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Research Article

Evaluation of Antioxidant Potential and Phytochemical Composition of Carrageenan Extracts from Nine Edible Seaweed Species

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Abstract

Seaweeds have become a subject of interest due to their dual role, not only in providing functional attributes, such as gelling, thickening, and stabilizing in food products, but also for their potential antioxidant properties. Currently, a growing body of research supports the idea that supplementation with antioxidants is a valuable approach in preventing oxidative stress, which can lead to cancer, diabetes, cardiovascular, and neurological diseases, where free radicals are implicated. This study aimed to determine the antioxidant activity and phytochemical composition of carrageenan extracts from nine algae collected from the Western Visayas region. Antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and the phytochemical composition was analyzed by thin-layer chromatography. Eight of the nine algal extracts exhibited antioxidant activity. The most active extracts were observed in *S. crassifolium* (IC_{50} = 559 µg/mL), followed by *S. moniliformis* (IC_{50} = 573 µg/mL) and *E. muricatum* (IC_{50} = 629 µg/mL), with no significant difference from the positive control. The abundance of flavonoids, phenols, alkaloids, saponins, tannins, steroids, essential oils, and other phenolic compounds was observed across all extracts, indicating significant antioxidant activity. These findings imply that the integration of natural antioxidants from algae as a dietary supplement could prove beneficial in mitigating oxidative stress, thus holding significance in a wide array of disease prevention strategies, such as cancer, diabetes, cardiovascular diseases, and neurological disorders, all of which are intricately linked to the role of free radicals.

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INTRODUCTION

Driven by a shift in global consumer preferences toward natural, plant-based ingredients, carrageenan has garnered significant attention for its dual utility: providing vital functional attributes in food matrices while also exhibiting promising intrinsic antioxidant properties. This interest is reinforced by an expanding body of clinical evidence implicating free radicals and reactive oxygen species (ROS) in severe disruptions to human health, including oncogenesis[1], accelerated aging[2,3], cardiovascular diseases[4], insulin-resistant diabetes[5], gastrointestinal disorders[6], and various autoimmune or inflammatory pathologies[7]. When a physiological imbalance occurs, elevated ROS levels can induce genetic mutations and single- or double-strand DNA breaks, ultimately impairing critical macromolecules, including cellular DNA, functional proteins, and structural lipids. This pathological cascade culminates in systemic oxidative stress and drives diverse disease states[4], underscoring the importance of targeted deployment of antioxidant compounds to minimize endogenous oxidative damage[8].

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The human antioxidant defense network operates via a collaborative dual framework consisting of endogenous substances synthesized directly within the body, such as reduced glutathione and specialized antioxidant enzymes, including catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase, complemented by exogenously sourced compounds, such as antioxidant vitamins A, C, and E, which possess the capacity to reduce reactive metabolites into significantly less reactive chemical forms[9]. Nevertheless, an excessive or unregulated intake of dietary supplements can actively disrupt the delicate equilibrium of this inherent defense system. Because standard recommended daily allowances are frequently insufficient to fully mitigate severe oxidative stress, individuals often turn to concentrated formulations; however, consuming substantial quantities of high-potency antioxidant supplements can paradoxically induce toxic pro-oxidant effects or trigger an uncompensated physiological state known as antioxidative stress[10].

The clinical challenges and side effects associated with prolonged use of conventional synthetic therapies have spurred the global scientific community to explore alternative medical approaches centered on safe, biocompatible, and naturally occurring antioxidant agents. Presently, significant research attention is directed toward the systematic exploration of abundant marine biodiversity, which serves as an underutilized reservoir of diverse bioactive compounds with global pharmaceutical and nutraceutical implications. The Western Visayas region of the Philippines possesses exceptionally rich aquatic resources, according to the Food and Agriculture Organization (FAO). Among these diverse marine life forms, seaweeds have become a primary focal point of pharmacological investigation as a prominent source of diverse biological activities, including antibacterial[11], anti-angiogenesis[12], and anti-hypertensive[13] properties, attributes that are heavily mediated by their unique secondary metabolite profiles and high local availability.

