BIOPROSPECTING OF *Turbinaria ornata* (Fucales, Phaeophyceae) FOR COSMETIC APPLICATION: ANTIOXIDANT, TYROSINASE INHIBITION AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Brown macroalgae are rich sources of polyphenols with diverse pharmacological activities relevant to formulation of novel cosmetic products. This study evaluated the antioxidant, tyrosinase inhibition, and antibacterial properties of a brown macroalga, *Turbinaria ornata* (Turner) J. Agardh. The seaweed extract had a total phenolic content of 18.50 ± 0.17 mg GAE/g. Antioxidant efficiency of *T. ornata* exerted high copper reduction antioxidant capacity and potent DPPH radical scavenging activity in a dose-dependent manner with EC_{50} value of 24.34 μg GAE/mL and 22.10 μg GAE/mL, respectively showing its potential as an alternative source of effective natural antioxidant. *T. ornata* has an excellent tyrosinase inhibitory activity (EC_{50} of 67.50 μg GAE/mL), more effective than that of commercially available skin-lightening ingredient, kojic acid with EC_{50} of 109.8 μg/mL. *In vitro* antibacterial activity using microdilution technique assay demonstrated *T. ornata* possessed strong inhibitory activity against common bacterial skin pathogen such as *Staphylococcus aureus* (MIC = 125 μg/mL), *Staphylococcus saprophyticus* (MIC = 125 μg/mL), *Staphylococcus epidermidis* (MIC = 250 μg/mL) and *Serratia marcescens* (MIC = 500 μg/mL). The current study is the first report in the Philippines exploring the biological properties of *T. ornata* as potential alternative source of skin lightening active ingredient important for cosmetic application.

Key words: algae, lightening ingredient, marine, melanogenesis, polyphenols, seaweeds

INTRODUCTION

Melanin is a pigment produced by skin cells that serve as a primary defense against ultraviolet light to prevent skin damages. The presence of tyrosinase enzyme and UV light (ultraviolet B (UV-B) and ultraviolet A (UV-A)) induces the production of melanin that can result in hyperpigmentation (excessive browning of skin) and melanoma. Tyrosinase is a polyphenol oxidase copper-containing enzyme important in melanin synthesis. This enzyme catalyzes a two-step reaction in melanin synthesis, monophenolase reaction (hydroxylation of L-tyrosine to produce L-3,4-dihydroxyphenylalanine (L-DOPA)) and diphenolase reaction (oxidation of L-DOPA to DOPAquinone). Accumulation and overproduction of melanin in skin can result to several disorders such as melanoma, melisma, melanosis and senile lentigos (Petrillo et al. 2016). Thus, inhibition of this enzyme using depigmenting agents (tyrosinase inhibitors) has become an important line of research in medicine.

Tyrosinase inhibitors act as active skin lightening ingredients for cosmetic application. Recently, hydroquinone, kojic acid, and arbutin have been reported to cause toxic side effects, allergy and loss or inconsistent efficacy during long-term use. The use of kojic acid and hydroquinone at high concentration may cause erythema and allergic contact dermatitis leading to skin cancer (Deveci et al. 2018). Thus, the search for an effective, safe, cheap and natural alternative source such as seaweeds for skin lightening ingredient is considered as a strategic solution to address such issue. Seaweeds are marine organisms that are known for their diverse bioactive metabolites characterized by a wide array of pharmacological activities (Cha et al. 2011; Arguelles and Martinez-Goss 2019; Rushdi et al. 2020). These organisms are currently being tapped as new source of active compounds that have skin care properties such as antibacterial, whitening, antioxidant, anti-wrinkle and anti-aging effects. *T. ornata* is a brown macroalga commonly found in the marine ecosystem of the Philippines. The use of this seaweed for synthesis of new drugs and other important medicinal products has long been studied (Rushdi et al. 2020). However, no documented studies were conducted in the Philippines regarding the use of *T. ornata* as an alternative source of skin depigmenting ingredient for cosmetic application. This macroalga have metabolites such as polyphenols, phlorotannins, fucoidan, quercetin, gallic acid, fucoxanthin and epicatechin with therapeutic properties important in developing novel skin care products (Azam et al. 2017).

