



Biochemical composition, phytochemical profile, antioxidant activity, and cytotoxicity of the seagrass *Halodule pinifolia* from Pasiagan, Bongao, Tawi-Tawi, Philippines

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Abstract. The biochemical composition of the seagrass *Halodule pinifolia* collected from Pasiagan, Bongao, Tawi-Tawi, Philippines was investigated to assess its potential nutraceutical and pharmacological applications. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, phenolics, saponins, steroids, and triterpenoids. Proximate analysis demonstrated considerable nutritional value, with protein (12.46%), carbohydrates (52.82%), lipids (0.85%), and ash (33.87%), with the high ash content suggesting substantial mineral content. Cytotoxicity assessment yielded an LC_{50} of $867.32 \pm 0.028 \mu\text{g mL}^{-1}$ (mean $\pm$ SD), indicating low cytotoxicity. Quantitative analyses showed a total phenolic content (TPC) of $0.869 \pm 0.014 \text{ mg GAE g}^{-1}$ and a total flavonoid content (TFC) of $0.34 \pm 0.091 \text{ mg QE g}^{-1}$. Antioxidant assays demonstrated free-radical-scavenging activity in both DPPH ($0.725 \pm 0.021 \text{ mg Trolox g}^{-1}$) and ABTS ($0.196 \pm 0.017 \text{ mg Trolox g}^{-1}$) systems. These findings indicate that *H. pinifolia* contains nutritionally relevant constituents and phytochemicals associated with antioxidant activity. The species, therefore, warrants further investigation as an underexplored marine resource for potential functional food and nutraceutical applications.

Keywords: assay, free-radical, methanol, nutraceutical, proximate.

Introduction. Seagrasses are marine angiosperms that thrive in inhabit shallow coastal waters and provide essential ecosystem services, including sediment stabilization, nutrient cycling, carbon sequestration, and habitat provision for diverse marine organisms (Hemminga & Duarte, 2000; Duarte, 2002). Beyond their ecological importance, seagrasses are increasingly recognized as sources of bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial properties (Putri Gono et al., 2022; Ameen et al., 2024). However, their biochemical and pharmacological potential remains comparatively understudied in Southeast Asia, particularly in the Philippines.

Halodule pinifolia (Miki) den Hartog is a slender, perennial seagrass commonly found in shallow intertidal and subtidal habitats, where it occurs on sandy or muddy substrates, particularly in sheltered coastal environments (den Hartog, 1970; Phillips & Menez, 1988). The species is widely distributed throughout the tropical and subtropical Indo-Pacific, including the Philippines, and is recognized as an important component of shallow coastal seagrass communities (den Hartog, 1970; Short et al., 2007). Its broad distribution and occurrence across intertidal and subtidal habitats make *H. pinifolia* a relevant species for investigating the nutritional and bioactive properties of seagrass resources.

Pasiagan, Bongao, Tawi-Tawi, was selected as the study site because of the occurrence of established seagrass communities within its shallow coastal waters. Given the limited information on the biochemical properties of locally occurring seagrasses in Tawi-Tawi, this study aimed to characterize the nutritional composition, phytochemical

constituents, and antioxidant potential of *H. pinifolia* collected from the area. The findings may provide baseline information on its biochemical characteristics and potential as a source of naturally derived nutraceutical and bioactive compounds.

H. pinifolia is commonly distributed along tropical Asian coasts and occurs from the upper littoral to subtidal zones, where it grows on sandy or muddy substrates (den Hartog, 1970; Phillips & Menez, 1988). Morphologically, the species is characterized by a creeping rhizome approximately 0.4-0.8 mm thick, bearing 1-6 roots and an upright stem approximately 1-30 mm tall at each node, with internodes measuring 10-53 mm in length. The leaves occur in clusters of two to three per stalk, with leaf sheaths measuring approximately 6-34 mm in length and leaf blades measuring 60-166 mm in length and 0.4-0.8 mm in width. The midrib is conspicuous and may become enlarged or split toward the leaf tip. Two inconspicuous intramarginal veins terminate in small teeth, while mature leaf tips are characteristically unevenly serrated (den Hartog, 1970). These morphological characteristics are useful for the field identification and taxonomic characterization of *H. pinifolia*.

