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Khon Kaen University, Thailand***In vitro* antioxidant, alpha-glucosidase inhibition and antibacterial properties of *Turbinaria decurrens* Bory (Sargassaceae, Ochrophyta)**Eldrin DLR. Arguelles^{1,*}, Arsenia B. Sapin²¹Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, Laguna, Philippines²Food Laboratory, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, Laguna, Philippines

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Abstract

The genus *Turbinaria* is a chemically diverse group of seaweeds containing several phytochemicals with unique pharmacological activities useful in the treatment and management of diseases. This study was done to evaluate the bioactive potential of a brown macroalga, *Turbinaria decurrens* Bory from Pagkalitan, Batangas. The acidified methanolic extract of *T. decurrens* has a total phenolic content of 27.84 ± 0.12 mg gallic acid equivalents (GAE)/g. Antioxidant efficacy of *T. decurrens* exerted potent radical scavenging activity against 2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals as well as high reducing capability of copper ions in a dose-dependent manner with half maximal inhibitory concentration (IC₅₀) value of 49.31 µg GAE/mL, 69.91 µg GAE/mL and 17.2 µg GAE/mL, respectively. *In vitro* study of α -glucosidase inhibition showed that *T. decurrens* has potent inhibitory activity (IC₅₀ of 11 µg/mL) as compared to that of acarbose and metformin (anti-diabetic drugs) with IC₅₀ values of 7960 µg/mL and 49.66 mg/mL, respectively. Assessment of the antibacterial activities using microtiter plate dilution assay showed that *T. decurrens* have strong activity against Methicillin-resistant *Staphylococcus aureus*, minimum inhibitory concentration (MIC=125 µg/mL) followed by Penicillin-acylase producing *Bacillus cereus* (MIC=250 µg/mL), *Staphylococcus epidermidis* (MIC=250 µg/mL), and *Pseudomonas aeruginosa* (MIC=500 µg/mL). This investigation is one of the pioneering research studies in the Philippines showing the potential of *T. decurrens* extract as a cheap source of biologically active compounds with potential use for the treatment of drug-resistant bacterial infection and diabetes.

Keywords: Algae, Bacterial pathogens, Bioactive compounds, Biological activity, Diabetes, Macroalgae, Philippines, Polyphenols, Phytochemicals, Seaweed, *Turbinaria*

1. Introduction

The diminishing therapeutic effect of standard drug agents and its side effects during the treatment of a disease is a worldwide problem in health and medicine [1]. Although, several novel bioactive metabolites have been documented from traditional sources such as plants in the past years, health-related problems are still now a primary issue and immediate practical solutions are required to address it. The use of other existing natural resources such as seaweed is considered as one of the strategic solutions to address such issues. Seaweeds are known in producing diverse types of bioactive compounds with a wide array of biological activities [2,3]. These bioactive compounds are products of their adaptive strategy as a defense mechanism against drastic environmental change [3,4,5]. Substances such as phenols, polysaccharides, halogenated ketones, terpenoids, bioactive peptides, sterols, dietary fibers, and fucoidans are synthesized by these organisms which are known to have antifungal, antioxidant, antibacterial, anti-inflammatory and anti-diabetic activities [2]. Also, seaweeds can produce other bioactive products that can act as growth enhancers in a plant as well as a food supplements (feeds) in several marine fish cultures [2]. Important precursors in the production of cosmetic and pharmaceutical products such as

laminaran, alginate, phloroglucinol, squalene, and fucoxanthin can also be derived from extracts of these marine organisms [3].