In this study, nine distinct varieties of marine seaweeds, including *Eucheuma muricatum*, *Gracilaria coronopifolia*, *Galaxaura oblongata*, *Scinaia moniliformis*, *Sargassum crassifolium*, *Acanthophora spicifera*, *Gracilaria eucheumioides*, *Laurencia okamurae*, and *Titanophora weberae*, were strategically collected from the coastal waters of Western Visayas, Philippines. The secondary metabolite compositions and concurrent antioxidant capacities of these nine seaweed samples were evaluated through systematic screening processes. The explicit purpose of this investigation was to characterize the distinct bioactive compounds present within these seaweeds and to evaluate their specific antioxidant capacities as contributing factors to their broader biological profiles. To date, this work constitutes the first formal report detailing the comparative biological activities and rigorous chemical characterization of these distinct seaweed species native to these specific islands.

MATERIALS AND METHODS

Materials

The biological matrix used in this investigation comprised fresh seaweed specimens encompassing nine distinct samples: *E. muricatum* (EM/A), *G. coronopifolia* (GC/B), *G. oblongata* (GO/C), *S. moniliformis* (SM/D), *S. crassifolium* (SC/E), *A. spicifera* (AS/F), *G. eucheumioides* (GE/G), *L. okamurae* (LO/H), and *T. weberae* (TW/I) (**Figure 1**). These macroalgal specimens were collected from the coastal waters of Western Visayas, Philippines, at the coordinates 11°42'22" N, 122°21'52" E. Taxonomic authentication of the specimens was conducted by Prof. Andres Tungpalan at Mariano Marcos State University, Philippines. Ground tissue preparation was performed using an Osterizer blender, and the resulting dry powders were stored in light-resistant amber glass containers.

The chemical reagents used for the extraction, neutralization, and phytochemical profiling phases included anhydrous sodium carbonate, distilled water, 1 M hydrochloric acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, and 95% analytical-grade ethanol. Ascorbic acid was utilized as the standard reference antioxidant compound. Quantitative absorbance screenings and pH tracking were monitored using a calibrated laboratory pH meter and a microplate spectrophotometer. Chromatographic profiling was performed using precoated silica gel thin-layer chromatography (TLC) plates. The specialized chemical spray reagents used for secondary metabolite visualization included antimony(III) chloride, potassium ferricyanide-ferric chloride, Dragendorff's reagent, magnesium acetate, Van Urk-Salkowski reagent, and vanillin-sulfuric acid.

