDLR, Sapin AB. Bioactive properties of *Sargassum siliquosum* J. Agardh (Fucales, Ochrophyta) and its potential as source of skin-lightening active ingredient for cosmetic application. J Appl Pharm Sci. 2020;10(7):51-58.

[3] Arguelles EDLR. Evaluation of antioxidant capacity, tyrosinase inhibition, and antibacterial activities of brown seaweed, *Sargassum ilicifolium* (Turner) C. Agardh 1820 for cosmeceutical application. J Fisher Environ. 2021;45(1):64-77.

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

[5] Arguelles EDLR, Sapin AB. *In vitro* antioxidant, alpha-glucosidase inhibition, and antibacterial properties of *Turbinaria decurrens* Bory (Sargassaceae, Ochrophyta). Asia Pac J Sci Technol. 2020;25(3):1-9.

[6] Mekinić IG, Šimat V, Botić V, Crnjac A, Smoljo M, Soldo B, et al. Bioactive phenolic metabolites from Adriatic brown algae *Dictyota dichotoma* and *Padina pavonica* (Dictyotaceae). Foods. 2021;10:1187.

[7] Parveen S, Nadumane VK. Anti-angiogenesis and apoptogenic potential of the brown marine alga, *Chnoospora minima*. Future J Pharm Sci. 2020;6:19.

[8] Arguelles EDLR, Monsalud RG, Sapin AB. Chemical composition and *In vitro* antioxidant and antibacterial activities of *Sargassum vulgare* C. Agardh from Lobo, Batangas, Philippines. J ISSAAS. 2019;25(1):112-122.

[9] Nelson WA, Duffy CAJ. *Chnoospora minima* (Phaeophyta) in Port Underwood, Marlborough - a curious new algal record for New Zealand. NZJ Bot. 1991;29(3):341-344.

[10] Galway: National University of Ireland. AlgaeBase, http://www.algaebase.org [accessed 30 March 2021].

[11] Trono Jr GC. Field guide and atlas of the seaweed resources of the Philippines. Manila: Bookmark; 1997.

[12] Association of Official Analysis Chemistry. Official methods of analysis of AOAC international. 18th ed. Gaitherburg: AOAC International; 2011.

[13] Gao L, Wang S, Oomah BD, Mazza G. Wheat quality: antioxidant activity of wheat millstreams. In: Ng P, Wrigley CW, editors. Wheat quality elucidation. 1st ed. Minnesota: AACC International; 2002. p. 219-233.

[14] Re R, Pellegrine N, Proteggente A, Pannala A, Yang M, Evans RC. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26:1231-1237.

[15] Plaza M, Benavent AM, Castillo MD, Ibáñez E, Herrero M. Facts about the formation of new antioxidants in natural samples after subcritical water extraction. Food Res Int. 2010;43:2341-2348.

[16] Moon JY, Yim EY, Song G, Lee NH, Yun CG. Screening of elastase and tyrosinase inhibitory activity from Jeju Island plants. Eur Asian J Biosci. 2010;4:41-53.

[17] Jayakody MM, Vanniarachchy MPG, Wijesekara I. Composition analysis of selected Sri Lankan seaweeds. J Trop For Environ. 2019;9(2):93-100.

[18] Kokilam G, Vasuki S, Sajitha N. Biochemical composition, alginic acid yield and antioxidant activity of brown seaweeds from Mandapam region, Gulf of Mannar. J Appl Pharm Sci. 2013;3(11):99-104.

[19] Ahmad F, Sulaiman FR, Saimon W, Yee CF, Matanjun P. Proximate compositions and total phenolic contents of selected edible seaweed from Semporna, Sabah, Malaysia. Borneo Sci. 2012;31:85-96.

[20] Arguelles EDLR, Sapin AB. Chemical composition and bioactive properties of Sargassum aquifolium (Turner) C. Agardh and its potential for pharmaceutical application. Philipp J Sci. 2021;151(S1):9-24.

