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Research Article

Bioactive Properties of *Halymenia durvillei* Bory 1828 for Pharmaceutical Application: Antioxidant, Antidiabetic, Antiwrinkling and Skin-Whitening Activities

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Abstract: Seaweeds are known sources of bioactive compounds that provide diverse health benefits. However, knowledge on the potential pharmaceutical application of some economically important seaweeds such as *Halymenia durvillei* is still limited. In this study, the bioactive properties of *H. durvillei* were studied. The results showed that the macroalga contains a total phenolic content (TPC) of 6.77 ± 0.03 mg GAE/g. Antioxidant activity of *H. durvillei* exhibited potent $ABTS^+$ radical scavenging activity and high copper reduction capacity with IC_{50} value of 106 µg GAE/mL and 20.44 µg GAE/mL, respectively. *In vitro* assessment of tyrosinase and elastase inhibition properties of *H. durvillei* extract showed that the alga has potent inhibitory activity with IC_{50} of 40 µg GAE/mL and IC_{50} of 696 µg GAE/mL, respectively more effective than kojic acid and tocopherol. In addition, evaluation of α-amylase inhibition properties showed that *H. durvillei* extract has potent inhibitory activity with IC_{50} value of 56 µg GAE/mL more effective than acarbose (standard anti-diabetic drug) with IC_{50} of 101 µg/mL. The current investigation shows the potential of *H. durvillei* for the pharmaceutical application, which can be utilized for the synthesis of novel drugs.

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Farmasötik Uygulama İçin *Halymenia durvillei* Bory 1828'in Biyoaktif Özellikleri: Antioksidan, Antidiyabetik, Kırışıklık Önleyici ve Cilt Beyazlatıcı Aktiviteler

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Anahtar Kelimeler

Biyolojik aktivite,
Kimyasal bileşim,
Deniz,
Filipinler,

Öz: Deniz yosunları, çeşitli sağlık yararları sağlayan biyoaktif bileşiklerin bilinen kaynaklarıdır. Bununla birlikte, *Halymenia durvillei* gibi ekonomik açıdan önemli bazı deniz yosunlarının potansiyel farmasötik uygulamalarına ilişkin bilgiler hala sınırlıdır. Bu çalışmada H. durvillei'nin biyoaktif özellikleri incelenmiştir. Sonuçlar, makroalganın 6.77 ± 0.03 mg GAE/g toplam fenolik içerik (TPC) içerdiğini gösterdi. H. durvillei'nin antioksidan aktivitesi, sırasıyla 106 µg GAE/mL ve 20.44 µg GAE/mL IC_{50} değeri ile güçlü $ABTS^+$ radikal süpürme aktivitesi ve yüksek bakır indirgeme kapasitesi sergiledi. *H. durvillei* ekstraktının tirozinaz ve elastaz inhibisyon özelliklerinin in vitro değerlendirmesi, alg'in IC_{50}'si 40 µg GAE/mL ve IC_{50}'si 696 µg GAE/mL 'lik güçlü inhibitör aktiviteye sahip olduğunu ve kojik asit ve tokoferolden daha etkili olduğunu gösterdi. Ek olarak,

Yosunlar

α-amilaz inhibisyon özelliklerinin değerlendirilmesi, *H. durvillei* ekstraktının IC_{50} değeri 56 µg GAE/mL olan güçlü inhibitör aktiviteye sahip olduğunu ve IC50 değeri 101 µg/mL olan akarbozdan (standart anti-diyabetik ilaç) daha etkili olduğunu göstermiştir. Mevcut araştırma, yeni ilaçların sentezi için kullanılabilen farmasötik uygulama için *H. durvillei*'nin potansiyelini göstermektedir.

1. Introduction

Over the past few years, there has been an increasing demand to develop natural, cheap, and novel bioactive compounds that will address emerging diseases such as cancer, diabetes, and microbial infections (Sobuj et al., 2021). Natural bioactive compounds currently being screened for this purpose includes phenolic compounds, polysaccharides, essential oils, and bioactive peptides derived from seaweeds. Marine algae are rich sources of bioactive compounds since these organisms live in extreme environments where temperature, light, and salinity are constantly fluctuating (Arguelles and Sapin, 2021). Such conditions allowed seaweeds to develop diverse secondary metabolites that possess unique and potent biological activities. These metabolites are considered valuable bioactive substances that can be used as functional ingredients for food, industrial, and medical applications (Sari et al., 2019; Sobuj et al., 2021).