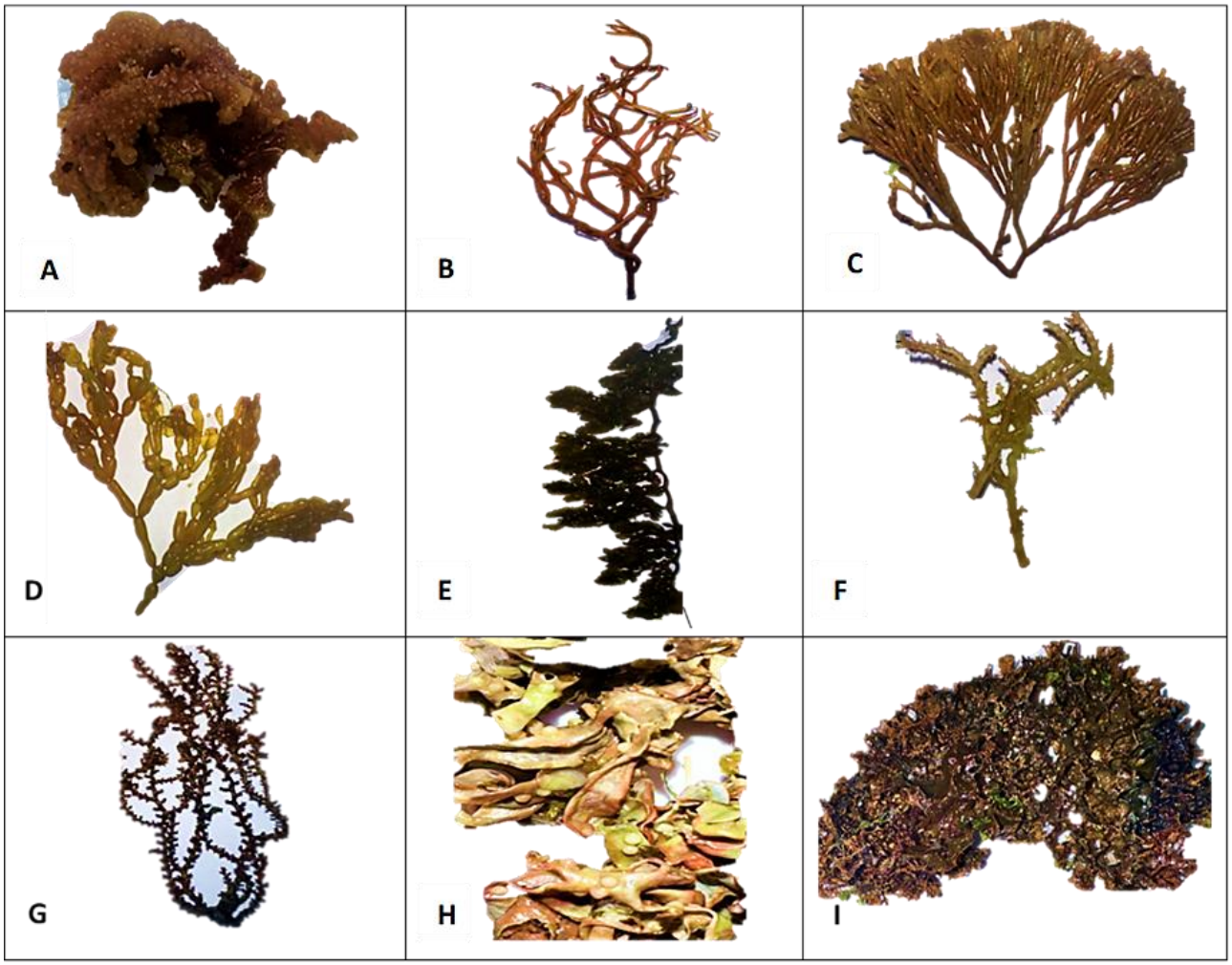

Figure 1. The actual photo of *E. muricatum* (A), *G. coronopifolia* (B), *G. oblongata* (C), *S. moniliformis* (D), *S. crassifolium* (E), *A. spicifera* (F), *G. eucheumioides* (G), *L. okamurae* (H), *T. weberae* (I) collected in Western Visayas, Philippines.

Methods

Extraction procedure

The finely powdered macroalgal samples were separately extracted with a solution of anhydrous sodium carbonate and distilled water until the extraction medium reached a consistently alkaline pH greater than 8. The samples were heated at 50°C for 10 minutes in a controlled water bath, then filtered to separate the insoluble cellular residue from the liquid phase. The filtrates from all nine samples were neutralized by dropwise addition of 1 M hydrochloric acid until the pH was below 7. These neutralized filtrates were then completely lyophilized to remove residual water. A total of nine distinct crude extracts were subsequently evaluated at three target concentrations (1, 100, and 1,000 µg/mL) during the phytochemical screening phases to evaluate the secondary metabolite fractions within the extracts.

DPPH free-radical-scavenging assay

The antioxidant capacity of the macroalgal extracts was quantitatively assessed by measuring their radical-scavenging activity against stable DPPH radicals, which serves as an indicator of their intrinsic hydrogen-donating ability, following an established method[14]. For the experimental run, a 20 µL aliquot of each extract and standard solution was dispensed into designated wells of a multi-well plate, followed by the immediate addition of a 0.004% DPPH solution dissolved in 95% analytical-grade ethanol. After incubating the reaction mixtures in the dark at room temperature for exactly 30 minutes, the relative absorbance was measured at 517 nm using a microplate spectrophotometer. This identical protocol was systematically applied across all seaweed samples at varying concentration intervals to thoroughly assess their scavenging impact at both low and high dose thresholds. Each independent experiment was performed in triplicate, and the percentage

mean radical inhibition was calculated using the following **Equation 1**, in which A_{DPPH} represents the average absorbance value recorded for the control DPPH radical solution, whereas A_{Sample} denotes the average absorbance value computed for each individual seaweed extract, with the latter value strictly adjusted by deducting the average baseline absorbance of the blank ethanol control.

$$\%inhibition = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100 \quad [1]$$

Secondary metabolite composition by thin-layer chromatography

Phytochemical screening was performed using TLC according to a previously described methodology[12]. A small aliquot of the specific macroalgal extract that demonstrated the highest baseline antioxidant capacity was carefully applied near the lower origin section of a pre-coated silica gel plate. The spotted plate was then positioned vertically inside a shallow chromatographic chamber equilibrated with the developing solvent system. Following separation, the secondary metabolite bands were visualized using the prepared series of specialized spray reagents, including antimony (III) chloride, potassium ferricyanide-ferric chloride, Dragendorff's reagent, magnesium acetate, Van Urk-Salkowski reagent, and vanillin-sulfuric acid.