Brown seaweeds are among the most dominant species of macroalgae in the coast of Pagkalitan, Batangas. Among this group of seaweeds, *Turbinaria decurrens* Bory (a member of the family Sargassaceae) is abundantly available in the sampling area throughout the year. *T. decurrens* is a common tropical brown seaweed characterized by having a firm leaf structure that is peltate and turbinate in shape (triangular in the surface). The leaves are arranged in a densely, monopodial radially branched system spreading from the main axes having air vesicles instilled allowing it to float in the water [3]. The use of seaweed-derived constituents in the formulation of pharmacologically important products has long been studied with other known seaweeds (such as *Codium*, *Eucheuma*, and *Kappaphycus*). However, only a few documented pieces of research were conducted in the Philippines on the use of brown seaweeds (such as *T. decurrens*) for the formulation of pharmacological products [2,6]. Even though *T. decurrens* has not attracted the most attention in current seaweed natural products research, biological and therapeutic properties of this seaweed such as cytotoxic, antidiabetic, antiviral, anti-inflammatory, antimicrobial, and antitumor activities are promising [3]. These properties are attributed to active metabolites such as fucoidan, polyphenols, embelin, tanacetol A, fucoxanthin, and phlorotannin reported in *T. decurrens* with unusual structural properties as well as interesting pharmacological activities [3]. The marine ecosystem of the Philippine is known to have diverse species of *Turbinaria* with promising bioactive properties yet to be explored. However, only a handful of research was documented about the antibacterial, antioxidant, and antidiabetic potential of this macroalga and to date, studies focusing on the therapeutic potential of *Turbinaria* species is still few in the country [6]. Previously, *T. ornata* collected from Baybay, Leyte was studied for its bioactive potential. The result of this study suggests that the ethanolic extract of *T. ornata* exhibited cytotoxic (against *Saccharomyces cerevisiae*) and antiangiogenic (100% vascular inhibition in duck chorioallantoic (CAM membrane samples) activities [6]. The current study is one of the pioneering research investigations in the Philippines investigating the bioactive properties of a brown macroalga, *Turbinaria decurrens* Bory with potential use for the treatment of drug-resistant bacterial infection and diabetes. The study was done to analyze the total phenolic content, anti-diabetic, and antibacterial activities of *T. decurrens*. The antioxidant activity was done using three assays and correlation analysis on the phenolic content of the algal extract and its antioxidant activities were established.

2. Materials and methods

2.1 Sample collection

The brown macroalga *Turbinaria decurrens* Bory was collected on 18 January 2020 from Pagkalitan (Lat. 13° 38' 4.2" N; Long. 121° 2' 39' E), Batangas, Philippines. The macroalga was identified using morphological features based on the standard reference material for seaweed identification and AlgaeBase (<https://www.algaebase.org>) [7,8]. The sample was cleaned several times using seawater to remove sand debris and other associated organisms such as planktons and other microorganisms. The seaweed sample was kept in a sterile autoclavable plastic bag and promptly transferred to the laboratory. *T. decurrens* was then washed repeatedly with distilled water and dried using a blotting paper to clean off the excess water. The seaweed sample was air-dried, chopped, and pulverized using a mixer grinder [2].

2.2 Seaweed extract preparation

The powdered *T. decurrens* (1 g) were immersed in 30 mL acidified methanol (1 HCl: 80 CH₃OH: 10 H₂O) and extracted for 30 min using an ultrasonic bath and stirred for 1 h [9]. The extract was then centrifuged at a speed of 12,000 rpm at 20 °C for 20 min [10]. The algal extract was concentrated through a rotary evaporator 1adjusted at a temperature of 40 °C under reduced pressure. The concentrated seaweed extract obtained was kept at 4 °C to maintain its activity [11,12].

2.3 Determination of total phenolic content

Analysis of the total phenolic content of *T. decurrens* was done using Folin-Ciocalteu's reagent [12,13]. Briefly, a portion (0.5 mL) of the diluted seaweed extract [1:12 (extract: diluent (distilled water))] was added with an equal volume (0.5 mL) of 10% sodium carbonate solution and Folin-Ciocalteu's reagent for 1 min. The solution was placed in room temperature and set aside for 5 min. The volume of the reaction solution was then adjusted by pouring 5 mL of sterile distilled water. Optical density (OD) reading of the solution was taken at 720 nm using an Ultraviolet-Visible (UV-Vis) spectrophotometer. Construction of a calibration curve was done using gallic acid with prepared range of concentrations (20.0, 40.0, 60.0, 80.0 and 100.0 µg/mL; $y = 0.0061x - 0.0124$, $R^2 = 0.99984$). The total phenolic concentration of the extract was reported as mg gallic acid equivalents (GAE)/g of the seaweed sample [12,13].