Halymenia durvillei Bory 1828 is a widely distributed seaweed belonging to Rhodophyta. It is characterized by having soft, flattened, and cartilaginous branches with cylindrical stipe. The branch of this seaweed is red to orange in color and is usually arranged as alternate or pinnate forming bushy clumps on solid rock surfaces (Trono, 1997). *H. durvillei* is considered as a high value marine algae because of its carrageenan content (Trono and Largo, 2019). This commercially important seaweed can also be an alternative source of active metabolites such as proteins, carotenoids, phenolic compounds, and arachidonic acid. These compounds have biological activities such as antioxidant, anti-diabetic, and antimicrobial activities that can be harnessed for pharmaceutical application. In addition, the natural pigment of *H. durvillei,* such as phycoerythrin, can also be produced and utilized as natural colorants for commercial application (Trono and Largo, 2019).

The Philippines, being at the center of the Coral Triangle, is known for its rich marine algal flora with diverse bioactive metabolites. However, only limited reports are available on the bioactive properties of seaweeds in the Philippines (Arguelles 2020; Magdugo et al., 2020; Arguelles and Sapin 2020a, b, c; Arguelles 2021b; Arguelles and Sapin, 2021). Thus, the current study aims to document some of the important biological properties of *H. durvillei* for potential medical application. The study specifically aims to evaluate the total polyphenolic content (TPC), antioxidant (using $ABTS^{+}$ radical scavenging and copper reduction antioxidant capacity (CUPRAC) assays), anti-diabetic, as well as tyrosinase and elastase inhibition properties of *H. durvillei*. In addition, correlation analysis on the phenolic content of the seaweed extract and its antioxidant activities were also established.

2. Material and Methods

2.1. Seaweed sampling and collection

Fresh algal biomass of *Halymenia durvillei* Bory was collected on 08 March 2021 in the coast of Nasugbu (Lat. 14° 5' 31.4484" N; Long. 120° 37' 21.8382" E), Batangas, Philippines (Figure 1). The biomass was rinsed with sterile distilled water several times to remove epiphytes and sand particles in the algal sample. *Halymenia durvillei* was oven-drying at 60 °C for 12 hours and was pulverized (250–500 µm) before subjecting it for solvent extraction. The taxonomic identification of the seaweed was done using the taxonomic keys of Guiry and Guiry (2021) on Algae Base (web site: www.algaebase.org) and Trono (1997). The seaweed was verified by the algae curator of the National Institute of Molecular Biology and Biotechnology (BIOTECH), Laguna, Philippines.

Figure 1. Thallus morphology of *H. durvillei* from the coast of Nasugbu, Batangas.

2.2. Preparation of seaweed extract

Powdered algal biomass of *H. durvillei* (1 gram) was subjected to solvent extraction following the protocol of Gao *et al*., (2002). The biomass was extracted using 30 mL acidified methanol (1 HCl: 80 CH_3OH: 10 H_2O) in an ultrasonic bath for 30 minutes with continuous stirring for 1 hour. The algal mixture was then centrifuged at 12,000 rpm for 20 minutes at a temperature of 20 °C. The harvested algal extract was further concentrated via rotary evaporator (BUCHI Rotavapor®) set at 40 °C under reduced pressure. The algal extract was placed under refrigerated conditions (4 °C) to preserve its biological activity for use in different biological assays included in the current study (Arguelles and Sapin, 2020a). The yield extract of *H. durvillei* was calculated using the equation:

$$Yield\ (\%) = \left(\frac{Weight\ of\ the\ algal\ extract\ (g)}{Weight\ of\ the\ dried\ algal\ biomass\ (g)}\right) \times 100 \quad (1)$$

