Research Article

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Galaxaura rugosa (J. Ellis & Solander) J.V. Lamouroux for Cosmeceutical Application:Antioxidant, Antibacterial, Tyrosinase and Elastase Inhibition Properties

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ABSTRACT

Red seaweeds are considered novel sources of natural products with diverse biological activities that can be harness for drug synthesis. The bioactive properties of red macroalga, *Galaxaura rugosa* were studied. Results showed that the macroalga contain a total phenolic content (TPC) of 12.11 ± 0.22 mg GAE/g. The antioxidant activity of *G. rugosa* exhibited potent $ABTS^+$ radical scavenging activity (IC_{50} = 81.00 µg GAE/ml) and high copper reduction capacity (IC_{50} = 19.26 µg GAE/ml). Assessment of tyrosinase and elastase inhibition properties of *G. rugosa* extract showed that the alga has effective inhibitory activity with IC_{50} of 88.00 µg GAE/ml and IC_{50} of 243.00 µg GAE/ml, respectively which are more efficient than kojic acid and tocopherol. The seaweed extract exhibited potent antibacterial activities against common bacterial skin pathogens like *Staphylococcus aureus* (Minimum Inhibitory Concentration (MIC) = 125 µg/ml), Methicillin-resistant *Staphylococcus aureus* (MIC = 125 µg/ml), and *Staphylococcus epidermidis* (MIC = 250 µg/ml). This study is considered a pioneering investigation that shows the potential of *G. rugosa* for cosmeceutical application.

Keywords: Biological Activity, Cosmetic Application, Phenolic Compounds, Seaweed, Skin Aging

1. Introduction

Seaweeds are considered novel sources of promising bioactive metabolites with diverse applications in therapeutics and cosmetics. These organisms have been documented to possess bioactive properties with multiple applications in the cosmetic industry [1-3]. Primary and secondary metabolites from seaweeds such as vitamins, polysaccharides, essential amino acids, fatty acids, fucoxanthin, polyphenol sulphated polysaccharide, catechins, fucosterol, flavonols, and fucoidan, were documented to have anti-aging, antioxidant, anti-tyrosinase, antibacterial, and anticancer properties [1,4]. These reported biological properties highlights the special role of seaweed as important medicinal constituent in managing skin disease and aging from several countries around the world.

Skin aging is a natural biological process that results to dryness, sagging, thickness, pigmentation and loss of skin elasticity [4-5]. This process occur because of physiological (metabolic and hormonal changes) and environmental factors (such as UV radiation exposure) [5-6]. To address skin aging, bioactive compounds extracted from seaweeds are currently being tapped as important therapeutic ingredients in several cosmeceutical products. Recently, seaweed-derived phenolic compounds were proven to prevent hyperpigmentation and other skin disorders like cancer and melanoma (melanin-related skin diseases) when used as active ingredient for topical creams [5-6]. In addition, these substances render skin protecting properties like anti-tyrosinase and anti-aging effects proving the use of these organisms as alternative source of new drugs [5-6]. These bioactive properties of seaweeds are associated to the adaptive response of these organisms to unfavorable environmental conditions (such as allelopathy, grazing, and oxygen fluctuations) in the ecosystem. Exposure to such conditions can lead to development of protective (bioactive) substances that allow the seaweed to resist the effects of toxic free radicals and oxidizing substances [2-3,5-7]. Bacterial pathogens associated to skin diseases such as *Staphylococcus aureus* and *Staphylococcus epidermidis* is considered a primary concern to cosmeceutical industry. These organisms may cause skin diseases such as impetigo, cellulitis, folliculitis, erythrasma, and boils [2,5-7]. Thus, development of products with antibacterial activities against known medically important skin pathogens is a must in several topical creams and other cosmeceutical products. Seaweeds contains polyphenols, alkaloids, and terpenes that possess biological activities against bacterial infections [5-7]. Some of the most active chemical compounds in seaweeds against bacterial pathogens are phlorotannins and terpenoids. These compounds are capable of inhibiting infection by causing cytotoxic effects and cell lysis in bacterial cells [2,5]. In this study, reference bacterial pathogens were obtained from PNCM, BIOTECH-UPLB. Some of these bacterial strains are known pathogens associated to different skin diseases. Thus, antibacterial assay was done to assess the effectivity of the seaweed extract against these organisms. In the Philippines, limited studies have been documented regarding the use of seaweeds as a natural alternative source of skin care active ingredients. To date, only a few species of Philippine seaweeds (*Sargassum ilicifolium, Sargassum aquifolium, Sargassum siliquosum*, and *Turbinaria ornata*) have been reported to possess antibacterial, antioxidant as well as tyrosinase and elastase inhibition properties [2-3,5,7]. Therefore, additional studies that will include isolation (of bioactive constituents) and screening (of skin protecting biological properties) of other species of seaweeds (such as the red and green seaweed species) is timely and very relevant.