Previous studies in the Philippines have begun to characterize its nutritional and phytochemical profile. Tangon et al. (2020a) reported its proximate composition and phytochemical constituents from Agusan del Norte, highlighting its nutritional and medicinal relevance. Similarly, Mary et al. (2022) confirmed its antioxidant capacity, while Baehaqi et al. (2017) demonstrated solvent-dependent variations in antioxidant activity. Wagey (2014) examined chlorophyll variation in *Halodule* species in Central Visayas, revealing their physiological adaptability to environmental stressors. Other Philippine seagrass species have also been studied: Cordova et al. (2022) identified phenolics and flavonoids in *E. acoroides* with significant antioxidant activity, while Tangon et al. (2019) documented phytochemicals and proximate composition in *E. acoroides* and *T. hemprichii*. Tangon et al. (2020b) further quantified flavonoids and phenolics, linking them to antioxidant defense mechanisms. These studies collectively suggest that Philippine seagrasses harbor diverse bioactive compounds, yet systematic investigations remain limited in scope and species coverage.

Comparative research outside the Philippines reinforces these findings and expands the pharmacological relevance of seagrasses. Amudha et al. (2017) reported phytochemical diversity and antioxidant activity in *E. acoroides* from Tamil Nadu, India, while Lau et al. (2019) demonstrated similar biochemical and antioxidant properties in Malaysian seagrasses. Santoso et al. (2023) confirmed bioactive compound diversity in tropical *H. pinifolia*, and Thiraviyam et al. (2025) highlighted antibacterial and antioxidant activities in *Cymodocea serrulata*, underscoring the pharmacological relevance of seagrasses in South Asia. More recent reviews emphasize their nutraceutical potential: Mathakala et al. (2024) demonstrated seagrass-derived compounds as modulators of pro-inflammatory markers, while De Leo et al. (2025) identified *Cymodocea nodosa* as a promising candidate for nutraceutical applications. Collectively, these studies highlight the global recognition of seagrasses as underexplored marine resources with significant biomedical promise.

Despite these advances, research on Philippine seagrasses remains fragmented, with most studies focusing on ecological roles rather than biochemical and pharmacological applications. This gap is particularly evident for *H. pinifolia*, which, despite its ecological ubiquity, has not been comprehensively evaluated for its nutraceutical and therapeutic potential. Addressing this gap is critical, as seagrasses may represent sustainable sources of bioactive compounds that can contribute to functional food development and marine-derived pharmaceuticals. The present study aims to systematically evaluate the biochemical composition and antioxidant potential of *H. pinifolia* from Pasiagan, Bongao, Tawi-Tawi, Philippines, thereby advancing the understanding of seagrasses as valuable marine resources with both ecological and biomedical significance.

Material and Method

Study site and sample collection. The *Halodule pinifolia* samples were collected from an established seagrass meadow in Pasiagan, Bongao, Tawi-Tawi, Philippines (approximately 5.0085° N, 119.7508° E). The sampling site was located in a shallow coastal area characterized by suitable conditions for the growth of *H. pinifolia*.

Fresh specimens of *H. pinifolia* were collected during the lowest tide in the second week of September 2025 from an established seagrass meadow in Pasiagan, Bongao, Tawi-Tawi, Philippines (Figure 1). The sampling site consisted of shallow coastal reef flats characterized by sandy to muddy substrates and dense stands of *H. pinifolia*. The sampling site was located approximately at 5.0085° N latitude and 119.7508° E longitude, as determined using a Global Positioning System (GPS). The site represents a shallow coastal seagrass habitat consistent with environments in which *H. pinifolia* has been reported to occur. Previous studies of Philippine and tropical seagrass meadows have documented environmental conditions generally characterized by warm tropical water temperatures, marine salinity, and near-neutral to slightly alkaline pH. For example, a study in Sarangani Province, Philippines, where *H. pinifolia* was among the dominant seagrasses, reported water temperatures of approximately 31.3°C and salinities of 35-38 ppt.