2.4 Antioxidant assays

2.4.1 ABTS radical scavenging assay

Scavenging activity of *T. decurrens* extract to ABTS free radical was done using a method that is based on the decolorization of 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) [14]. In this antioxidant assay, ABTS is changed to its radical cation by adding sodium persulfate (bluish in color) capable of absorbing light at a wavelength of 734 nm. The ABTS radical cation is sensitive and reactive in almost all antioxidants (such as polyphenols) and converts the ABTS radical cation (bluish in color) to its neutral form (colorless). A quantitative relationship exists between the concentration of antioxidants existing in the algal extract as well as the lowering of absorbance at 734 nm. Briefly, 40 μ L of algal extract prepared at different phenolic concentrations (12.5, 25.0, 37.5, 50.0, and 62.5 μ g GAE/mL) and 40 μ L of 90% methanol for the control were mixed to 3 mL of ABTS radical mixture with an initial absorbance of 0.72 ± 0.05 at 734 nm. The mixtures were stirred and set aside for 5 min. Then, the optical density was read at 734 nm making use of water to zero the instrument. Ascorbic acid (prepared in different concentrations: 37.5, 75.0, 112.5, 150.0, and 187.5 μ g GAE/mL) was used as the positive control in this study. IC_{50} of *T. decurrens* extract was calculated by interpolation to show the concentration (expressed in μ g GAE/mL) of the seaweed extract that can exhibit a 50% inhibition of the ABTS free radical. The ABTS inhibition was computed using the formula below:

$$\text{ABTS Inhibition (\%)} = \frac{\text{Abs}_{734}(\text{control}) - \text{Abs}_{734}(\text{sample})}{\text{Abs}_{734}(\text{control})} \times 100$$

2.4.2 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging assay was analyzed making use of ascorbic acid as reference antioxidant in the assay [12,15]. Briefly, 100 μ L of the seaweed extract (prepared at varying concentrations: 20.0, 40.0, 60.0, 80.0, and 100.0 μ g GAE/mL) was mixed to 5.0 mL of 0.1 mM DPPH solution. The reaction solution was thoroughly mixed using a vortex mixer and set aside for 20 min at ambient temperature. The optical density of the algal sample was measured using UV-Vis spectrophotometer at a wavelength of 517 nm [12,15]. Ascorbic acid (prepared at different concentrations: 20.0, 40.0, 60.0, 80.0, and 100.0 μ g GAE/mL) was used as the positive control in this assay. IC_{50} of *T. decurrens* extract was calculated by interpolation to show the concentration (expressed in μ g GAE/mL) of the seaweed extract that can exhibit a 50% inhibition of DPPH radical. The DPPH scavenging activity (%) was estimated using the formula below:

$$\text{DPPH Inhibition (\%)} = \frac{\text{Abs}_{517}(\text{control}) - \text{Abs}_{517}(\text{sample})}{\text{Abs}_{517}(\text{control})} \times 100$$

2.4.3 Copper reduction antioxidant capacity (CUPRAC) assay

The copper ion reducing capacity of the seaweed extract was analyzed using a colorimetric method [12,16]. Briefly, an aliquot (0.5 mL) of the different prepared concentration of the seaweed extract (5.0, 10.0, 15.0, 20.0, and 25.0 μ g GAE/mL) was mixed with 1mL each of 0.0075 M neocuproine, ammonium acetate ($C_2H_3O_2NH_4$) buffer (1M, pH 7.0) solution, and 0.01 M $CuCl_2$ into a sterile test tube. The solutions were set-aside for 30 min at ambient temperature. The optical density of each reaction mixture was measured at 450 nm and recorded [12,16]. IC_{50} of *T. decurrens* extract was computed by interpolation. It is the effective concentration of the algal extract that gives a CUPRAC value of 0.5 absorbance reading at 450 nm.

2.5 α -glucosidase inhibition assay

The capacity of *T. decurrens* extract to inhibit α -glucosidase was analyzed through a spectrophotometric assay making use of *p*-nitrophenyl- α -glucopyranoside (*p*NPG) as a substrate [17]. The existence of bioactive substances with α -glucosidases activity in an extract transforms *p*NPG (substrate) to *p*-nitrophenol (*p*NP), a detectable molecule that is measured spectrophotometrically at 410 nm. If α -glucosidase is inhibited, low optical density (absorbance) at 410 nm will be measured. The reaction mixture consisting of 75 μ L of α -glucosidase (2.5 U/mL), 100 μ L of seaweed extract or 100 μ L of 0.1 M phosphate buffer pH 6.8 (for the case of the control) were mixed in test tubes. The volume of the solution was modified to 500 μ L by adding 295 μ L buffer and 30 μ L of 10 mM *p*-nitrophenyl- α -D-glucopyranoside (Sigma N1337) before incubation. The prepared mixtures were incubated at 37 $^{\circ}$ C for 12 min after which 3 mL of 50 mM NaOH were added in the solution. The optical density of each sample