The Philippine coast is known to have diverse species of seaweeds with promising biological activities yet to be explored (Arguelles et al. 2019). However, only a handful of research were documented about the antibacterial, and antioxidant potential of these organisms and to date, studies focusing on the potential cosmetic application of *Turbinaria* species are non-existent in the country. The current study is the first report in the Philippines exploring the biological properties of *T. ornata* as potential source of skin lightening active ingredient important for cosmetic application. The study was done to analyze the total phenolic content, tyrosinase inhibition, antibacterial and antioxidant activities of *T. ornata*. The antioxidant activity was done using two methods (copper reduction antioxidant capacity (CUPRAC) and DPPH radical scavenging assays). Also, correlation analysis on the phenolic content of the algal extract and its antioxidant activities were established.

MATERIALS AND METHODS

Seaweed sampling and collection. The marine brown macroalga, *T. ornata* was collected on 24 November 2019 from Catanauan (Lat. 13° 36’ 20.88’ N; Long. 122° 14” 18.24’ E), Quezon, Philippines. The seaweed was identified using relevant morphotaxonomic features according to Trono (1997) and Algae Base (web site: www.algaebase.org). The alga was washed immediately several times with seawater and cleaned thoroughly using soft brush bristles to remove animal castings, detritus, excess sand particles and necrotic parts of the alga (Arguelles 2019). The cleaned seaweed was immediately transferred to the laboratory and repeatedly washed using a sterile distilled water to take away excess salt on the algal surface. The seaweed sample was air-dried (for six days), cut into small portions and pulverized into a powder before extraction and biological activity assay (Arguelles et al. 2019).

Seaweed extraction. The pulverized *T. ornata* biomass (1 gram) was immersed in a 30 mL acidified methanol (1 HCl: 80 CH_3OH: 10 H_2O). The algal sample was extracted for 30 mins with stirring for 1 hour using an ultrasonic bath. The mixture was centrifuged for 20 mins at a speed of 12,000 rpm at 20°C . The seaweed extract was evaporated under reduced pressure (at 40 °C) and was maintained at 4°C to keep its activity before use in different bioassays (Arguelles and Sapin 2020).

Total phenolic content. The phenolic content of *T. ornata* extract is expressed as milligram of gallic acid equivalent (GAE) per gram of the seaweed sample. This was obtained using a calibration curve that was constructed using gallic acid (97.5-102.5% (titration), Sigma-Aldrich) with prepared range of concentrations of 20 - 100 μg/mL. The phenolic content was analyzed using Folin-Ciocalteu reagent

following the protocol of Nuñez-Selles et al. (2002). An aliquot (0.5 ml) of the seaweed extract was mixed with Folin-Ciocalteau's reagent and 10% sodium carbonate solution in equal volume for 1 minute. The reaction mixture was set-aside at ambient temperature for 5 mins. Sterile distilled water (5 mL) was then added to the mixture. The optical density (OD) reading was recorded at 720 nm using a Shimadzu UV 1601 (Japan) ultraviolet-visible spectrophotometer. .

DPPH radical scavenging assay. The free radical scavenging activity of *T. ornata* extract was analyzed spectrophotometrically using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Ribeiro et al. 2008) with few modifications. An aliquot (100 μl) of the seaweed extract mixed with 5.0 ml of 0.1 mM DPPH methanolic solution. The reaction mixture was then homogenized using a vortex mixer. The solution was set-aside for 20 mins at ambient room temperature. The optical density (OD) readings were recorded at 517 nm using a UV-VIS spectrophotometer (Deveci et al. 2018). The percent scavenging inhibition activity (%) was calculated using the formula:

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

where: $\text{Abs}_{\text{control}}$ = optical density of the control (DPPH solution without sample) and $\text{Abs}_{\text{sample}}$ = optical density of the test sample (DPPH solution plus test sample). Gallic acid was used as the control in this assay. The effective concentration (EC_{50}) that can inhibit 50% of the DPPH radicals in the algal extract as well as the control were determined upon analysis of DPPH inhibition and is expressed in μg/ml (Arguelles 2020).