Data analysis

A two-way independent analysis of variance (ANOVA) was employed to systematically assess statistical variation in antioxidant activity across the different experimental concentrations and treatment groups, using a verified significance threshold of $\alpha = 0.05$. For all analytical findings that demonstrated statistically significant differences, subsequent pairwise diagnostic evaluations were performed using Tukey's Honestly Significant Difference (HSD) post hoc test to make precise comparisons of the average radical scavenging activity across different concentration levels.

RESULTS AND DISCUSSION

The chemical interplay between the stable radical DPPH and an exogenous free-radical scavenger initiates the formation of reduced DPPH (DPPH-H). This molecular conversion exhibits a significantly lower optical absorbance than the parent radical, due to the reduction of its radical center via hydrogen or electron donation. The liberated radicals undergo a distinct colorimetric shift from deep violet to pale yellow upon decolorization, indicating electron accumulation and the corresponding pairing of the lone-pair sheet. Spectrophotometric screening of the macroalgal extracts against DPPH radicals showed that eight of the nine collected samples exhibited antioxidant activity. The radical-scavenging effects of these samples collected from Western Visayas were strongly dose-dependent. Among the 27 evaluated concentration parameters, the 1,000 µg/mL extract of *S. crassifolium* exhibited the highest overall radical inhibition, followed closely by identical concentrations of *S. moniliformis* and *E. muricatum*. Evaluating these datasets with a two-way independent ANOVA demonstrated no statistically significant difference between the peak percentage inhibition achieved by the crude extracts of *S. crassifolium*, *S. moniliformis*, and *E. muricatum* relative to the commercial reference standard, ascorbic acid.

The quantitative tracking in **Figure 2** shows that the macroalgae's baseline antioxidant capacity increased progressively with increasing concentration. The lowest radical-scavenging activity was observed at a dose of 1 µg/mL, while minimal inhibition profiles were recorded at the 100 µg/mL concentration threshold for *G. coronopifolia*, *G. oblongata*, *A. spicifera*, and *T. weberae*. Conversely, *L. okamurae* extract exhibited a dose-dependent pro-oxidant inversion profile at elevated concentrations. This biphasic behavior is likely driven by the presence of redox-active trace metal ions and complex halogenated or phenolic components that undergo rapid auto-oxidation at high concentrations. These auto-oxidation pathways can generate secondary ROS or chemically interfere with the DPPH assay reagents, resulting in negative calculated antioxidant values. Consequently, while low doses of *L. okamurae* may provide mild radical-scavenging protection, higher concentrations shift the thermodynamic balance toward oxidative reactions. As shown in **Figure 2**, the *S. crassifolium* extract displayed superior therapeutic efficacy compared to the other macroalgal treatments, a trend further supported by its lower IC_{50} values. The overall antioxidant efficacy was ranked in the following descending order: *S.*

crassifolium (IC_{50} = 559 µg/mL) is greater than *S. moniliformis* (IC_{50} = 573 µg/mL) is greater than *E. muricatum* (IC_{50} = 629 µg/mL) is greater than *G. eucheumioides* (IC_{50} = 989 µg/mL) is greater than *G. oblongata* (IC_{50} = 1,841 µg/mL) is greater than *G. coronopifolia* (IC_{50} = 3,005 µg/mL) is greater than *A. spicifera* (IC_{50} = 3,240 µg/mL) is greater than *T. weberae* (IC_{50} = 3,994 µg/mL).