Samples were collected manually during low tide. Three biological replicates were obtained at approximately 5-m intervals within the established seagrass meadow to account for small-scale spatial variability. Each biological replicate consisted of intact *H. pinifolia* plants collected from the designated sampling area. The plants were carefully collected while retaining the stolons and roots and minimizing disturbance to the surrounding seagrass meadow. The collected specimens were placed in clean polyethylene bags, transported to the laboratory under cooled conditions, and processed immediately upon arrival.

Upon arrival at the laboratory, the samples were manually cleaned and thoroughly rinsed with seawater followed by distilled water to remove adhering sediments, epiphytes, and excess salts. The cleaned plant material was air-dried at room temperature until a constant weight was achieved. The dried specimens were subsequently homogenized into a fine powder using a clean mortar and pestle. The powdered samples were transferred into clean, airtight containers and stored under appropriate conditions until further biochemical, phytochemical, cytotoxicity, and antioxidant analyses.

For the extraction of bioactive constituents, the powdered *H. pinifolia* samples were extracted using methanol as the extraction solvent. The resulting methanolic extracts were used for phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), cytotoxicity, and antioxidant assays.

All quantitative analyses were performed in triplicate. The replicate measurements were averaged, and the results were expressed as mean±standard deviation (SD). Where the three measurements represented repeated determinations of the same sample, these were treated as technical replicates. Where samples were independently collected from separate portions of the meadow, these were treated as biological replicates. This replication procedure was used to improve the reliability and reproducibility of the quantitative measurements.



Figure 1. Pasiagan, Bongao, Tawi-Tawi, Philippine.

Preparation of extract. The powdered *H. pinifolia* samples were extracted using analytical-grade absolute methanol as the extraction solvent. The extraction procedure was adapted from previously published methods for *H. pinifolia* and other tropical seagrasses. Methanol has been widely used for extracting polar and moderately polar phytochemical constituents from seagrass tissues.

Briefly, 10 g of dried and powdered *H. pinifolia* material from each biological replicate was transferred into a clean extraction vessel and mixed with 100 mL of absolute methanol at a sample-to-solvent ratio of 1:10 (w/v). The mixture was allowed to be extracted for 24 h at room temperature with continuous agitation using an orbital shaker. After extraction, the mixture was filtered through Whatman No. 1 filter paper. The remaining plant residue was rinsed with methanol, and the rinsate was combined with the original filtrate to maximize recovery of extractable constituents.

The combined methanolic filtrate was concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 40 °C to minimize thermal degradation of heat-sensitive constituents. The resulting crude methanolic extract was weighed, and the extraction yield was calculated as:

$$\text{Extraction yield (\%)} = \left(\frac{\text{Weight of dried crude extract}}{\text{Weight of dried plant material}} \right) \times 100$$

The dried crude extract was transferred into amber-colored, airtight containers and stored at 4°C until further phytochemical, cytotoxicity, total phenolic, total flavonoid, DPPH, and ABTS analyses.

The extraction procedure was performed independently for each biological replicate. Published studies on *H. pinifolia* have likewise employed methanol extraction at room temperature for approximately 24 h, although solvent-to-sample ratios and extraction procedures vary among studies.

Phytochemical screening. Qualitative phytochemical screening of the methanolic *H. pinifolia* extract was performed following established procedures described by Harborne (1998) and Sofowora (2008). The crude methanolic extract was reconstituted in methanol to a concentration of 10 mg mL⁻¹ prior to analysis. Alkaloids were screened using Dragendorff's reagent; phenolic compounds and tannins were detected using ferric chloride; flavonoids were assessed using the magnesium ribbon-hydrochloric acid test; steroids and triterpenoids were evaluated using the Liebermann-Burchard reaction; and

saponins were detected using the frothing test. The results were recorded qualitatively based on characteristic color changes, precipitate formation, or persistent frothing.

Proximate composition. The proximate composition of *H. pinifolia* was determined according to the AOAC Official Methods of Analysis (AOAC International, 2005). Protein content was determined using the Kjeldahl method, which involved sample digestion with concentrated sulfuric acid, followed by neutralization, distillation, and titration. Carbohydrate content was calculated by difference. Lipid content was determined by Soxhlet extraction using petroleum ether as the extraction solvent. Ash content was determined by incinerating the dried sample in a muffle furnace at 550°C until a constant weight was obtained. Results were expressed as percentages of the dry sample weight.