Copper reduction antioxidant capacity (CUPRAC) assay. The cupric-reducing antioxidant capacity was done following the procedure of Alpinar et al. (2009). Briefly, 1 mL each of 0.0075 M neocuproine, 1 M ammonium acetate buffer (pH 7) and 0.01 M $CuCl_2$ solutions were mixed thoroughly in a sterile test tube (Arguelles et al. 2017). Thereafter, 0.5mL of *T. ornata* extract prepared at varying concentrations (5, 10, 15, 20 and 25 μg GAE/mL) as well as ascorbic acid standard solutions (10, 20, 30, 40 and 50 μg/mL) were added to the initial reaction mixture. The overall volume for each prepared concentrations were adjusted using sterile distilled water up to 4.1 mL. The mixtures were kept for 30 mins at ambient room temperature and the OD readings was recorded at 450 nm (Arguelles et al. 2017).

Tyrosinase inhibition assay. The tyrosinase enzyme inhibitory activity of *T. ornata* extract was measured *in vitro* using a microplate reader following the protocols of Hapsari et al. (2012) with few modifications. Mushroom tyrosinase was utilized in the assay, while L-DOPA was used as substrates for the reaction. Mixture of 0.1M potassium phosphate buffer (pH 6.5), 5mM DOPA (3,4-dihydroxy-L-phenylalanine, Sigma D-9628), and mushroom tyrosinase (250 units/mL, Sigma T- 3824) were prepared. Using a microtiter well-plate, 40 µL DOPA is mixed with 40 µL of *T. ornata* extract and 40 µL buffer (in the case of the control). Phosphate buffer was added to the reaction mixture to have a total volume of 160 µL and 40 µL of mushroom tyrosinase was added last. The blank used in the assay was the optical density without the enzyme solution. The reaction mixtures were set-aside for 15 mins at ambient room temperature. The optical density was recorded at 490 nm using a microplate reader. Tyrosinase enzyme inhibition was computed using the equation below:

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

where: Ab is the absorbance reading of the blank, As is the absorbance reading of the sample, and Ac is the absorbance reading of the control.

Tests microorganisms. Four pathogenic Gram-negative bacteria (*Escherichia coli* BIOTECH 1825, *Enterobacter aerogenes* BIOTECH 1145, *S. marcescens* BIOTECH 1748 and *Pseudomonas aeruginosa* BIOTECH 1824) and five Gram-positive bacteria (*S. epidermidis* BIOTECH 10098, *Listeria monocytogenes* BIOTECH 1958, *S. aureus* BIOTECH 1823, *S. saprophyticus* BIOTECH 1802 and *Bacillus cereus* BIOTECH 1509) were procured from the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), Philippines. The type cultures were pre-cultured using Luria Bertani (LB) Broth medium and stored at 37ºC with shaking for 24 h. The purity of each pathogenic bacterial strain was maintained by continuous checking of the biochemical tests and morphological characteristics (Arguelles 2018).