solution was taken at 410 nm [17]. Anti-diabetic drugs (acarbose and metformin) were used as positive controls for the alpha-glucosidase inhibition assay. The percent α -glucosidase inhibition was computed using the formula below:

$$\alpha - \text{Glucosidase Inhibition (\%)} = \frac{\text{Abs}_{410} (\text{control}) - \text{Abs}_{410} (\text{sample})}{\text{Abs}_{410} (\text{control})} \times 100$$

2.6 Antibacterial assay

2.6.1 Test microorganisms

Pathogenic strains of four Gram-negative bacteria (Penicillin acylase – producing *Escherichia coli* BIOTECH 1634, *Pseudomonas aeruginosa* BIOTECH 1824, *Aeromonas hydrophila* BIOTECH 10089, and *Klebsiella aerogenes* BIOTECH 1145), and three Gram-positive bacteria *Staphylococcus epidermidis* BIOTECH 10098, Penicillin acylase-producing *Bacillus cereus* BIOTECH 1635, and Methicillin-resistant *Staphylococcus aureus* BIOTECH 10378) were obtained from the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB). The pathogenic bacteria were pre-cultured utilizing Luria Bertani (LB) medium incubated at 37 °C with shaking overnight. The biochemical tests and morphological characteristics of each pathogen were continuously monitored to ensure purity [10,12].

2.6.2 Micro-dilution antibacterial assay

The antibacterial activity of *T. decurrens* extract against different pathogenic bacteria was analyzed using a two-fold serial dilution technique [2,12,18]. Using a 96-well microtiter plate, 100 μ L of prepared bacterial cultures (each with a cell density of 1×10^5 cells/mL) were mixed with 100 μ L of *T. decurrens* extract in different prepared dilutions (1000 μ g/mL - 7.8125 μ g/mL). Acidified methanol was also added in the assay as the negative control. The microtiter assay plate was kept at 35 °C in for 12 h, after which minimum inhibitory concentration (MIC) for each pathogenic bacteria were recorded. MIC is regarded as the minimum concentration of *T. decurrens* extract that will inhibit bacterial growth after an incubation time of 12 h. The minimum bactericidal activity (MBC) of the extract was checked by plating a loopful of the sample (about 10 μ L) from each MIC wells (that exhibited growth inhibition of bacteria) into freshly prepared culture (tryptic soy agar) medium [10,12]. The plates were kept at 35 °C in an incubator for 24 h and were examined for visible colony growth for each dilution subculturing. No visible bacterial growth would mean that *T. decurrens* extract was bactericidal at that specific dilution [10,12].

2.7 Statistical analyses

Each assay was done at three replicates and results are presented as means $\pm$ standard deviations (mean $\pm$ SD). The statistical tests for the correlation analysis were analyzed using linear correlation coefficient obtained using Microsoft Office Excel 2007.

3. Results and discussion

3.1 Total phenolic content

The total phenolic concentration (TPC) detected in the macroalgal extract was evaluated using the Folin-Ciocalteu method and is presented in gallic acid equivalent (calibration curve equation: $y = 0.0061x - 0.0124$, $R^2 = 0.99984$). The results indicated that the total phenolic concentration in *T. decurrens* extract is 27.84 ± 0.12 mg GAE/g. The phenolic content obtained from *T. decurrens* extract is greater than that observed from other species of seaweeds found in the coast of Ceará, Brazil such as *Dictyota dichotoma* (19.94 mg GAE/g), *Amansia multifida* (18.46 mg GAE/g), *Caulerpa cupressoides* (18.04 mg GAE/g), *Sargassum vulgare* (17.97 mg GAE/g), *Cryptonemia crenulata* (11.61 mg GAE/g), and *Ulva fasciata* (10.49 mg GAE/g) [4]. Likewise, lower TPC was noted in methanol extracts of *Turbinaria conoides* and *Turbinaria ornata* with a TPC value of 16.64 ± 0.10 mg GAE/g and 3.42 ± 0.35 mg GAE/g respectively [19]. Generally, the concentration of polyphenolic compounds in brown macroalga is greater than that obtained from green and red seaweeds [2,5]. Also, the amount of phenolic content of a sample is influenced by the solvent's polarity during the extraction process. In general, polyphenolic compounds have greater solubility in polar solvents (such as acidified methanol) that results to high yield of these active compounds in the extract [12].