Brine shrimp lethality assay. The cytotoxicity of the crude methanolic *H. pinifolia* extract was evaluated using the brine shrimp (*Artemia salina*) lethality assay based on the method of Meyer et al. (1982), with modifications. *Artemia* cysts were hatched in artificial seawater under continuous aeration and illumination for approximately 24–48 h until active nauplii were obtained.

Ten actively swimming nauplii were transferred into each test vial containing artificial seawater. The crude methanolic extract was prepared as a stock solution and tested at final concentrations of 10, 100, 500, and 1,000 µg mL⁻¹. Each concentration was tested in triplicate, with 10 nauplii per replicate, giving a total of 30 nauplii per concentration. Artificial seawater containing the corresponding solvent without extract served as the negative control. A standard cytotoxic compound was used as the positive control.

The test vials were maintained at room temperature under illumination for 24 h. After exposure, the numbers of surviving and dead nauplii were recorded. Nauplii were considered dead when no visible movement was observed during direct observation. Percentage mortality was calculated for each concentration, and the median lethal concentration (LC₅₀) was estimated using probit analysis. The LC₅₀ value was expressed in µg mL⁻¹ together with its corresponding variability or confidence interval.

Total phenolic content. TPC was determined using the Folin-Ciocalteu colorimetric method. The crude methanolic extract was reconstituted in methanol at 1 mg mL⁻¹. An aliquot of the extract was mixed with Folin-Ciocalteu reagent and sodium carbonate solution and allowed to react for 30 min at room temperature in the dark. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Gallic acid was used as the reference standard to construct the calibration curve. A reagent blank containing all reagents except the extract was prepared for baseline correction. Measurements were performed in triplicate, and TPC was expressed as mg gallic acid equivalents per gram of extract (mg GAE g⁻¹).

Total flavonoid content. TFC was determined using the aluminum chloride colorimetric method. The methanolic extract was prepared at 1 mg mL⁻¹ and reacted with aluminum chloride and potassium acetate according to the selected colorimetric procedure. After the specified reaction period, absorbance was measured at 415 nm using a UV-Vis spectrophotometer. Quercetin was used as the reference standard for construction of the calibration curve. A reagent blank was included for baseline correction. All measurements were conducted in triplicate, and TFC was expressed as mg quercetin equivalents per gram of extract (mg QE g⁻¹).

DPPH radical-scavenging assay. DPPH radical-scavenging activity was determined using a methanolic DPPH solution following the method of Yen and Chen (1995), with modifications. The crude *H. pinifolia* extract was prepared in methanol at a concentration range of 25–400 µg mL⁻¹. An aliquot of the extract solution was mixed with 0.16 mM DPPH solution in methanol. The reaction mixture was vortexed and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm. Methanol was used as the solvent blank, while the DPPH solution without extract served as the reaction control. Trolox

was used as the reference antioxidant. A sample blank containing extract without DPPH was included to correct for background absorbance. Each concentration was analyzed in triplicate. Antioxidant activity was expressed as mg Trolox equivalents per gram of extract (mg TE g⁻¹).

ABTS radical-scavenging assay. ABTS radical-scavenging activity was determined using the ABTS radical cation method. ABTS solution was reacted with potassium persulfate and maintained in the dark at room temperature for 12-16 h to generate the ABTS•⁺ radical. The resulting solution was diluted with methanol until an absorbance of approximately 0.70±0.02 was obtained at 734 nm. The *H. pinifolia* extract was prepared in methanol at a concentration range of 25-400 µg mL⁻¹ and reacted with the ABTS•⁺ solution. The reaction mixture was incubated in the dark at room temperature for 6 min, after which absorbance was measured at 734 nm. Methanol was used as the solvent blank, while ABTS•⁺ solution without extract served as the reaction control. Trolox was used as the reference antioxidant. A sample blank containing extract without ABTS•⁺ was included where necessary to correct for background absorbance. All measurements were performed in triplicate, and antioxidant activity was expressed as mg Trolox equivalents per gram of extract (mg TE g⁻¹).