Micro-dilution antibacterial assay. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *T. ornata* extract were analyzed using two-fold serial dilution technique following the procedures done by Arguelles et al. (2017). An aliquot (100 μl) of test bacterial cultures (cell density of 1x 10^6 cells/mL) was added to a 100 μl of *T. ornata* extract in a 96-well microtiter plate prepared in varying dilutions (1000 μg/mL - 7.8125 μg/mL). The assay was done in triplicate and the microdilution plates were incubated for 12 h at 35°C, after which MICs of the seaweed extract against the test microorganisms were determined. On the other hand, MBC was determined by plating a loopful of sample obtained from each MIC well that exhibited bacterial growth inhibition into a new sterile culture (tryptic soy agar) medium (Arguelles 2018). The culture plates were kept in an incubator set at 35°C for 24 h. After incubation, the petri plates were assessed for bacterial colony growth for each dilution subculturing. No bacterial growth would mean that the algal extract was bactericidal at that particular dilution. MBC value is the minimum concentration of *T. ornata* extract at which no bacterial growth on agar subculture was observed (Arguelles and Sapin 2020).

Statistical analyses. The experimental data for antibacterial, antioxidant and tyrosinase inhibitor activity assays are presented as means ± standard deviations (mean ± SD) of three parallel measurements. The statistical test for the linear correlation and coefficient correlation analysis were evaluated using MS Office Excel 2007.

RESULTS AND DISCUSSION

Total phenolic content (TPC). Polyphenols derived from seaweeds are active compounds with diverse pharmacological activities. These compounds are being used to treat human diseases such as diabetes, melanoma, inflammatory and other cardiac diseases (Chakraborty et al. 2013). The total phenolic content (TPC) of the acidified methanolic *T. ornata* extract was analyzed using Folin-Ciocalteu's phenol reagent and is presented in terms of gallic acid equivalent (GAE). The analysis showed that the TPC of *T. ornata* extract was 18.50 ± 0.17 mg GAE/g. The concentration of the phenolic substances obtained in this study was greater than that observed by Oucif et al. (2017) from selected seaweeds from Algerian west coast such as *Cystoseira compressa* (10.24 ± 0.09 mg GAE/g), *Enteromorpha compressa* (3.94 ± 0.28 mg GAE/g DW), *Ulva lactuca* (2.25 ± 0.05 mg GAE/g DW) and *Porphyra umblicalis* (3.80 ± 0.05 mg GAE/g DW). The recommended effective extraction compounds are polar solvents like ethanol, methanol and acetone since polyphenols are more soluble in these substances (Oucif et al. 2017). Polar organic solvents are capable of extracting phenolic compounds attached in saponins, sugars, glycosides, proteins, salts, mucus and organic acids (Cho et al. 2007). Other works for *Turbinaria* species reported different phenolic contents. Chakraborty et al. (2013) observed lower amount in *T. ornata* and *T. conoides* methanol extracts with 16.64 ± 0.10 mg GAE/g and 3.42 ± 0.35 mg GAE/g, respectively. Differences in the amount of TPC from different seaweed species might be caused by extrinsic factors (such as salinity, irradiation, season, sampling and nutriments) as well as intrinsic factors (such as stage of reproduction and growth of seaweeds).

Antioxidant activities. The development of a novel skin-lightening product for cosmetic application would require versatile antioxidant compounds to enhance its activity. Thus, two methods of *in vitro* antioxidant activity assays were used to analyze the antioxidative potential of *T. ornata* extract used in this study. The antioxidant properties of the extract were analyzed using DPPH radical scavenging activity assay and copper reduction antioxidant capacity (CUPRAC) assay.