[21] Heffernan N, Smyth TJ, Villa SA, Fitzgerald RJ, Brunton NP. Phenolic content and antioxidant activity of fractions obtained from selected Irish macroalgae species (Laminaria digitata, Fucus serratus, Gracilaria gracilis and Codium fragile). J Appl Phycol. 2015;27:519-530.

[22] Machu L, Misurcova L, Ambrozova VJ, Orsavova J, Mlcek J, Sochor J, et al. Phenolic content and antioxidant capacity in algal food products. Molecules. 2015;20:1118-1133.

[23] Cox S, Ghannam AN, Gupta S. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int Food Res J. 2010;17:205-220.

[24] Boonchum W, Peerapornpisal Y, Kanjanapothi D, Pekkoh J, Pumas C, Jamjai U, et al. Antioxidant activity of some seaweed from the Gulf of Thailand. Int J Agric Biol. 2011;13(1):95-99.

[25] Tu PTB, Tiwata S. Anti-oxidant, anti-aging, and anti-melanogenic properties of the essential oils from two varieties of Alpinia zerumbet. Molecules. 2015;20:16723-16740.

[26] Jiménez JT, O'Connell S, Lyons H, Bradley B, Hall M. Antioxidant, antimicrobial, and tyrosinase inhibition activities of acetone extract of Ascophyllum nodosum. Chem Pap. 2010;64(4):434-442.

[27] Kang HS, Kim HR, Byun DS, Son BW, Nam TJ, Choi JS. Tyrosinase inhibitors isolated from the edible brown alga Ecklonia stolonifera. Arch Pharm Res. 2004;27:1226-32.

[28] Sari DM, Anwar E, Nurjanah N, Arifianti AE. Antioxidant and tyrosinase inhibitor activities of ethanol extracts of brown seaweed (Turbinaria conoides) as lightening ingredient. Pharmacog J. 2019;11(2):379-382.

[29] Arifianti AE, Anwar E, Nurjanah. Aktivitas penghambatan tirosinase dan antioksidan serbuk rumput laut dari Sargassum plagyophyllum segar dan kering. J Pengolah Has Perikan Indones. 2017;20(3):488-493.

[30] Chang VS, Teo SS. Evaluation of heavy metal, antioxidant and anti-tyrosinase activities of red seaweed (Eucheuma cottonii). Int Food Res J. 2016;23(6):2370-2373.

[31] Susano P, Silva J, Alves C, Martins A, Gaspar H, Pinteus S, et al. Unravelling the dermatological potential of the brown seaweed Carpomitra costata. Mar. Drugs. 2021;19:135.

[32] Freitas R, Martins A, Silva J, Alves C, Pinteus S, Alves J, et al. Highlighting the biological potential of the brown seaweed Fucus spiralis for skin applications. Antioxidants. 2020;9(7):1-21.

[33] Phasanasophon K, Kim SM. Antioxidant and cosmeceutical activities of Agarum cribrosum phlorotannin extracted by ultrasound treatment. Nat Prod Comm. 2018;13(5):565-570.

[34] Mashjoor S, Yousefzadi M, Esmaeili MA, Rafiee R. Cytotoxicity and antimicrobial activity of marine macro algae (Dictyotaceae and Ulvaceae) from the Persian Gulf. Cytotechnology. 2016;68:1717-1726.
[35] Ertürk Ö, Ta B. Antibacterial and antifungal effects of some marine algae. Kafkas Univ Vet Fak Derg. 2011;17:121-124.
[36] Arguelles EDLR. Bioactive properties of Halymenia durvillei Bory 1828 for pharmaceutical application: antioxidant, antidiabetic, antiwrinkling and skin-whitening activities. Yuz Yil Univ J Agric Sci. 2022;32(1): 57-68.
[37] Arguelles EDLR, Sapin AB. Bioactive properties and therapeutic potential of Padina australis Hauck (Dictyotaceae, Ochrophyta). Int J Agric Technol. 2022;18(1):13-34.
[38] Arguelles EDLR, Sapin AB. Bioprospecting of Turbinaria ornata (Fucales, Phaeophyceae) for cosmetic application: antioxidant, tyrosinase inhibition and antibacterial activities. J ISSAAS. 2020;26(2):30-41.