Statistical analysis. All quantitative measurements were performed using three independent biological replicates, with each analytical determination conducted in triplicate. Results were expressed as mean ± standard deviation (SD). Descriptive statistics were used to summarize the biochemical composition, TPC, TFC, and antioxidant activity of the samples.

For analyses involving comparisons among multiple concentrations or treatment groups, data were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test when a significant overall difference was detected. Statistical significance was set at $p < 0.05$. The LC₅₀ of the brine shrimp lethality assay was estimated using probit analysis based on the concentration-mortality relationship.

Table 1

Biochemical composition of *Halodule pinifolia* from Pasiagan, Bongao, Tawi-Tawi, Philippines

Parameter	Result / Presence (Unit/Notes)
Bioactive components	Alkaloids, tannins, flavonoids, phenolics, saponins, steroids, and triterpenoids - Present (qualitative)
Proximate composition	Protein: 12.46% (% dry weight), Lipid: 0.85% (% dry weight), Carbohydrate: 52.82% (% dry weight), Ash: 33.87% (% dry weight)
Cytotoxicity	LC ₅₀ = 867.32±0.028 µg mL ⁻¹ — non-toxic
Total phenolic content	0.869±0.014 mg GAE g ⁻¹
Total flavonoid content	0.34±0.091 mg QE g ⁻¹
Antioxidant properties	DPPH: 0.725±0.021 mg Trolox g ⁻¹ ABTS: 0.196±0.017 mg Trolox g ⁻¹

Results and Discussion

Phytochemical composition. Phytochemical screening of *H. pinifolia* collected from Pasiagan, Bongao, Tawi-Tawi, revealed the presence of alkaloids, tannins, flavonoids, phenolic compounds, saponins, steroids, and triterpenoids. The detection of these metabolite groups indicates that *H. pinifolia* possesses a diverse secondary-metabolite profile that may contribute to its biological activity. Phenolic compounds and flavonoids are particularly relevant to antioxidant activity because of their capacity to donate electrons or hydrogen atoms and interact with reactive species. Other detected metabolites, including alkaloids, saponins, steroids, and triterpenoids, have also been associated with antimicrobial, anti-inflammatory, and other biological activities (Harborne, 1998; Sofowora, 2008).

The phytochemical profile observed in the present study is consistent with reports from other tropical seagrasses. Similar classes of secondary metabolites have been reported in *Enhalus acoroides*, *Thalassia hemprichii*, and *H. pinifolia* from Philippine and Southeast Asian waters (Tangon et al., 2019; Santoso et al., 2023). Variations in phytochemical composition among seagrass populations may arise from differences in species, geographic location, tissue age, environmental stress, nutrient availability, and extraction conditions. Thus, the profile observed in *H. pinifolia* may reflect both species-specific characteristics and the environmental conditions of the Pasiagan habitat.

Proximate composition. The proximate analysis showed that carbohydrates constituted the largest component of *H. pinifolia* dry biomass (52.82%), followed by ash (33.87%), protein (12.46%), and lipids (0.85%). The high carbohydrate content indicates that carbohydrates constitute a major fraction of the dry matter and may reflect the substantial contribution of structural and storage polysaccharides in the plant. As a photosynthetic marine angiosperm, *H. pinifolia* actively fixes carbon, which may contribute to carbohydrate accumulation.

The protein content of 12.46% indicates a moderate contribution of nitrogen-containing compounds to the overall nutritional composition. Protein levels in seagrasses may vary with species, developmental stage, nutrient availability, and environmental conditions. In particular, nitrogen availability in the surrounding water and sediment can influence plant nitrogen assimilation and consequently tissue protein content. Similar carbohydrate-dominant profiles have been reported in Philippine seagrasses, although differences among species and sampling locations have also been observed (Tangon et al., 2019).