Galaxaura rugosa, a red seaweed (Florideophyceae), is abundantly distributed in the Philippine coastal areas. The thallus of this alga is dark red-brown; compact, stiff, and thick capable of forming hemispherical mounds. In addition, it is characterized to have irregularly dichotomous branching pattern with moderate calcification. This macroalgae, yet poorly studied, has been reported to have antibacterial and antioxidant properties [8,9]. Therefore, the current study aims to document other biological properties of this seaweed for cosmeceutical application. The study specifically aims to assess the TPC, antibacterial, antioxidant, as well as tyrosinase and elastase inhibition activities of *G. rugosa*.

2. Material and Methods

2.1. Seaweed Sampling And Collection

G. rugosa was collected on 08 May 2021 in the coast of Nasugbu (Lat. 14° 5' 31.4484" N; Long. 120° 37' 21.8382" E), Batangas, Philippines (Figure 1). The algal biomass was washed several times (using sterile distilled water) and gently scrubbed, to remove

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sand, and other epiphytes from the algal sample. It was then subjected to oven-drying at 60°C for 12 hours. The dried sample was pulverized before subjecting it for solvent extraction. The taxonomic identification was done using taxonomic keys of Trono [10] as well as Algae Base (web site: www.algaebase.org) and was verified by the algae curator of BIOTECH, UPLB, Laguna, Philippines [11].

2.2. Seaweed Extract Preparation

Dried and pulverized biomass of *G. rugosa* (1 g) was subjected to solvent extraction 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 [12]. The sample mixture was then centrifuged at 12,000 rpm for 20 minutes at a temperature of 20°C. The seaweed extract was further concentrated using a rotary evaporator (BUCHI Rotavapor®) set at 40 °C under reduced pressure. The concentrated seaweed extract was then kept under refrigerated condition (4°C) to preserve its biological activity [2,5]. The yield extract of *G. rugosa* was determine using the formula:

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

2.3. Total Phenolic Content

The TPC of *G. rugosa* was analyzed using Folin-Ciocalteu assay (calibration curve equation used in the assay: $y = 0.006415x - 0.0140$, $R^2 = 0.99978$) and expressed as milligram of gallic acid equivalent per gram of the seaweed [13]. Briefly, 0.5 ml of both Folin-Ciocalteau's reagent and 10% sodium carbonate solution were mixed with 0.5 ml of *G. rugosa* crude extract for 1 min. The reaction mixture was thoroughly mixed and was set-aside at ambient normal temperature for 5 min. The volume of the reaction (experimental) mixture was further adjusted using 5 ml distilled water. The absorbance (OD) of the sample mixtures and the control were noted using a spectrophotometer (Ultraviolet-Visible, Shimadzu, Kyoto, Japan) at 720 nm wavelength.

Figure 1. Thallus morphology of *G. rugosa* from the coast of Nasugbu, Batangas.

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

The ability of *G. rugosa* to scavenge $ABTS^+$ was done following the procedure done by Re et al. (1999) [14]. Briefly, 40 µl of *G. rugosa* extract prepared in different phenolic concentrations (30.0 – 150.0 µg GAE/ml) and 40 µl of the control (90% methanol) were mixed with $ABTS^+$ radical mixture (3 ml) with an initial absorbance of 0.72 ± 0.05 at 734 nm. The reaction mixtures were mixed and incubated for 5 minutes at normal room temperature. The absorbance readings of the prepared reaction sample solutions were taken at 734 nm. The percent (%) $ABTS^+$ inhibition was noted using the equation:

$$ABTS^+ \text{ Inhibition (\%)} = \frac{Abs_{734}\,(\text{control}) - Abs_{734}\,(\text{sample})}{Abs_{734}\,(\text{control})} \times 100$$