DPPH radical scavenging activity. The human skin is constantly exposed to UV-radiation and other environmental pollutants that are capable of generating reactive oxygen species and free radicals that lead to skin damage and aging (Poljšak and Dahmane 2012). UV radiation causes photochemical synthesis of reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydroxyl radical (OH^-), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). These free radicals are suppressed by natural antioxidants in skin (such as melanin) and other antioxidants obtained from food (Poljšak and Dahmane 2012). The potential of *T. ornata* extract to scavenge free radicals were assessed using DPPH radical scavenging assay. *T. ornata* extract exhibited potent hydrogen-donating properties that can serve as an effective antioxidant. The antioxidative activity of the algal extract increases in a concentration-dependent manner with EC_{50} value of 22.10 μg GAE/mL, more potent than the positive control, ascorbic acid, with EC_{50} value of 23.38 μg/mL (Table 1). The results suggested that higher antioxidant activity was observed in extract with higher phenolic concentration that is in agreement with Boonchum et al. (2011). The antioxidant activities of seaweeds from Danish coast such as *Fucus spiralis*, *Sargassum muticum*, and *Dictyota dichotoma* have DPPH scavenging ability with EC_{50} of 71.50 μg GAE/mL, 457.10 μg GAE/mL, and 928.60 μg GAE/mL, respectively. These results are less effective in collation to that of *T. ornata*, which showed the potential of the seaweed as novel source of natural antioxidant that could be used as a replacement to commercially available antioxidants (Arguelles and Sapin 2020). Antioxidant properties of brown macroalgae are associated with the existence of substances such as pigments, vitamins, fucoxanthin, phlorotannins, terpenoids, peptides and other flavonoids with anti-melanogenic properties that can be used as active ingredients for cosmetic products (Azam et al. 2017).

Table 1. DPPH free radical scavenging activity and EC_{50} value of phenolics from *T. ornata* and ascorbic acid.

Sample	**Phenolic concentration (μg GAE/mL)**					**EC_{50}***
	10	**20**	**30**	**40**	**50**	
	DPPH inhibition (%)					μg/mL
Turbinaria ornata	29.96 ± 0.95	47.28 ± 0.61	60.24 ± 0.78	68.31 ± 1.50	74.82 ± 0.28	22.10
	Concentration (μg/mL)					
	8	**16**	**24**	**32**	**40**	
	DPPH inhibition (%)					
Ascorbic Acid	17.65 ± 0.005	34.21 ± 0.44	51.32 ± 0.05	68.58 ± 0.00	83.86 ± 0.00	23.38

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

Copper reduction antioxidant capacity (CUPRAC). CUPRAC assay was done to assess the reducing antioxidant ability of *T. ornata* extract against free radicals. It is a technique that measures

the capability of the algal extract to reduce bis (neocuproine) copper (II) chelate to colored Cu (I)-chelate product. *T. ornata* extract showed dose-dependent copper ion reduction ability (Table 2). *T. ornata* had higher phenolic concentration than ascorbic acid indicating greater antioxidant activity. Moreover, *T. ornata* extract exhibited potent antioxidant activity in collation to that of ascorbic acid with EC_{50} of 24.34 μg GAE/mL and EC_{50} = 46.30 μg/mL, respectively. The results suggested that *T. ornata* extract could inhibit oxidation through metal chelation mechanism that can be ascribed to polyphenolic compounds (such as phlorotannins, bromophenols, quercetin and fucoxanthin) present in the seaweed extract (Ponnan et al. 2017).

Table 2. Copper reduction antioxidant capacity (CUPRAC) and EC_{50} value of phenolics from *T. ornata* and ascorbic acid.

Sample	**Phenolic concentration (μg GAE/mL)**					**EC_{50}***
	5	**10**	**15**	**20**	**25**	
	CUPRAC (Absorbance at 450 nm)					µg/mL
Turbinaria ornata	0.105 ± 0.001	0.207 ± 0.001	0.304 ± 0.001	0.396 ± 0.002	0.516 ± 0.001	24.34
	Concentration (μg/mL)					
	10	**20**	**30**	**40**	**50**	
	CUPRAC (Absorbance at 450 nm)					
Ascorbic Acid	0.112 ± 0.002	0.213 ± 0.007	0.328 ± 0.004	0.429 ± 0.012	0.542 ± 0.011	46.30

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

The reduction potential exhibited by *T. ornata* extract (acting as antioxidant) enhances the tyrosinase inhibition activity of polyphenols present in the extract. These compounds are capable of interacting with *O*-quinones causing inhibition in the synthesis of intermediate compounds important in melanin production (Namjooyan et al. 2019). The findings of this assay substantiate the potential of *T. ornata* as a promising antioxidant useful in cosmetic product formulation.