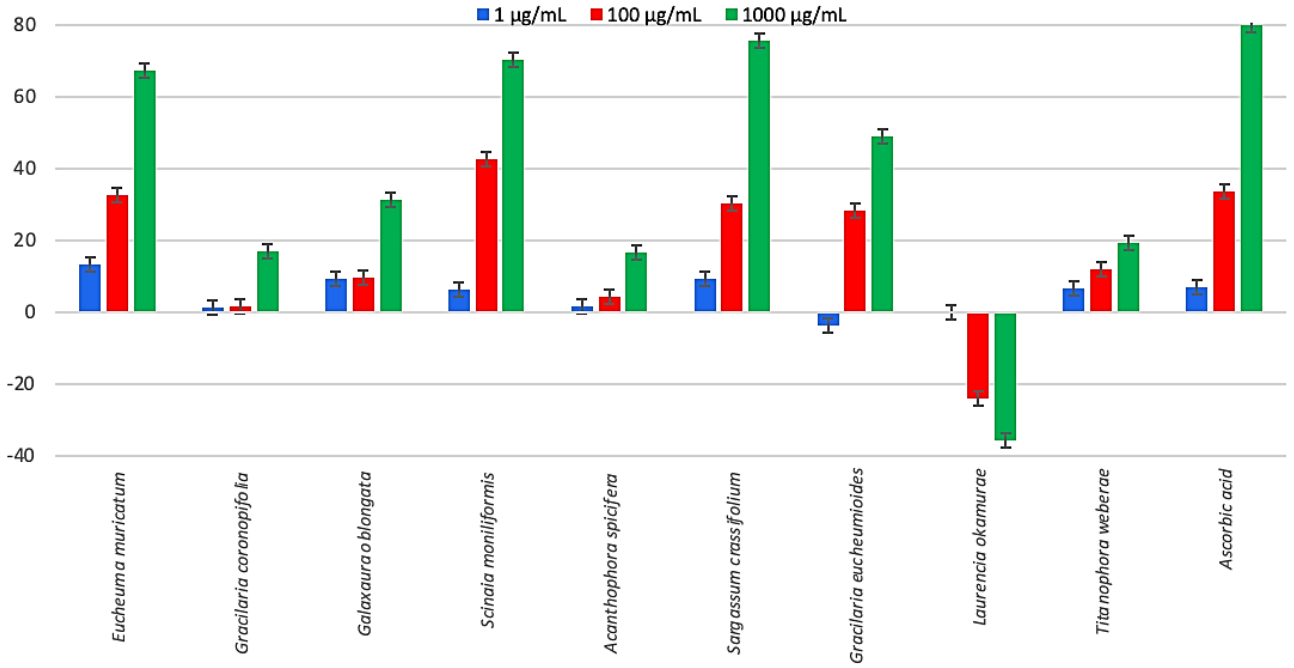

Figure 2. The effect of *E. muricatum*, *G. coronopifolia*, *G. oblongata*, *S. moniliformis*, *S. crassifolium*, *A. spicifera*, *G. eucheumioides*, *L. okamurae*, and *T. weberae* at concentrations of 1, 100, and 1,000 µg/mL against free radicals using Ascorbic acid as a reference standard.

A two-way independent ANOVA was performed to systematically assess variation in radical-scavenging activity across the three concentration thresholds (1, 100, and 1,000 µg/mL) and across individual macroalgal extracts at an established significance level of $\alpha = 0.05$. Based on the data in **Table I**, the total variation in antioxidant performance can be attributed to differences in concentration levels ($F = 1{,}932.36$, $p < 0.001$). Similarly, statistically significant differences in antioxidant capacity were observed across the different treatment types ($F = 5.86$, $p < 0.001$). The statistical parameters listed in the header include the sum of squares (SS), degrees of freedom (df), mean square (MS), F-statistic (F), and the calculated probability value (p). In this parametric framework, because the calculated p-values are lower than the predetermined significance threshold of $\alpha = 0.05$, the observed mean differences across both experimental axes are considered highly significant.

Table I. Mean difference in antioxidant activity of each algal concentration and treatment types.