2.5. Copper Reduction Antioxidant Capacity (CUPRAC) Assay

The CUPRAC assay was done using the methods of Alpinar et al. (2009) [15]. Initially, 1 ml each of the different prepared solutions (1 M ammonium acetate buffer (pH 7), 0.01 M $CuCl_2$ solution, and 0.0075 M neocuproine) were thoroughly mixed in clean test tubes with 0.5 ml of *G. rugosa* extracts (phenolic concentrations: 5.0 - 25.0 µg GAE/ml) as well as ascorbic acid (standard antioxidant). The overall volume of each sample mixtures were adjusted to a total of 4.1 ml using a distilled water and were incubated for 30 minutes at ambient room temperature. The absorbance (OD) for both the *G. rugosa* extract and ascorbic acid concentrations against a reagent blank was noted at 450 nm [3,5,7].

2.6. Antibacterial Assay

Three Gram-negative bacteria (*Serratia marcescens* BIOTECH 1748, *Pseudomonas aeruginosa* BIOTECH 1824, and *Enterobacter aerogenes* BIOTECH 1145) and three Gram-positive bacteria (*Staphylococcus aureus* BIOTECH 1823, *Staphylococcus epidermidis* BIOTECH 10098, and Methicillin-Resistant *Staphylococcus aureus* BIOTECH 10378) were tested against *G. rugosa* crude extract. These test organisms were obtained from PNCM, BIOTECH-UPLB. Initially, these pathogens were grown using Luria Bertani (LB) Broth and was incubated at 37°C for 24 hours with shaking. The purity of each bacterial cultures were regularly monitored by conducting morphological characterization and biochemical tests [16].

Microtiter plate dillution assay was used to know the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *G. rugosa* extract [16]. Briefly, 100 µl of each bacterial cultures were mixed with 100 µl of *G. rugosa* extract at varying dilutions (7.8125 µg/ml – 1000 µg/ml) in a 96-well microtiter plate. The microtiter plate was placed at 35°C for 12 hours, after which the MIC for each bacteria were noted. The MIC of *G. rugosa* extract is the lowest concentration of the extract that showed bacterial growth inhibition after 12 hours incubation period. On the other hand, minimum bactericidal activity (MBC) of *G. rugosa* extract was determined by inoculating a loopful of the sample (from MIC wells that showed no visible bacterial growth) into freshly prepared tryptic soy agar. The plates were incubated at 35°C for 24 hours and were examined for bacterial growth for each dilution subculturing. Absence of colony growth would mean that the algal extract was bactericidal to the pathogen at that specific dilution [16].

2.7. Tyrosinase Inhibition Assay

The whitening property of *G. rugosa* extract was evaluated *in vitro* using tyrosinase inhibition assay following the methods of Hapsari et al. (2012) [17]. Solutions of mushroom tyrosinase (250 units/ml, Sigma T- 3824), 0.1M potassium phosphate buffer (pH 6.5), and 5mM DOPA (3,4-dihydroxy-L-phenylalanine, Sigma D-9628) were prepared. Briefly, an aliquot of 40 µl DOPA is mixed with 40 µl of *G. rugosa* extract (at different concentrations) or 40 µl buffer (for the control) in a 96-well microtiter plate. The overall volume of each sample mixture was adjusted to 160 µl by adding 40 µl of phosphate buffer and mushroom tyrosinase. The microtiter plate containing the mixtures was placed at ambient room temperature for 15 min. The absorbance reading was taken using a microtiter plate reader at a wavelength of 490 nm [17]. The percent tyrosinase inhibition was computed using the formula:

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

where A_{blank} is the absorbance reading of the blank, A_{sample} is the absorbance reading of the sample (seaweed extract), and $A_{control}$ is the absorbance reading of the control. The positive control used in the assay is kojic acid.