Correlation study between total phenolic content and antioxidant activity. Polyphenols are bioactive substances in seaweeds that are capable of exhibiting antioxidant activities. Correlation analysis between phenolic concentration and antioxidant activity of *T. ornata* using DPPH scavenging and CUPRAC assays are presented in Figure 1. The results showed a positive correlation for DPPH (R^2 =0.95993) and CUPRAC (R^2 =0.99811) exists between antioxidant activities and phenolic concentration of the *T. ornata* extract. Previous studies showed that positive correlations among phenolic content and antioxidant activities have been documented to several species of seaweeds (Wang et al. 2009; Ponnan et al. 2017; Arguelles, 2020). The correlation among DPPH and CUPRAC values and the phenolic concentrations in *T. ornata* extract proved that polyphenols might have a significant contribution for the antioxidant activity of the alga. Identification of active phenolic compounds in *T. ornata* extract is essential to further understand the reaction mechanism and correlation of polyphenols and its antioxidant properties (Arguelles et al. 2017).

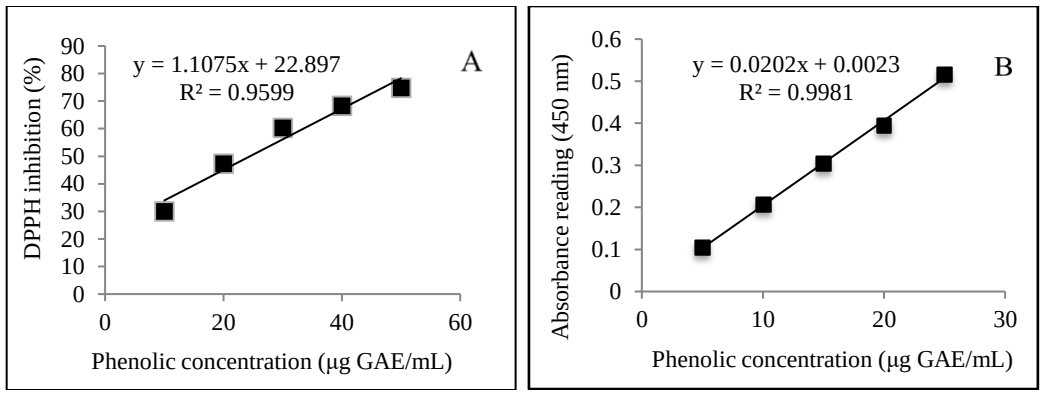

Fig. 1. Simple regression correlation between phenolic content and antioxidant activity via DPPH radical scavenging (A) and copper reduction antioxidant capacity (B) assay of *T. ornata.*

Tyrosinase inhibition activity. *T. ornata* extract exhibited potent activity as tyrosinase enzyme inhibitor with EC_{50} value of 67.50 μg GAE/mL in collation to that of the positive control (kojic acid) with EC_{50} of 109.8 μg GAE/mL. This result showed that *T. ornata* extract is more effective than kojic acid and that this extract contains bioactive substances with anti-melanogenic activities (Table 3). The activity of the *T. ornata* extract was also more effective than other previously reported seaweeds with tyrosinase inhibition properties such as *Turbinaria conoides* (EC_{50} = 188.85 μg /mL), *Eucheuma cottonii* (EC_{50} = 234.44 μg /mL), *Ecklonia stolonifera* (EC_{50} = 345.0 μg /mL), and *Sargassum plagyophyllum* (EC_{50} = 4.97 mg/mL) (Kang et al. 2004; Chang and Teo 2016; Arifianti et al. 2017; Sari et al. 2019).

Table 3. Tyrosinase inhibitor activity and EC_{50} value of phenolics from *T. ornata* and kojic acid.