2.3. Determination of total phenolic content (TPC)

The TPC of *H. durvillei* was analyzed via Folin-Ciocalteu assay following the methods of Nuñez-Selles et al., (2002) and expressed as microgram (μg) of gallic acid equivalent (GAE) per gram of the seaweed biomass (calibration curve equation: y = 0.006415x – 0.0140, R^2 = 0.99978). Briefly, about 0.5 mL of *H. durvillei* extract was mixed with 0.5 mL 10% sodium carbonate solution and 0.5 mL of Folin-Ciocalteau's reagent for 1 minute. The sample mixture was mixed and set aside from at room temperature for 5 minutes. The volume of the mixture was adjusted using 5 mL distilled water. The absorbance reading of the sample and control were taken using an Ultraviolet-Visible spectrophotometer at a wavelength set at 720 nm.

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

The $ABTS^+$ scavenging assay for *H. durvillei* was done following the procedure of by Re et al. (1999) with a few modifications in the procedure. Briefly, 40 μL of *H. durvillei* extract prepared in different concentrations (30.0 – 150.0 μg GAE/mL) and 40 μL of 90% methanol (control) were mixed with 3 mL of $ABTS^+$ radical mixture with an initial absorbance reading of 0.72 ± 0.05 at 734 nm. The reaction mixtures were mixed and placed at ambient room temperature for 5 min. The absorbance readings of each prepared reaction sample solution were taken at 734 nm. $ABTS^+$ inhibition (%) was noted using the equation:

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

Where A_{sample} is the absorbance reading of the sample (algal extract) and $A_{control}$ is the absorbance reading of the control (ascorbic acid). The $ABTS^+$ inhibition activity (%) was plotted with different prepared concentrations of *H. durvillei* extract. IC_{50} of the seaweed extract is the concentration the seaweed extract that exhibited 50% $ABTS^+$ radical scavenging activity.

2.5. Copper reduction antioxidant capacity (CUPRAC) assay

The copper reduction antioxidant capacity assay for *H. durvillei* extract was made using the methods of Alpinar *et al*., (2009). In this assay, 1 mL each of 0.01 M $CuCl_2$ solution, 0.0075 M neocuproine, and 1 M ammonium acetate buffer (pH 7) were mixed in sterile test tubes containing 0.5 mL of *H. durvillei* extract (at a different prepared phenolic concentration) and ascorbic acid (control) (Arguelles et al., 2019). The volume of the sample mixtures was adjusted (to 4.1 mL) using sterile distilled water and was kept at room temperature for 30 min. The absorbance reading for both the *H. durvillei* extract and ascorbic acid concentrations was noted at 450 nm (Arguelles, 2021a).

2.6. Tyrosinase inhibition assay

The whitening property of *H. durvillei* extract was evaluated *in vitro* using tyrosinase inhibition assay following the methods of Hapsari *et al*., (2012). Solutions of mushroom tyrosinase (250 units/mL, Sigma T- 3824), 5mM DOPA (3,4-dihydroxy-L-phenylalanine, Sigma D-9628), and 0.1M potassium phosphate buffer (pH 6.5) were prepared. Briefly, an aliquot of 40 µL DOPA is mixed with 40 µL of *H. durvillei* extract (at varying concentration: 15.0, 30.0, 45.0, 60.0, and 75.0 µg GAE/mL) or 40 µl buffer (for the control) in a microtiter plate. The total volume of each reaction sample mixture was adjusted to 160 µL by adding 40 µL of phosphate buffer and mushroom tyrosinase. The microtiter plate containing the mixtures was incubated at ambient temperature for 15 min. The absorbance reading was taken using a microtiter plate reader at a wavelength of 490 nm (Hapsari et al., 2012). The percent tyrosinase inhibition was computed using the equation below:

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

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

2.7. Elastase inhibition assay

The anti-wrinkling property of *H. durvillei* extract was evaluated using elastase inhibition activity assay following the procedure of Moon *et al*., (2010). Initially, solutions of elastase from porcine pancreas (50 ug/mL, Sigma E-7885), N-succinyl-$(ALA)_3$-p-nitroanilide (25 mM, Sigma S-4760), and 0.2M TRIS-HCl buffer (pH 8.0) were prepared. An aliquot (40 µL) of the *H. durvillei* extract or 40 µL buffer (control) was thoroughly mixed with 40 µL N-succinyl-$(ALA)_3$-p-nitroanilide in sterile test tubes. The volume of the reaction mixture was adjusted to 1 mL using phosphate buffer, and 40 µL elastase was added last in the solution. The blank (control) tube was the one that did not contain the enzyme solution. After 20 minutes of incubation, 2 mL of TRIS-HCl buffer were put in the reaction mixtures, and the absorbance reading of each sample was taken at 410 nm wavelength. The percent elastase inhibition was determined using the equation:

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

Where $A_{control}$ is the absorbance reading of the control, A_{blank} is the absorbance reading of the blank, and A_{sample} is the absorbance reading of the sample (algal extract). Tocopherol was used as the positive control in the assay.

2.8. α-amylase inhibition assay

The anti-diabetic properties of *H. durvillei* extract were assessed *in vitro* using α-amylase inhibition assay using the procedures of Phoboo (2015) with slight modifications. Initially, solutions of alpha-amylase from porcine pancreas (0.5 mg/mL, Sigma A3176), 0.02 M Sodium-phosphate buffer, pH 6.9 with 0.006M NaCl and 1% starch solution were prepared. Varying concentrations of *H. durvillei* extract (20.0, 40.0, 60.0, 80.0, and 100.0 μg GAE/mL) were prepared by dilution with water. To 50 µL of the alpha-amylase solution, 25 µL of *H. durvillei* extracts or 25 µL buffer (for the control) were thoroughly mixed in sterile test tubes. The total volume of the sample mixtures was adjusted up to 250 µL by adding 175 µL phosphate buffer. These mixtures were then added (at timed intervals) with 250 µL starch solution and were kept for 20 minutes. After incubation, the mixture was halted by adding 400 µL of dinitrosalicylic acid (DNS) color reagent (also added at timed intervals). The blank (control) used in the assay was consisted of 400 µL DNS reagent and 500 µL buffer. The reaction mixtures (in test tubes) were subjected to a boiling water bath for 5 minutes, cooled, and were further diluted with 5 mL sterile distilled water. The absorbance reading of the sample mixtures and control were taken at a 540 nm wavelength. The percent (%) inhibition was determined using the equation:

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

2.9. Statistical Analyses

The assays used in this study were done in three replicates, and the data were given as means ± standard deviations. The correlation analysis among antioxidant activity and the phenolic concentration of *H. durvillei* extract using Pearson's correlation coefficient (r) was evaluated using Microsoft (MS) Office Excel 2007.

3. Results and Discussion

3.1. Extraction yield and TPC

Halymenia durvillei was extracted using acidified methanol (1:30) with stirring for 1 hour using an ultrasonic bath. To obtain the crude seaweed extract, the liquid soluble active constituents of the extract were concentrated and dried via a rotary evaporator. In this study, crude extract of *H. durvillei* was observed to have a reddish to brown color, which can be attributed to algal pigments such as phycoerythrin and other algal pigments present in the algal biomass (Sobuj et al., 2021). The extraction yield of *H. durvillei* crude extract is 14.91 ± 0.02%. This yield is higher than those obtained by Boonchum *et al*., (2017) from *Sargassum binderi*, *Tubinaria conoides*, *Amphiroa* sp., and *Halimeda macroloba* with extraction yields of 12.25%, 6.41%, 2.94%, and 2.52%, respectively. Variations in the extraction yield of *H. durvillei* as compared to other seaweeds previously reported may be due to factors such as extraction method and polarity of the solvent extractant, which are critical for large scale use of bioactive products that can be harnessed to *H. durvillei* (Boonchum et al., 2017).

Table 1. Total phenolic content and extraction yield of *Halymenia durvillei* acidified methanolic extract

Sample	Extract	Extraction Yield (%)[1]	Total Phenolic Content (mg GAE/g)[1]
Halymenia durvillei	Acidified Methanol	14.91 ± 0.02	6.77 ± 0.03

[1]Mean± Standard deviation.