3.2 Antioxidant activities

Brown seaweeds are known to possess diverse kinds of polyphenols with a broad range of antioxidant activities. Thus, screening and utilization of these natural polyphenols for possible use in drug synthesis and the development of pharmaceutical products should be documented. The antioxidant activities of *T. decurrens* extract from Pagkalitan, Batangas were evaluated using DPPH, CUPRAC, and ABTS assays. The use of only one assay in the evaluation of the antioxidant potential of seaweeds is insufficient for determining the mechanisms involve in antioxidant activity. Thus, three antioxidant assays were used in the study. *Turbinaria decurrens* exerted radical scavenging activity against free radicals (ABTS and DPPH) showing dose-dependent percent inhibition (Table 1 and 2). The ability of the macroalgal extract to scavenge ABTS and DPPH increases when the concentration of *T. decurrens* extract is also increased. *T. decurrens* extract exhibited strong ABTS antioxidant activity than the ascorbic acid with IC_{50} of 49.31 μ g GAE /mL and IC_{50} = 147.9 μ g/mL, respectively. Furthermore, *T. decurrens* extract showed comparably similar DPPH antioxidant activity than the ascorbic acid (positive control) with IC_{50} of 69.91 μ g GAE/mL and IC_{50} = 59.6 μ g/mL, respectively. The results of antioxidant assays show that *T. decurrens* extract exhibits radical potent scavenging activity that can be compared with ascorbic acid. Thus, the possibility of using *T. decurrens* as a natural source of antioxidants that can be used as a substitute for artificial antioxidants (such as butylated hydroxyanisole and butylated hydroxytoluene), which are known to have carcinogenic effects when using at high concentration [2,20].

Table 1 ABTS radical scavenging activity and IC_{50} value of *T. decurrens* extract and ascorbic acid.

Sample	Extract concentration (μ g GAE/mL)					$IC_{50}^*(\mu$ g/mL)
	12.5	25.0	37.5	50.0	62.5	
	ABTS inhibition (%)					
<i>Turbinaria decurrens</i>	17.77 $\pm$ 0.31	28.10 $\pm$ 0.10	40.12 $\pm$ 0.21	50.59 $\pm$ 0.63	61.21 $\pm$ 1.25	49.31
	Concentration (μ g/mL)					
	37.5	75.0	112.5	150.0	187.5	
	ABTS inhibition (%)					
Ascorbic acid	11.70 $\pm$ 1.54	24.56 $\pm$ 0.62	36.70 $\pm$ 0.51	50.87 $\pm$ 0.82	64.53 $\pm$ 1.64	147.9

* 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.

Table 2 DPPH free radical scavenging activity and IC_{50} value of *T. decurrens* extract and ascorbic acid.

Sample	Extract concentration (μ g GAE/mL)					$IC_{50}^*(\mu$ g/mL)
	20.0	40.0	60.0	80.0	100.0	
	DPPH inhibition (%)					
<i>Turbinaria decurrens</i>	21.21 $\pm$ 0.006	34.88 $\pm$ 0.23	44.55 $\pm$ 0.28	55.65 $\pm$ 0.11	61.51 $\pm$ 0.11	69.91
	Concentration (μ g/mL)					
	20.0	40.0	60.0	80.0	100.0	
	DPPH inhibition (%)					
Ascorbic acid	16.64 $\pm$ 0.00	34.11 $\pm$ 0.61	50.34 $\pm$ 0.00	67.32 $\pm$ 0.61	83.58 $\pm$ 0.67	59.6

* IC_{50} is the effective concentration that inhibits DPPH free radical by 50%. Computed by interpolation.

Table 3 Copper reduction antioxidant capacity (CUPRAC) and IC_{50} value of *T. decurrens* extract and ascorbic acid.