Sample	**Phenolic concentration (μg GAE/mL)**					**EC_{50}*** μg /mL
	25	**50**	**75**	**100**	**125**	
	Tyrosinase inhibition (%)					
Turbinaria ornata	26.05 ± 0.45	36.08 ± 0.26	56.10 ± 0.35	62.04 ± 0.30	66.96 ± 0.39	67.50
	Concentration (μg/mL)					
	50	100	150	200	250	
	Tyrosinase inhibition (%)					
Kojic Acid	30.79 ± 0.11	47.22 ± 0.25	61.66 ± 0.27	69.81 ± 0.04	77.01 ± 0.25	109.8

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

The enzyme inhibition activity of *T. ornata* is primarily caused by polyphenols such as fucoidan, phlorotannins, fucoxanthin, and flavonoids present in the algal extract that can serve as competitive enzyme inhibitors of tyrosinase (Namjooyan et al. 2019). These compounds have diverse pharmacological activities and are known to possess anti-aging compounds useful for cosmetic application. Fucoxanthin caused a significant reduction in the synthesis of reactive oxygen species (ROS) in human fibroblasts when exposed to UV-B rays (Hoe et al. 2009). This polyphenol reduced cellular damage and increased cell survival, showing the capability of fucoxanthin to protect skin from harmful effects of UV radiation. Antioxidant compounds present in the algal extract could have

anti-tyrosinase activity by inactivating the active site of the enzyme through hydrogen bond formation with copper. Polyphenols such as fucoidan present in several species of brown seaweeds are capable of reacting with copper (in the active site of tyrosinase) through sulfide atoms, a new mechanism involved in inhibiting the production of melanin in skin cells (Namjooyan et al. 2019). Also, other factors such as extraction temperature can enhance the tyrosinase inhibition properties of an algal extract. Twenty species of red seaweeds showed that temperature during algal extraction had an effect on tyrosinase inhibition activity of the algae. It was reported that increasing the temperature during extraction process caused an enhancement (from 9.95 to 90.75% percent inhibition) in the inhibitory activities of the seaweed extracts (Cha et al. 2011). In this investigation, *T. ornata* showed promising anti-tyrosinase activity that can be used as whitening agents for formulation of new skin care products.

Previous studies in the Philippines showed that *T. ornata* exhibited antioxidant activity. However, anti-melanogenic property such as tyrosinase inhibitor activity was not documented. The current study is the first report in the Philippines exploring the biological properties of *T. ornata* as a potential source of skin lightening active ingredient important for cosmetic application. Nevertheless, further studies on *T. ornata* extract are recommended to identify the active compounds and elucidate its reaction mechanism as a tyrosinase inhibitor.

Antibacterial activities. In the Philippines, only few studies on antimicrobial activities of marine seaweeds have been conducted and to date, no documented studies were done on the antibacterial activities of *T. ornata* extract against common skin bacterial pathogens. Thus, this study was done to evaluate the bioactive properties of this macroalga as a potential source of active compounds for cosmetic application. Of the nine bacterial strains tested, four bacterial test organisms were inhibited by the algal extract (Table 4). *T. ornata* showed potent activity against *S. aureus S. saprophyticus, S. epidermidis* and *S. marcescens* having MIC of 125 μg/mL, 125 μg/mL, 250 μg/mL, and 500 μg/mL, respectively. On the other hand, inhibitory activity was not detected against *E. aerogenes*, *B. cereus*, *E. coli*, *P. aeruginosa*, and *L. monocytogenes.* Minimum bactericidal concentration (MBC) against *S. saprophyticus,* and *S. aureus* is 250 μg/mL. It was more potent than that of *S. epidermidis* and *S. marcescens*, with MBC value of 500 μg/mL and 1000 μg/mL, respectively. The antibacterial properties of *T. ornata* are in agreement with those previous antimicrobial studies done for *Turbinaria* (Marudhupandi et al. 2013; Rattaya et al. 2015; Tye et al. 2016). Antibacterial activity against *S. aureus* of seaweeds found in the Red sea in Hurghada, Egypt such as *Sargassum hystrix*, *Caulerpa racemosa* and *Codium fragile* showed MIC value of 20 mg/mL, 50 mg/mL, and 20 mg/mL, respectively (Salem et al. 2011). On the other hand, antibacterial activities of dichloromethane *T. ornata* extract against *S. aureus*, *S. saprophyticus* and *S. epidermidis* exhibited MIC value of 4 mg/mL, 1 mg/mL, and 2 mg/mL respectively (Tye et al. 2016). These reported inhibition concentrations are less effective in collation to the antibacterial activity of *T. ornata* extract obtained in this study.