Seaweeds are good sources of phenolic compounds with potential pharmacological use. Phenolic compounds such as catechins (catechin gallate and epicatechin), flavonols, and phlorotannins were documented from red and brown seaweeds (Yoshie-Stark et al., 2003). These compounds are

considered valuable because of the reported antioxidant and antibacterial activities that can be used as active ingredients for novel drug synthesis (Yoshie-Stark et al., 2003; Mekinić et al., 2019). In addition, these biologically active algal metabolites act as defense mechanisms for adaptation against harsh conditions in the marine environment (Mekinić et al., 2019). The TPC of *H. durvillei* is 6.77 ± 0.03 mg GAE/g extract (Table 1). This result was greater than those observed from other seaweeds such as *Sargassum polycystum, Zonaria tournefortii*, *Fucus serratus,* and *Himanthalia elongata* with TPC of 0.37 mg GAE/g, 0.78 mg GAE/g, 4.0 mg GAE/g, and 5.48 mg GAE/g, respectively (O'Sullivan et al., 2011; Fu et al., 2015; Belda et al., 2016; Fellah et al., 2017). On the other hand, Chakraborty et al., (2013) showed that dichloromethane extracts of *Turbinaria conoides* and *Turbinaria ornata* have higher TPC than *H. durvillei*, which are 51.47 mg GAE/g, and 12.72 mg GAE/g, respectively. In general, the amount and kinds of phenolic compounds in algal biomass is highly affected by the age and maturity of the seaweed as well as the type and polarity of solvent used in the extraction (Mekinić et al., 2019).

3.2. $ABTS^+$ scavenging activity

Phenolic compounds are potent free-radical scavengers since these compounds possess reducing properties that serve as electron or hydrogen donating agents (Boonchum et al., 2011; Orak, et al., 2021). In this study, *H. durvillei* exhibited potent $ABTS^+$ radical scavenging activity, more efficient than ascorbic acid (Table 2). The $ABTS^+$ scavenging activity of the algal extract exhibited inhibition of $ABTS^+$ free radicals in a concentration-dependent manner. The computed effective concentration (IC_{50}) of *H. durvillei* extract is 106 µg/mL, which is more effective as compared to ascorbic acid with IC_{50} value of 151 µg/mL. This antioxidant activity is considered more potent than that obtained for methanol extracts of *Padina tetrastromatica* (IC_{50} value of 1.33 ± 0.09 mg/mL) and *Gracilaria tenuistipitata* (IC_{50} value of 3.01 ± 0.10 mg/mL) (Sobuj et al., 2021). However, $ABTS^+$ scavenging activity of *H. durvillei* is less effective as compared to *Turbinaria decurrens* with IC_{50} value of 49.31 µg/mL (Arguelles and Sapin, 2020a). The polarity of the solvent extractant and the method of extraction are critical factors in determining the amount and activity of polyphenols that can be obtained from a seaweed sample. In general, polar solvents (e.g. methanol) are effective extractants of phenolic compounds since these solvent can inhibit and suppress the activity of polyphenol oxidase (Boonchum et al., 2011; Sobuj et al., 2021).

Table 2. $ABTS^+$ radical scavenging activity and IC_{50} value of *H. durvillei* extract and ascorbic acid

Halymenia durvillei		**Ascorbic Acid****	
Phenolic concentration (µg GAE/mL)	**$ABTS^+$ Inhibition (%)[1]**	**Concentration (µg/mL)**	**$ABTS^+$ Inhibition (%)[1]**
30	18.00 ± 1.19	37.5	14.34 ± 0.49
60	34.04 ± 0.00	75.0	25.82 ± 0.00
90	45.15 ± 0.80	112.5	38.07 ± 0.20
120	54.36 ± 1.29	150.0	49.69 ± 0.10
150	64.70 ± 0.99	187.5	62.70 ± 0.79
IC_{50}*	**106 µg/mL**	**IC_{50}***	**151 µg/mL**

*IC_{50} is the concentration that effectively inhibits the activity of $ABTS^+$ cation radical by 50%. Computed by interpolation.
**A reference antioxidant.
[1]Mean± Standard deviation.