Sample	Extract concentration (μ g GAE/mL)					$IC_{50}^*(\mu$ g/mL)
	5.0	10.0	15.0	20.0	25.0	
	CUPRAC (Absorbance at 450 nm)					
<i>Turbinaria decurrens</i>	0.166 $\pm$ 0.001	0.289 $\pm$ 0.000	0.431 $\pm$ 0.004	0.584 $\pm$ 0.009	0.719 $\pm$ 0.008	17.2
	Concentration (μ g/mL)					
	10.0	20.0	30.0	40.0	50.0	
	CUPRAC (Absorbance at 450 nm)					
Ascorbic acid	0.112 $\pm$ 0.002	0.213 $\pm$ 0.007	0.328 $\pm$ 0.004	0.429 $\pm$ 0.012	0.542 $\pm$ 0.011	46.3

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

The copper ion reducing power of *T. decurrens* extract and ascorbic acid at varying concentrations is shown in Table 3. *Turbinaria decurrens* extract exhibited a dose-dependent copper ion reduction capability (Figure 3). In this antioxidant assay, higher OD reading would mean greater antioxidant capacity. The highest reducing activity was observed at 25 µg GAE/mL of the prepared phenolic concentration of the algal extract. The observed trend in this antioxidant assay is comparable to those observed from ABTS and DPPH assay where 62.5 µg GAE/mL and 100 µg GAE/mL (from the prepared concentrations of *T. decurrens* extract) exhibited the highest activity. *T. decurrens* extract exhibited potent copper ion reduction capability than ascorbic acid with IC₅₀ of 17.20 µg GAE/mL and IC₅₀ = 46.30 µg/mL, respectively. The strong antioxidant activity of *T. decurrens* extract in contrast to ascorbic acid shows the potential of the seaweed as a natural alternative source of bioactive compounds valuable in the development of pharmacologically important products. Results of the antioxidant assays suggest that *T. decurrens* extracts can inhibit oxidation through free radical scavenging and metal chelation mechanisms. These metal-chelating antioxidant properties can be ascribed to polyphenolic compounds such as phlorotannins, quercetin, fucoxanthin, bromophenols, catechins, and flavonoids that are present in the algal extract [12,21].

Table 4 Correlation between phenolic content and antioxidant activities of *T. decurrens* extract.

Antioxidant Assay	Regression Equation	Correlation Coefficient (R)
ABTS radical scavenging assay	$y = 0.875x + 6.747$	0.99972
DPPH radical scavenging assay	$y = 0.5069x + 13.149$	0.99201
Copper reduction antioxidant capacity (CUPRAC) assay	$y = 0.028x + 0.0175$	0.99941

The correlation coefficient (R) among antioxidant activity of *T. decurrens* (using ABTS, DPPH, and CUPRAC assay) and phenolic concentration are presented in Table 4. It is evident from these results that a good positive correlation for ABTS (R=0.99972) DPPH (R=0.99201), CUPRAC (R=0.99941) exists linking the total phenolic concentration and antioxidant activities of the acidified methanolic extract of *T. decurrens*. Positive correlations among antioxidant activities and phenolic contents have been documented in *Turbinaria* species [19,20-21]. This positive correlation proves that polyphenols existing in the sample play a crucial part for the antioxidant activities of the macroalgal extract. Polyphenols are substances that preserve seaweed thalli against photodestruction caused by UV radiation and are known to display antioxidant properties [5,20].