The genera *Staphylococcus* is considered as the most abundant skin bacteria in humans that can cause nosocomial and skin infections. Thus, antibacterial activity against *Staphylococcus* and other bacterial skin pathogens (such as *Serratia* and *Pseudomonas*) is an important property of an active skin-lightening ingredient for the development of new cosmetic product. In this study, *T. ornata* extract exhibited excellent antibacterial activities towards *Staphylococcus* and *Serratia* proving its potential for cosmetic application. The antibacterial activities of *T. ornata* extracts can be ascribed to bioactive metabolites such as fucoidans, phlorotannins, fucoxanthin, ectoine, terpenoids, tanacetol A, potassium alginate and other bioactive peptides present in *T. ornata* extract with reported diverse antimicrobial properties (Rushdi et al. 2020).

Table 4. Antibacterial activity of *T. ornata* extract.

Test organism	Minimum inhibitory concentration (μg/ml)	Minimum bactericidal concentration (μg/ml)
Gram-positive bacteria		
Staphylococcus aureus BIOTECH 1823	125.00	250.00
Staphylococcus saprophyticus BIOTECH 1802	125.00	250.00
Staphylococcus epidermidis BIOTECH 10098	250.00	500.00
Bacillus cereus BIOTECH 1509	>1000.00	ND
Listeria monocytogenes BIOTECH 1958	>1000.00	ND
Gram-negative bacteria		
Serratia marcescens BIOTECH 1748	500.00	1000.00
Pseudomonas aeruginosa BIOTECH 1824	>1000.00	ND
Escherichia coli BIOTECH 1825	>1000.00	ND
Enterobacter aerogenes BIOTECH 1145	>1000.00	ND

*ND = None detected

The results showed that Gram-positive bacteria are more vulnerable to *T. ornata* crude extract that is in agreement with earlier studies (Chellaram et al. 2012; Tye et al. 2016; Arguelles and Sapin, 2020) showing the effect of differences in the cell wall composition of the test bacteria. Gram-negative bacteria possess an outer membrane as well as a thick murine layer that acts as a barrier preventing the entry of antibiotics or bioactive substances into the cell (Chellaram et al. 2012; Arguelles et al. 2018; Arguelles and Sapin 2020). Variations in the general antibacterial activity of *T. ornata* observed in this investigation and the results obtained from other studies can be ascribed to factors such as seasonal variation, type of extraction solvent, and method of extraction resulting to variation on the kind of active compounds recovered (Arguelles 2020). Additional studies focusing on the cultivation as well as purification and identification of bioactive substances is recommended to further understand and utilize the potential of *T. ornata* for large-scale production.

CONCLUSION

Bioprospecting of seaweed resources of the Philippines offer several important pharmacological benefits related to improvement and development of cosmetic products. The current study showed that *T. ornata* extract contains bioactive compounds with potent tyrosinase inhibitor, antibacterial and antioxidant activities that can be used for cosmetic application. It is the first report in the Philippines exploring the biological properties of *T. ornata* as a potential alternative source of skin lightening active ingredient important for cosmeceutical application. Future studies regarding the identification and structure elucidation of bioactive compounds present in *T. ornata* extract is important to understand the reaction mechanism of the target compounds. Mass cultivation and

optimization studies are recommended to maximize the potential use of this seaweed for commercial and industrial purposes.

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