3.3. Copper reduction antioxidant capacity (CUPRAC)

CUPRAC method is a redox reduction assay between CUPRAC reagent (cupric neocuproine) and antioxidants (in the algal extract) producing Cu(I)-neocuproine chromophore, which is measured spectrophotometrically (Arguelles, 2021a). *Halymenia durvillei* extract exhibited a concentration-dependent copper ion reduction ability which is similar to that observed for $ABTS^+$ scavenging assay wherein at high phenolic concentration, the highest $ABTS^+$ free radical inhibition was observed. Table 3 shows that *H. durvillei* extract exhibited potent antioxidant activity more effective than ascorbic acid with IC_{50} value of 20.44 µg/mL. Also, *H. durvillei* is more effective than that obtained for *Turbinaria ornata* from the coast of Catanauan, Quezon, with IC_{50} value of 24.34 µg/mL (Arguelles and Sapin, 2020c). The study shows the potential of *H. durvillei* extract as an effective inhibitor of oxidation (via

metal chelation mechanism). This activity is possibly associated with polyphenols (e.g. quercetin and gallic acids) present in *H. durvillei* extract, which are known metal chelators (Arguelles and Sapin, 2020c).

Table 3. Copper reduction antioxidant capacity and IC_{50} value of *H. durvillei* extract and ascorbic acid

Halymenia durvillei		**Ascorbic Acid****	
Phenolic concentration (µg GAE/mL)	**CUPRAC value (Absorbance at 450 nm)[1]**	**Concentration (µg/mL)**	**CUPRAC value (Absorbance at 450 nm)[1]**
5.0	0.114 ± 0.002	10.0	0.114 ± 0.001
10.0	0.263 ± 0.003	20.0	0.232 ± 0.002
15.0	0.382 ± 0.001	30.0	0.347 ± 0.001
20.0	0.490 ± 0.004	40.0	0.454 ± 0.008
25.0	0.603 ± 0.000	50.0	0.566 ± 0.005
IC_{50}*	**20.44 µg/mL**	**IC_{50}***	**44.12 µg/mL**

*IC_{50} is the concentration that gives a CUPRAC value of 0.5 at a wavelength of 450 nm. Computed by interpolation.
**A reference antioxidant.
[1]Mean± Standard deviation.

3.4. Correlation study between TPC and antioxidant activity

The correlation analysis among phenolic concentrations in the algal extract and antioxidant activities of *H. durvillei* using $ABTS^+$ scavenging and CUPRAC assays are presented in Table 4. Results showed a positive correlation exists for $ABTS^+$ (r =0.9938) and CUPRAC (r =0.9979) and phenolic concentration of the *H. durvillei* extract. This correlation is similar to previous studies that reported positive correlations among antioxidant activities and phenolic concentration from several species of seaweeds such as *Sargassum ilicifolium, Codium intricatum, Sargassum siliquosum, Sargassum vulgare,* and *Turbinaria decurrens* (Arguelles et al., 2019; Arguelles, 2020; Arguelles, 2021b; Arguelles and Sapin, 2020a,b). Furthermore, the positive correlation between CUPRAC and $ABTS^+$ antioxidant activities and phenolic concentrations in *H. durvillei* extract shows that polyphenols are responsible for the antioxidant activity of the seaweed. Thus, isolation and identification of these compounds are necessary to deepen our understanding of the potent antioxidant activities of the algal extract.

Table 4. Correlation between phenolic content and antioxidant activities of *Halymenia durvillei* extract

Antioxidant Assay	**Correlation Coefficient (r)**	**p-value***
$ABTS^+$ Radical Scavenging Assay	0.9938	0.000592
Copper Reduction Antioxidant Capacity (CUPRAC) Assay	0.9979	0.000116

*Correlation is significant at $p<0.05$.