Source of Variation	SS	df	MS	F	p
Concentration	12.385	2	6.193	1932.36	less than 0.001
Treatment	0.188	10	0.019	5.86	less than 0.001
Residuals	0.276	86	0.003		

Following the primary variance analysis, a post hoc Tukey HSD test was performed to identify specific differences in average antioxidant expression across the individual concentration steps. The pairwise metrics revealed a highly significant disparity in average radical-scavenging capacity between the 1 µg/mL group (where $M = 0.89$ and $SD = 0.08$), the 100 µg/mL group (where $M = 0.32$ and $SD = 0.08$, with p less than 0.001), and the 1,000 µg/mL maximum dose group (where $M = 0.04$ and $SD = 0.03$, with p less than 0.001). Within **Table II**, the variable abbreviations represent the arithmetic mean (M), the sample standard deviation (SD), and the calculated probability value (p). The variance testing confirms that since each computed p-value falls well below the significance threshold of $\alpha = 0.05$, the observed differences in mean antioxidant values between all paired concentration steps are statistically significant.

Table II. Pairwise comparison of the average antioxidant activity of each concentration.

Concentration (µg/mL)	M	SD	Tukey HSD p-value: I	Tukey HSD p-value: II
1	0.89	0.08		
100	0.32	0.08	less than 0.001	
1,000	0.04	0.03	less than 0.001	less than 0.001

The presence of specialized secondary metabolites within marine extracts plays a major role in driving vital biological activities, including selective cytotoxicity against cancer cell lines[15], systemic anti-inflammatory responses[16], antioxidant and antimicrobial defenses[17], anti-infective properties[18], analgesic effects[19], anti-diabetic modulation[20], anti-Parkinsonism traits[21], and broad immunomodulatory or antispasmodic activities[22]. For example, indole-piperazine chemical scaffolds have been characterized as targeted neuroprotective agents in neurodegenerative models[23]. Thin-layer chromatography fingerprinting conducted in this study confirmed the presence of diverse secondary metabolite classes within the crude extracts of *S. crassifolium*, *S. moniliformis*, and *E. muricatum*. Optimized eluent solvent ratios consisting of 3 to 2 hexane to ethyl acetate for *S. crassifolium*, 12 to 1 dichloromethane to methanol for *S. moniliformis*, and 9 to 6 hexane to ethanol for *E. muricatum* achieved clear resolution and separation of the individual phytochemical bands. Qualitative evaluation of the plates confirmed that these extracts were rich in essential bioactive classes, including flavonoids, phenols, alkaloids, saponins, condensed tannins, phytosteroids, and complex volatile essential oils, as summarized in **Table III**.

Table III. Secondary metabolites present on *S. crassifolium*, *S. moniliformis*, and *E. muricatum* extracts.

Phytochemical Class	*Sargassum crassifolium*	*Scinaia moniliformis*	*Eucheuma muricatum*
Steroids	Strong	Moderate	Weak
Flavonoids	Strong	Strong	Moderate
Alkaloids	Moderate	Strong	Weak
Anthraquinones	Weak	Absent	Moderate
Indoles	Moderate	Weak	Strong
Higher Alcohols	Moderate	Moderate	Weak
Phenols	Strong	Strong	Strong
Essential Oils	Moderate	Weak	Weak

The high abundance of phytosteroids, flavonoids, alkaloids, anthraquinones, indoles, higher aliphatic alcohols, polyphenols, and essential oils within the extracts of *S. crassifolium*, *S. moniliformis*, and *E. muricatum* directly accounts for their powerful radical-scavenging performance. A large body of empirical evidence shows that highly developed phenolic moieties and flavonoids can directly neutralize roaming ROS and indirectly enhance cellular defenses by activating the protective nuclear factor erythroid 2-related factor 2 (Nrf2) transcription pathway[24-27]. Additionally, isolated plant alkaloids can reduce intracellular ROS accumulation in hydrogen peroxide-exposed cells by preventing hyperphosphorylation and modulating downstream signaling cascades involving protein kinase B and glycogen synthase kinase-3β[28]. Depending on the physiological microenvironment, alkaloids can exert both antioxidant and pro-oxidant cellular effects by targeting the Nrf2 pathway[29]. Recent synthesis work focusing on anthraquinone-based pyrimidine derivatives assembled via copper-catalyzed one-pot relay methods has highlighted their use as precise molecular probes for tracking real-time antioxidant activity[30]. Quantum chemical modeling has also demonstrated that the explicit structural orientation of hydroxyl groups on the anthraquinone nucleus controls radical-scavenging kinetics via a sequential proton-loss electron-transfer mechanism[31]. Similarly, indole derivatives show enhanced free-radical-scavenging activity when their structures include active electron-donating amine and thiol groups[32]. Density functional theory (DFT) simulations have further shown that the specific position of the phenolic O-H bond relative to local C-H bonds, the presence of sugar or catechol rings, and stable intramolecular hydrogen bonding are the primary structural factors governing the neutral radical-scavenging capacity of polyphenols[33]. The antioxidant performance of steroidal dihydropyrazole structures has been validated across multiple analytical platforms, including DPPH assays, ferric reducing/antioxidant power (FRAP), and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays[34]. The specific physical steps of DPPH radical scavenging, iron ion reduction, β-carotene oxidation inhibition, and protection of biomolecules against deoxyribose degradation remain highly relevant for industrial food preservation applications[35]. Finally, recent optimization trials have underscored the strong synergistic effects of combining volatile essential oils with polar phenolic extracts to boost total antioxidant activity[36], while related work has demonstrated the successful stabilization of antioxidant enzymes using active packaging systems during long-term cold storage[37].