3.3 α -glucosidase inhibition assay

The α -glucosidase is a digestive enzyme being released by brush border walls of the small intestine that reacts upon α -(1,4)-glycosidic bonds causing a breakdown of disaccharides and starch to small glucose units. The suppression of such an important enzyme is necessary for reducing postprandial blood glucose, hence an excellent focus on the management and regulation of diabetes [22]. Metformin and acarbose are widely used anti-hyperglycemic agents for the medication of Type 2 diabetes. These drugs have the ability to inhibit starch digestion enzymes (α -amylase and α -glucosidase). However, these anti-hyperglycemic agents are known to produce displeasing side effects (such as bloating, lactic acidosis, flatulence, nausea, abdominal distention, and vomiting) due to inhibition of starch digestion and bacterial fermentation in the colon [23]. Thus, searching for an alternative polyphenolic compound from other natural sources (such as seaweeds) that could inhibit α -amylase and α -glucosidase might give a solution to lessen these side effects, and provide more tolerable and cheaper alternatives. The α -glucosidase inhibitory activity of *T. decurrens* extract is presented in Table 5. The percentage inhibition of varying dose concentration of the algal extract showed a concentration-dependent reduction in α -glucosidase inhibition. *T. decurrens* extract exhibited high activity of α -glucosidase inhibition of $88.21 \pm 0.05\%$ at 20 µg GAE/mL concentration of the extract with a corresponding IC₅₀ value of 11 µg/mL. These inhibition values are relatively more potent than that of metformin and acarbose (anti-diabetic drugs), which gave an IC₅₀ value of 49660 µg/mL and 7960 µg/mL, respectively. The IC₅₀ value of *T. decurrens* extract in this study is stronger than that obtained for *T. decurrens* strain collected from the coast of Hurghada beach in Egypt wherein the seaweed has an IC₅₀ value 2.84 mg/mL for α -glucosidase inhibition [24]. Similar findings were also observed in *Turbinaria ornata* extract obtained from the Gulf of Mannar in South India wherein 87.6% inhibition of the enzyme was observed at 1000 µg/mL concentration of the algal extract with IC₅₀ value of 535.6 µg/mL [25]. The potent α -glucosidase inhibition property of *T. decurrens* extract shows the potential of this seaweed as an alternative source of a low glycemic index food ingredients that can be used in the regulation and management of blood glucose level in the body through slow digestion and absorption.

Table 5 α -glucosidase inhibition and IC₅₀ of *T. decurrens* extract in comparison to acarbose and metformin.

Sample	Extract concentration (μ g GAE/mL)					IC ₅₀ * (μ g/mL)
	4.0	8.0	12.0	16.0	20.0	
<i>Turbinaria decurrens</i>	α -glucosidase inhibition (%)					
	3.81 $\pm$ 0.10	22.23 $\pm$ 0.00	59.31 $\pm$ 0.14	75.73 $\pm$ 0.10	88.21 $\pm$ 0.05	11
	Concentration (μ g/mL)					
	2000	4000	6000	8000	10000	
Acarbose**	α -glucosidase inhibition (%)					
	13.16 $\pm$ 0.00	30.07 $\pm$ 0.00	41.25 $\pm$ 0.28	50.28 $\pm$ 0.07	57.22 $\pm$ 0.07	7960
	Concentration (mg/mL)					
	10.0	20.0	30.0	40.0	50.0	
Metformin**	α -glucosidase inhibition (%)					
	15.83 $\pm$ 0.51	18.30 $\pm$ 0.05	23.30 $\pm$ 0.15	29.86 $\pm$ 0.10	50.72 $\pm$ 0.41	49660

* IC₅₀ is the effective concentration that inhibits α -glucosidase activity by 50%.

**Reference α -glucosidase inhibitors and anti-diabetic drugs.

The anti-hyperglycemic activity exhibited by *T. decurrens* extract can be ascribed to bioactive compounds such as polyphenols that are present in the algal extract. These compounds are capable of binding in the active site of the α -glucosidase that changes the conformation (due to steric hindrances) and inhibit the catalytic activity of the enzyme. Also, presence of other known biologically active substances such as phlorotannins (eckestonol, and phlorofucofuroeckol A), bromophenols, hydroxybenzoic acid, epigallocatechin, p-coumaric acid, and dihydroxybenzoic acid and other polyphenols can also play an important part in the inhibitory activity of the macroalgal extract. These active compounds have anti-diabetic effects and are well known to have enzymatic inhibitory activity against α -glucosidase [3].