3.5. Tyrosinase inhibition activity

Seaweeds are known sources of skin whitening ingredients that can be tapped for cosmeceutical application (Sari et al., 2019). In this investigation, the whitening ability of *H. durvillei* extract was evaluated *in vitro* via inhibition of tyrosinase. Tyrosinase is an important enzyme that controls monophenolase and diphenolase reactions in melanin synthesis. Thus, inhibition of this enzyme can reduce the formation of melanin that leads to brown pigmentation (Arguelles, 2021b). *Halymenia durvillei* extract exhibited potent tyrosinase inhibition in a dose-dependent manner (Table 5). The computed IC_{50} of *H. durvillei* extract is 40 µg/mL, which is more effective than the control (kojic acid) with IC_{50} value of 113 µg/mL. *H. durvillei* extract is also more effective than those obtained from extracts of *Turbinaria ornata* and *Turbinaria conoides* with IC_{50} values of 67.5 µg/mL, and 188.5 µg/mL, respectively (Sari et al., 2019; Arguelles, 2021b). The result of this essay suggests that *H. durvillei* extract contains bioactive substances (such as phenolic compounds) with potent anti-melanogenic activities. Phenolic compounds are known tyrosinase inhibitors capable of causing conformational changes in the active site of tyrosinase, leading to the inactivation of the enzyme (Susano et al., 2021). The differences in the potency of the different seaweed extracts in inhibiting tyrosinase are

caused by factors such as extraction protocol, the polarity of the solvent, age, and maturity of the alga, which are being considered in the assay. Variations in these parameters can cause differences in the amount and types of bioactive substances that are present in the crude extract of the alga, causing differences in the activity and effectiveness of the extract (Dolorosa et al., 2019).

Table 5. Tyrosinase inhibition activity and IC_{50} value of *H. durvillei* extract and kojic acid

Halymenia durvillei		**Kojic Acid**[**]	
Phenolic concentration (µg GAE/mL)	**Tyrosinase Inhibition (%)**[1]	**Concentration (µg/mL)**	**Tyrosinase Inhibition (%)**[1]
15.0	25.84 ± 0.99	50.0	27.81 ± 0.77
30.0	38.19 ± 0.71	100.0	46.20 ± 0.36
45.0	55.43 ± 0.64	150.0	60.66 ± 0.22
60.0	66.37 ± 1.16	200.0	68.39 ± 0.31
75.0	75.93 ± 2.50	250.0	73.98 ± 0.01
IC_{50}[*]	**40 µg/mL**	**IC_{50}**[*]	**113 µg/mL**

[*] IC_{50} is the inhibitory concentration that effectively inhibits tyrosinase activity by 50%. Computed by interpolation.
[**] A reference tyrosinase inhibitor and known whitening agent.
[1] Mean± Standard deviation.

3.6. Elastase inhibition activity

Seaweeds that have collagenase and elastase inhibitory properties, which degrade collagen fibers and elastin, might play an important pharmaceutical application against skin aging (Sari et al., 2019). Bioactive compounds derived from these seaweeds can be further developed as cheap, alternative sources of active ingredients for cosmetic use. The anti-aging activity of *H. durvillei* extract was evaluated *in vitro* via elastase inhibition assay. Results showed that *H. durvillei* extract exhibited the highest inhibition activity at 700 µg GAE/mL with a percent inhibition of 50.40 ± 0.19% (Table 6). The IC_{50} of *H. durvillei* extract is 696 µg/ml, which is considered more potent than tocopherol with IC_{50} value of >2500 µg/mL. However, it is less potent than that observed for *Sargassum aquifolium,* which reported an IC_{50} value of 231 µg/mL (Arguelles and Sapin, 2021). Phenolic compounds derived from seaweeds are known elastase inhibitors. These compounds can inhibit metalloproteinase in human dermal fibroblast cells and prevents the synthesis of elastase in the skin (Jesumani et al., 2019). The current study shows that the crude extract of *H. durvillei* may contain phenolic compounds with potent elastase inhibition properties. Thus, suggesting that elastase inhibitors are promising target compounds that can be harnessed in *H. durvillei* for cosmeceutical application.

Table 6. Elastase inhibition activity and IC_{50} value of *H. durvillei* extract and tocopherol

Halymenia durvillei		**Tocopherol**[***]	
Phenolic concentration (µg GAE/mL)	**Elastase Inhibition (%)**[1]	**Concentration (µg/mL)**	**Elastase Inhibition (%)**[1]
300.0	9.83 ± 1.58	500.0	16.58 ± 0.19
400.0	17.65 ± 0.51	1000.0	19.35 ± 0.06
500.0	24.80 ± 0.37	1500.0	26.08 ± 1.13
600.0	39.64 ± 1.21	2000.0	31.03 ± 0.95
700.0	50.40 ± 0.19	2500.0	38.22 ± 0.37
IC_{50}[*]	**696 µg/mL**	**IC_{50}***	**>2500 µg/mL**[**]

[*] IC_{50} is the concentration that effectively inhibits elastase activity by 50%. Computed by interpolation.
[**] IC_{50} was not determined because 50% inhibition was not achieved at 2500 µg/mL concentration.
[***] A reference standard elastase inhibitor.
[1] Mean± Standard deviation.