2.8. Elastase Inhibition Assay

The anti-wrinkling properties of *G. rugosa* extract was evaluated using the method of Moon et al. (2010) [18]. Initially, solutions of 0.2M TRIS-HCl buffer, pH 8.0, elastase from porcine pancreas (50 ug/ml, Sigma E-7885), and N-succinyl-$(ALA)_3$-p-nitroanilide (25 mM, Sigma S-4760) were prepared. Aliquot (40 µl) of *G. rugosa* extract (at varying phenolic concentration: 100.0, 200.0, 300.0, 400.0, and 500.0 µg GAE/ml) or 40 µl buffer (for the control) were mixed with 40 µl N-succinyl-$(ALA)_3$-p-nitroanilide in sterile test tubes. The volume of the reaction mixture was adjusted to 1ml by adding first phosphate buffer and followed by 40 µl elastase in the solution. In this assay, the blank used is the reaction mixture lacking the enzyme solution. After 20 min, 2 ml of TRIS-HCl buffer were added in the reaction mixtures and the absorbance reading of each concentration (samples) was measured at 410 nm. The percent elastase inhibition was computed using the formula:

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

where A_{blank} is the absorbance of the blank, $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample (*G. rugosa* extract). The positive control used in the assay is tocopherol.

2.9. Statistical Analysis

The assays done in this investigation were done in three replicates and data were given as means ± standard deviations. The correlation analysis (Pearson's correlation coefficient) among antioxidant ac-

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tivities and the phenolic concentrations of *G. rugosa* extract were evaluated using Microsoft (MS) Office Excel 2019 [16].

3. Results and Discussion

3.1. Extraction Yield and TPC

Extraction yield is the portion of the crude seaweed extract that can be used from the sample [19]. In this study, crude extract of *G. rugosa* are observed to have a reddish brown color, which can be attributed to algal pigments such as phycoerythrin, carotenoids, and chlorophyll present in the seaweed crude extract [20]. The extraction yield of *G. rugosa* crude extract is 10.79 ± 0.03% which is higher as compared to that obtained from ethanol extracts of *S. horneri* (1.36±0.14%) but is lower than *Kappaphycus alvarezii* (15.469%) [19-20]. The differences in the extraction yield of *G. rugosa* documented in this study is associated with important factors such as polarity of the solvent and method of extraction used in the protocol [19-20]. Thus, optimization of the extraction condition and protocol is needed for mass production and use of natural products that can be derived from *G. rugosa*.

Phenolic compounds are secondary metabolites that are abundantly present in algal cell wall that are used mainly for defense against harsh environmental condition [5-6]. These compounds are present in several species of seaweeds and are being harnessed nowadays for cosmeceutical application because of the reported potent antioxidant, antibacterial, and tyrosinase inhibition activities [2-3,5-6]. The TPC of *G. rugosa* is 12.11 ± 0.22 mg GAE/g extract (Table 1). This result was greater than those observed from other seaweeds such as *Anthophycus longifolius* and *Padina pavonica* with TPC of 4.5 mg GAE/g and 10.55 mg GAE/g, respectively [21-22]. On the other hand, Bhuyar et al. [20] showed that ethanol extract of *K. alvarezii* has higher TPC than *G. rugosa*, which was 20.25 ± 0.03 mg GAE/g. Generally, extraction of phenolic compounds from algal biomass is highly affected by the type and polarity of solvent used in the extraction as well as solubility of algal polyphenols in the extraction solvents [19-20]. In addition, the age and maturity of the seaweeds as well as geographic location (where the seaweed was collected) can also affect the metabolic profile and kind of polyphenols that can be extracted from the macroalga [2].

3.2. $ABTS^+$ Radical Scavenging Activity

The $ABTS^+$ scavenging efficiency of *G. rugosa* extract exhibited a concentration-dependent inhibition of $ABTS^+$ free radicals (Table 2). Results of the analysis showed that *G. rugosa* has potent $ABTS^+$ radical scavenging activity, more effective than ascorbic acid (control). The computed effective concentration (IC_{50}) of *G. rugosa* extract is 81 µg/ml which is more potent as compared to ascorbic acid with IC_{50} value of 151 µg/ml. This antioxidant activity is considered more potent than that obtained for *S. aquifolium* (IC_{50} of 107 µg/ml) but is less effective as compared to *T. decurrens* (IC_{50} of 49.31 µg/ml) [2,23]. Phenolic compounds derived from seaweeds exhibit free-radical scavenging properties as a defense mechanism of the algal thalli against oxidative effects of UV radiation [24]. High concentration of this bioactive compound is accompanied with potent antibacterial and antioxidant activities [24].