This investigation was primarily limited to *in vitro* colorimetric antioxidant testing via the DPPH assay and qualitative thin-layer chromatographic profiling of the crude seaweeds. The lack of absolute quantitative metabolite measurements and in vivo biological validation limits the direct generalization of these results to complex physiological systems. Future research should focus on isolating and identifying the primary bioactive leads using high-resolution chromatographic and spectroscopic techniques, such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), and Nuclear Magnetic Resonance (NMR) spectroscopy.

Additionally, a broader range of antioxidant testing methods, including ABTS, FRAP, and Oxygen Radical Absorbance Capacity (ORAC) assays, should be used to fully confirm the chemical scavenging mechanisms. Evaluating the cytoprotective, biocompatibility, and anti-inflammatory pathways of the extracts in cell cultures or animal models will be necessary to better understand their medical relevance. Furthermore, conducting comparative screenings of seaweeds with lower antioxidant activity, such as Laurencia okamurae, could offer a broader perspective on macroalgal chemical diversity. Finally, sustainable aquaculture, cultivation methods, and green bioprocessing technologies for these active seaweeds should be explored to support their potential use in large-scale nutraceutical or pharmaceutical applications.

CONCLUSION

This study confirms that eight of the nine macroalgal varieties harvested from Western Visayas exhibit significant, dose-dependent free radical scavenging activity. The extracts of *S. crassifolium*, *S. moniliformis*, and *E. muricatum* demonstrated the highest efficacy, with radical inhibition levels statistically comparable to the ascorbic acid reference standard. Qualitative phytochemical analysis revealed that this bioactivity is driven by an abundance of flavonoids, phenols, alkaloids, steroids, and essential oils. These results indicate that integrating seaweed-derived antioxidants into functional supplements offers a viable strategy for mitigating systemic oxidative stress. While structural carrageenan polymers provide essential functional attributes, the associated secondary metabolites are primarily responsible for the potent radical-scavenging activity. Consequently, these marine resources hold significant potential for preventing chronic pathologies, including oncogenesis, diabetes, cardiovascular ailments, and neurological disorders, all of which are exacerbated by free radical-mediated damage.

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AUTHORS' CONTRIBUTION

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Formal analysis: Katrin Mae Mendoza Ortega
Funding acquisition: -
Investigation: Katrin Mae Mendoza Ortega
Methodology: Katrin Mae Mendoza Ortega, Eric Camilo Rubia Punzalan
Project administration: -
Resources: Katrin Mae Mendoza Ortega
Software: -
Supervision: Ma Carmen Ablan Lagman
Validation: Ma Carmen Ablan Lagman
Visualization: -
Writing - original draft: Katrin Mae Mendoza Ortega, Eric Camilo Rubia Punzalan, Ma Carmen Ablan Lagman

Writing - review & editing: Katrin Mae Mendoza Ortega, Eric Camilo Rubia Punzalan, Ma Carmen Ablan Lagman

DATA AVAILABILITY

Data and material accessibility were evaluated by scrutinizing online scientific journals. Solvents and other standard reagents were purchased from Sigma–Philippines.

CONFLICT OF INTEREST

The authors declared no conflict of interest related to this research. The funding institutions had no role in study design, data collection, analysis, interpretation, or manuscript preparation.

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