3.7. Anti-diabetic activity

Controlling blood glucose levels in patients with diabetes is an essential step in minimizing vascular complications. Hyperglycemia is controlled via inhibition of degrading starch enzymes such as α- amylase (Poulose et al., 2021). In this study, evaluation of the potential anti-diabetic property of *H. durvillei* was done *in vitro* via α-amylase inhibition assay. The inhibition property of *H. durvillei* extract

on the target enzyme is shown in Table 7. The algal extract exhibited a concentration-dependent reduction in α-amylase inhibition. *Halymenia durvillei* extract exhibited the highest inhibition of α-amylase (78.18 ± 0.15%) at phenolic extract concentration of 100 µg GAE/mL. *Halymenia durvillei* extract has potent α-amylase inhibition property (IC_{50} of 56 µg/mL) more efficient than the standard anti-diabetic drug, acarbose with IC_{50} of 101 µg/mL. The IC_{50} value of *H. durvillei* towards α-amylase is more potent as compared to that observed for *Gelidium spinosum* with IC_{50} value of 89.27 µg/mL. However, it is less effective than *Ascophyllum nodosum* extract, which exhibited IC_{50} of 53.6 µg/mL. (Lordan et al., 2013 ; Poulose et al., 2021). Generally, seaweeds are rich sources of antioxidants with anti-diabetic properties. Several compounds such as pigments (carotenoids and fucoxanthin) and phenolic compounds (i.e., tannins, and phenolic acids) are reported to have α -amylase inhibition activities (Kim et al., 2021). Thus, phenolic compounds in these seaweeds can be tapped as novel sources of bioactive substances for pharmaceutical application.

Table 7. α-amylase inhibition and IC_{50} of *H. durvillei* extract and acarbose

Halymenia durvillei		**Acarbose**[**]	
Phenolic concentration (µg GAE/mL)	**α-amylase inhibition (%)**[1]	**Concentration (µg/mL)**	**α-amylase inhibition (%)**[1]
20.0	16.25 ± 1.09	60.0	34.16 ± 0.76
40.0	39.18 ± 2.36	120.0	57.45 ± 0.20
60.0	51.81 ± 1.45	180.0	71.03 ± 0.66
80.0	63.83 ± 1.71	240.0	79.40 ± 0.20
100.0	78.18 ± 0.15	300.0	84.89 ± 0.40
IC_{50}[*]	**56 µg/mL**	**IC_{50}**[*]	**101 µg/mL**

*IC_{50} is the concentration that effectively inhibits α-amylase activity by 50%. Computed by interpolation.
**A reference standard antidiabetic drug.
[1]Mean± Standard deviation.

4. Conclusion

In the Philippines, biotechnological studies on the use of seaweeds for pharmacological application remain poorly understood. This study documented that *Halymenia durvillei* extract exhibited promising bioactivities such as alpha-amylase, elastase, and tyrosinase inhibition properties, as well as antioxidant activity. It is recommended that additional studies on the identification of the active compounds (via High Performance Liquid Chromatography or Liquid Chromatography-Mass Spectrometry), as well as elucidation of the reaction mechanisms, involved in these active substances, should be done to further support the findings of the study. The role of other compounds such as phycoerythrin should also be analyzed in the sample to show its relationship to the reported biological activities of *H. durvillei*. Correlation studies on the activity of phycoerythrin in relation to the biological activities exhibited by the extract can lead to a proposal of a possible mechanism by which these activities are exhibited by the seaweed. In addition, *in vivo* experimental trials are needed to confirm the safety and effectivity of *H. durvillei* extract as potential active ingredients for medicinal use.

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