3.3. Copper Reduction Antioxidant Capacity

G. rugosa extract exhibited a concentration-dependent reduction capacity of copper ion. The trend observed in this assay is like that of $ABTS^+$ scavenging assay wherein at high phenolic concentration, the highest $ABTS^+$ free radical inhibition was noted. Table 3 shows that the seaweed extract are characterized to have potent antioxidant property more effective than ascorbic acid with IC_{50} value of 19.26 µg/ml and 44.12 µg/ml, respectively. The copper reduction antioxidant capacity of *G. rugosa* is more effective than those obtained from *S. aquifolium* and *T. ornata* with IC_{50} values of 21.01 µg/ml and 24.34 µg/ml, respectively [2,7]. The findings of this study suggest that *G. rugosa* extract are capabale of inhibiting oxidation via metal chelation mechanism which is associated with phenolic compounds that are present in the algal extract [2,7].

Table 1. Total phenolic content and extraction yield of *G. rugosa* acidified methanolic extract.

Sample	Solvent	Extract Yield (%)	Total Phenolic Content (mg GAE/g)
G. rugosa	Acidified Methanol	10.79 ± 0.03	12.11 ± 0.22

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

G. rugosa		**Ascorbic Acid**	
Phenolic concentration (µg GAE/ml)	**$ABTS^+$ Inhibition (%)**	**Concentration (µg/ml)**	**$ABTS^+$ Inhibition (%)**
30	21.84 ± 0.10	37.5	14.34 ± 0.49
60	37.15 ± 1.09	75.0	25.82 ± 0.00
90	55.69 ± 0.50	112.5	38.07 ± 0.20
120	67.21 ± 0.89	150.0	49.69 ± 0.10
150	74.30 ± 1.59	187.5	62.70 ± 0.79
IC_{50}*	**81 µg/ml**	**IC_{50}***	**151 µg/ml**

*IC_{50} is the concentration that effectively inhibits the activity of $ABTS^+$ cation radical by 50%.

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

G. rugosa		**Ascorbic Acid**	
Phenolic concentration (µg GAE/ml)	**CUPRAC value**	**Concentration (µg/ml)**	**CUPRAC value**
5.0	0.145 ± 0.008	10.0	0.114 ± 0.001
10.0	0.276 ± 0.002	20.0	0.232 ± 0.002
15.0	0.398 ± 0.002	30.0	0.347 ± 0.001
20.0	0.518 ± 0.007	40.0	0.454 ± 0.008
25.0	0.638 ± 0.007	50.0	0.566 ± 0.005
IC_{50}*	**19.26 µg/ml**	**IC_{50}***	**44.12 µg/ml**

*IC_{50} is the concentration that gives a CUPRAC value of 0.5 at 450 nm.

3.4. Correlation Study Between TPC And Antioxidant Activity

Polyphenols derived from seaweeds are secondary metabolites that shows diverse antioxidant activities [5,7,23]. In this study, correlation analysis among phenolic concentrations of *G. rugosa* and antioxidant activities via $ABTS^+$ free radical scavenging and CUPRAC assays are presented in Table 4. The results showed a positive correlation exists between antioxidant activities [$ABTS^+$ (R=0.98722) and CUPRAC (R=0.99985)] and phenolic concentration of the *G. rugosa* extract. This observation is similar to previous studies that documented positive correlations among phenolic content and antioxidant activities among species of seaweeds such as *S. aquifolium, T. decurrens,* and *Laurencia intermedia* [2,23-24]. The correlation among CUPRAC and $ABTS^+$ values and the phenolic concentrations in *G. rugosa* extract suggest that polyphenols cause the potent antioxidant activities of the seaweed.

3.5. Antibacterial Activities

Seaweeds are cheap alternative source of active metabolites with antibacterial activities that are important in the cosmeceutical industry [2]. *G. rugosa* exhibited antibacterial activities against known bacterial skin pathogens (MRSA, *S. epidermidis* and *S. aureus*). These bacterial pathogens can cause superficial infections in skin tissues (sebaceous layer) causing acne and other known skin diseases [25-26]. The MIC of *G. rugosa* extract for MRSA and *S. au-*

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Table 4. Correlation between antioxidant activities and phenolic content of *G. rugosa* extract.