3.4 Antibacterial activity

The genus *Turbinaria* is a chemically diverse group of seaweeds containing several phytochemicals with unique pharmacological activities. Bioactive substances such as fucoidan, fucoxanthin, and phlorotannins were detected in this seaweed which is capable of inhibiting hydrolytic enzymes and other chemical interactions that may constitute to inactivation of bacterial adhesins and other protein envelopes, thus may act as an antibacterial agent [3]. Therefore, *T. decurrens* is considered as candidate marine algae possessing excellent biological activities that can be used for the synthesis of new antibiotics [3]. The results of the analysis of the antibacterial properties of *T. decurrens* extract against common and drug-resistant bacterial pathogens are presented in Table 6. Of the seven pathogenic bacteria tested, four test organisms were inhibited by *T. decurrens* extract by exhibiting pronounced activity against Methicillin-resistant *Staphylococcus aureus* (MIC of 125 μ g/mL), Penicillin acylase-producing *Bacillus cereus* (MIC of 250 μ g/mL), *Staphylococcus epidermidis* (MIC of 250 μ g/mL), and *Pseudomonas aeruginosa* (MIC of 500 μ g/mL). On the other hand, no inhibitory activity was observed against *Aeromonas hydrophila*, *Klebsiella aerogenes*, and Penicillin acylase-producing *Escherichia coli*. Among the tested bacterial pathogens, *T. decurrens* extract is most effective against Methicillin-resistant *S. aureus* having minimum bactericidal concentration (MBC) value of 250 μ g/mL. On the other hand, MBC value for *S. epidermidis* and Penicillin acylase-producing *B. cereus* (each with MBC of 500 μ g/mL) is greater than that obtained for *P. aeruginosa* (MBC of 1000 μ g/mL). The findings of this study proved that *T. decurrens* extract is more potent against Gram-positive bacteria than that of the Gram-negative bacteria because of the complex cell wall composition of the latter group of bacteria. Gram-negative bacteria possess a multilayered structure of cell wall that affects the entry and penetration of active substances inside the cells allowing additional protection and immunity for the bacteria [2,12,26]. The antibacterial activities exhibited by *T. decurrens* are similar to those earlier antimicrobial studies on the genus *Turbinaria* [19,27,28]. Two novel steroids 14, 15, 18, 20-diepoxyturbinarin and 3, 6, 17-trihydroxy-stigmasta-4, 7, 24 (28)-triene, as well as fucosterol isolated from cyclohexane extract of *T. conoides* from the Gulf of Mannar (India), exhibited potent inhibitory activities against *P. aeruginosa*, *C. albicans*, *E. coli*, *S. epidermidis*, *A. niger*, and *S. aureus* with MIC ranging between 2 to 16 μ g/mL [29]. Also, active compounds such as fucoxanthin isolated from *T. decurrens* extract showed good antibacterial activity against *Streptococcus agalactiae* with MIC value of 62.5 μ g/mL [3].

Table 6 Antibacterial activities of *T. decurrens* extract against bacterial pathogens.

Bacterial Pathogen	Minimum inhibitory concentration (µg/mL)	Minimum bactericidal concentration (µg/mL)
Gram-positive bacteria		
<i>Staphylococcus aureus</i> BIOTECH 10378	125.00	250.00
<i>Staphylococcus epidermidis</i> BIOTECH 10098	250.00	500.00
<i>Bacillus cereus</i> BIOTECH 1635	250.00	500.00
Gram-negative bacteria		
<i>Pseudomonas aeruginosa</i> BIOTECH 1824	500.00	1000.00
<i>Aeromonas hydrophila</i> BIOTECH 10089	>1000.00	ND
<i>Escherichia coli</i> BIOTECH 1634	>1000.00	ND
<i>Klebsiella aerogenes</i> BIOTECH 1145	>1000.00	ND

*ND = None Detected

At present, there are no research studies that document the antibacterial activities of *T. decurrens* in the Philippines. This study is the first report in the Philippines about antibacterial activity of *T. decurrens* strain against Methicillin-resistant *Staphylococcus aureus*, Penicillin-acylase producing *Bacillus cereus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*. The antibacterial activities of *T. decurrens* extract proved that this macroalga is a good candidate source of effective bioactive compounds against pathogenic, drug-resistant bacteria. Hence, additional studies should be conducted for the purification, identification, and mass production of these substances for medical applications [2,12,29].

4. Conclusions

Turbinaria decurrens Bory contain a high concentration of polyphenols and exhibited broad spectrum of antioxidant, α -glucosidase inhibition, and antibacterial activities. Additional studies focusing on the elucidation of the chemical structure and identity of bioactive compounds (using Gas Chromatography – Mass Spectrometry) present in the extract are needed to better understand the mechanisms involved in its biological activities which is important in large scale controlled studies and product development. *Turbinaria* is a rich resource that is abundantly available in the Philippine archipelago, thus the availability of this seaweed for commercial use.

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6. References

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