Antioxidant Assay	Regression Equation	Correlation Coefficient (R)
$ABTS^{+}$ Radical Scavenging Assay	y = 0.4499x + 10.744	0.98722
CUPRAC Assay	y = 0.0246x + 0.0266	0.99985

reus is 125 µg/ml while for *S. epiderimidis* is 250 µg/ml (Table 5). On the other hand, MBC value of *G. rugosa* extract is considered more potent in *S. aureus* and MRSA (250 µg/ml) than that observed for *S. epidermidis* (500 µg/ml). The antimicrobial property of *G. rugosa* extract against *S. aureus* is more potent than those observed from methanol extracts of macroalgae obtained from Adriatic Sea such as *Dictyota dichotoma, P. pavonica,* and *S. vulgare* with MIC value of 1.25, 1.25, and 2.5 mg/ml, respectively [25]. In addition, *G. rugosa* extract also exhibited a more effective antibacterial activity against *S. epiderimidis* as compared to seaweed species obtained from the coast of South Korea such as *Ecklonia clava, E. kurome,* and *Symphyocladia latiuscula* with reported MIC values of 2.5, 2.5 and 0.63 mg/ml, respectively [26]. This study is the first documented report of the antibacterial activities of *G. rugosa* extract against *S. epidermidis* and MRSA. Thus, documenting the potential biotechnological use of this red alga as novel alternative sources of bioactive compounds for cosmeceutical application. The antibacterial activity exhibited by *G. rugosa* in this study may be associated to known secondary metabolites in red seaweeds such as bromophenols and flavonoids which are toxic (can cause alteration in the cell membrane permeability leading to cell death) to several microorganisms such as bacteria and fungi [26].

The study also documented that *G. rugosa* extract are more potent in inhibiting Gram-positive bacterial strains than Gram-negative bacteria since no antibacterial activities were observed against *E. aerogenes, P. aeruginosa,* and *S. marcescens* (Table 5). Differences in the antibacterial activities of the two group of bacteria relies on the variation in the structure of bacterial cell wall. Gram-negative bacteria are known to have cell walls that are multilayered which act as supplemental barrier that protects the cells from antibiotics [2,23].

3.6. Tyrosinase Inhibition Activity

Tyrosinase is an enzyme responsible in melanin synthesis. Excessive production of this skin pigmenting enzyme will cause hyperpigmentation that may lead to skin cancer [5,7]. Thus, inhibitors of this enzyme is important in synthesis of novel drugs. Red seaweeds are known as sources of natural products that have moisturizing, antioxidant, and skin-whitening activities that are important in the cosmetic industry [24]. In this study, the whitening capacity of a red seaweed (*G. rugosa*) was evaluated *in vitro* using mushroom tyrosinase. Results showed that the seaweed extract exhibited a concentration-dependent activity against tyrosinase (Table 6). The computed IC_{50} of *G. rugosa* extract is 88 µg/ml which is more effective than kojic acid with IC_{50} value of 113 µg/ml. Also, *G. rugosa* extract is more potent than those obtained from extracts of other seaweeds from previous studies such as *T. ornata* and *S. ilicifolium* with IC_{50} values of 67.5 µg/ml, and 40.5 µg/ml, respectively [5,7]. The result of this analysis shows that *G. rugosa* extract contain bioactive substances with anti-melanogenic activities which can be harness as active ingredient for cosmetic application. Phenolic compounds are considered potent tyrosinase inhibitors. These compounds are reported to cause conformational changes in the active site of tyrosinase and are capable of competitive enzyme inhibition [5,7].

3.7. Elastase inhibition activity

Elastase and collagenase are enzymes responsible for degradation of skin collagen and elastin which causes wrinkling [27]. The anti-aging activity of *G. rugosa* extract was assessed *in vitro* via elastase inhibition assay. Results showed that *G. rugosa* extract exhibited highest inhibition activity at 500 µg GAE/ml with percent inhibition of 77.46 ± 0.31% (Table 7). The computed IC_{50} of *G. rugosa* extract is 243 µg/ml which is more effective than the control (tocopherol) with IC_{50} value of >2500 µg/ml. In addition, elastase inhibition activity of *G. rugosa* is more potent to that observed for Halopteris scoparia (IC_{50} value of 1.419 mg/ml) but is less effective than *S. aquifolium* (IC_{50} value of 231 µg/ml) [2,28,29]. Phenolic compounds derived from seaweeds are known elastase inhibitors. These compounds (such as phlorotannins,

Table 5. Antibacterial activities of *G. rugosa* extract.

Bacterial Pathogens	MIC values (µg/ml)	MBC values (µg/ml)
Gram-positive bacteria		
S. aureus BIOTECH 1823	125.00	250.00
Methicillin-Resistant *S. aureus* BIOTECH 10378	125.00	250.00
S. epidermidis BIOTECH 10098	250.00	500.00
Gram-negative bacteria		
E. aerogenes BIOTECH 1145	>1000.00	ND
P. aeruginosa BIOTECH 1824	>1000.00	ND
S. marcescens BIOTECH 1748	>1000.00	ND

*ND = None Detected; MIC= Minimum Inhibitory Concentration; MBC= Minimum Bactericidal Concentration

Table 6. Tyrosinase inhibition activity and IC_{50} value of *G. rugosa* extract and kojic acid.

G. rugosa		**Kojic Acid**	
Phenolic concentration (µg GAE/ml)	**Tyrosinase Inhibition (%)**	**Concentration (µg/ml)**	**Tyrosinase Inhibition (%)**
20.0	14.66 ± 1.91	50.0	27.81 ± 0.77
40.0	23.43 ± 0.44	100.0	46.20 ± 0.36
60.0	33.71 ± 2.18	150.0	60.66 ± 0.22
80.0	45.25 ± 0.67	200.0	68.39 ± 0.31
100.0	57.16 ± 0.52	250.0	73.98 ± 0.01
IC_{50}*	**88 µg/ml**	**IC_{50}***	**113 µg/ml**

* IC_{50} is the inhibitory concentration that effectively inhibits tyrosinase by 50%.

dioxinodehydroeckol, eckol, bieckol, and dieckol) are reported to cause inhibition of matrix metalloproteinase in human dermal fibroblast cells preventing the expression and synthesis of elastase in the skin [27,29]. The result of this analysis shows that *G. rugosa* extract contain bioactive substances with anti-aging activities which can be use as substitute active ingredient for drug synthesis and cosmetic use.

4. Conclusions

In conclusion, *G. rugosa* extract exhibited promising bioactivities such as antioxidant and antibacterial activities as well as elastase and tyrosinase inhibition properties that can be useful for cosmeceutical application. This study is considered as a preliminary data that describes the biotechnological use of *G. rugosa*. Thus, additional studies are recommended to identify the active compounds (via High Performance Liquid Chromatography or Liquid Chromatography-Mass Spectrometry) and know the reaction mechanisms involve in these active substances that are present in the algal extract. It is also recommended to do *in vivo*

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Table 7. Elastase inhibition activity and IC_{50} value of *G. rugosa* extract and tocopherol

G. rugosa		Tocopherol	
Phenolic concentration (µg GAE/ml)	**Elastase Inhibition (%)**	**Concentration (µg/ml)**	**Elastase Inhibition (%)**
100.0	24.73 ± 1.78	500.0	16.58 ± 0.19
200.0	41.05 ± 1.57	1000.0	19.35 ± 0.06
300.0	61.99 ± 0.37	1500.0	26.08 ± 1.13
400.0	73.23 ± 0.21	2000.0	31.03 ± 0.95
500.0	77.46 ± 0.31	2500.0	38.22 ± 0.37
IC_{50}*	**243 µg/ml**	**IC_{50}***	**>2500 µg/ml****

*IC_{50} is the concentration that effectively inhibits elastase by 50%.

**IC_{50} was not determined because 50% inhibition was not achieved at 2500 µg/ml concentration.

experimental trials to further confirm the effectivity and safety of *G. rugosa* extract for cosmeceutical application. In addition, mass production of the target active compounds found in *G. rugosa* extract should also be studied to further understand the mechanisms involve in other biological activities of the alga *in vivo*.

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Conflict of Interest

The author declare that they have no known conflict of interests that could have appeared to influence the work reported in this paper.

Statement of Contribution of Researchers

Concept of the study-EDLRA; Data collection -EDLRA, ABS; Literature review-EDLR; Data analysis-EDLR, ABS; Writing-EDLR; Supervision-EDLR.

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