The lipid content was comparatively low (0.85%), which is consistent with the generally low lipid fraction reported for many seagrass species. In contrast, the ash content was relatively high (33.87%), indicating a substantial proportion of inorganic residue in the dry biomass. This may be associated with mineral uptake and the physicochemical characteristics of the coastal habitat, including sediment composition and seawater chemistry. However, because individual mineral elements were not quantified in the present study, the ash value should be interpreted as total inorganic residue rather than evidence of enrichment with specific minerals. Elemental analysis would be necessary to determine the mineral composition and its nutritional significance.

Differences in proximate composition among seagrasses may also be influenced by environmental factors such as light availability, salinity, nutrient concentration, season, and plant developmental stage. Therefore, the values obtained in this study provide a site- and sampling-specific biochemical profile rather than a fixed nutritional composition for the species.

Cytotoxicity. The brine shrimp lethality assay produced an LC_{50} value of $867.32 \pm 0.28 \mu\text{g mL}^{-1}$. Based on commonly applied interpretations of the brine shrimp lethality assay, extracts with LC_{50} values below $100 \mu\text{g mL}^{-1}$ are considered highly toxic, values of 100 – $500 \mu\text{g mL}^{-1}$ indicate moderate toxicity, 500 – $1,000 \mu\text{g mL}^{-1}$ indicate weak or low toxicity, and values above $1,000 \mu\text{g mL}^{-1}$ are generally regarded as non-toxic (Meyer et al., 1982). Accordingly, the LC_{50} obtained for *H. pinifolia* falls within the weak or low-cytotoxicity category.

The relatively high LC_{50} indicates limited acute toxicity of the crude extract toward *Artemia salina* nauplii under the experimental conditions. This result is important because the extract demonstrated biological activity without exhibiting strong toxicity in this preliminary assay. The observed response may be related to the combined effects of the secondary metabolites detected during phytochemical screening. However, the brine shrimp lethality assay is a preliminary indicator of general cytotoxicity and cannot by itself establish safety for human consumption or therapeutic use. Further studies using mammalian cell lines, toxicity models, and compound-specific assays are required to determine the biological significance of the observed activity.

Total phenolic and flavonoid contents. The TPC of *H. pinifolia* was 0.869 ± 0.014 mg gallic acid equivalents (GAE) g^{-1} , while the TFC was 0.34 ± 0.091 mg quercetin equivalents (QE) g^{-1} . These quantitative results support the qualitative detection of phenolic compounds and flavonoids in the phytochemical screening.

The relatively modest phenolic and flavonoid contents may be influenced by environmental and physiological factors. Phenolic accumulation in plants can respond to light exposure, salinity, nutrient availability, herbivory, oxidative stress, tissue age, and other environmental conditions. Seagrasses growing under different environmental regimes may therefore exhibit considerable variation in their phenolic profiles. In addition, differences in extraction solvent, extraction time, temperature, and analytical procedures can substantially affect the measured concentrations.

The TPC and TFC values obtained in the present study are generally comparable with values reported for some tropical seagrasses, although direct numerical comparisons should be made cautiously because analytical protocols and reporting units vary among studies (Tangon et al., 2020a; Santoso et al., 2023). The presence of these compounds nevertheless provides a plausible chemical basis for the antioxidant activity observed in the DPPH and ABTS assays. However, phenolic and flavonoid contents alone cannot establish which specific compounds are responsible for the antioxidant response. Chromatographic and spectrometric analyses would be necessary to identify the individual compounds present.

Antioxidant activity. The *H. pinifolia* extract demonstrated radical-scavenging activity in both DPPH and ABTS assays. The DPPH assay produced an activity of 0.725 ± 0.021 mg Trolox equivalents (TE) g^{-1} , whereas the ABTS assay yielded 0.196 ± 0.017 mg TE g^{-1} . Thus, the apparent antioxidant activity was higher in the DPPH assay than in the ABTS assay under the conditions used.

The difference between the two assays may be explained by differences in radical chemistry, reaction mechanisms, and the interaction of antioxidant compounds with the assay systems. The DPPH assay primarily measures the ability of compounds to reduce a stable nitrogen-centered radical through hydrogen- or electron-transfer reactions. The ABTS assay, in contrast, measures the reduction of the ABTS radical cation and can respond to antioxidants with different polarity and reaction kinetics. Consequently, compounds present in the *H. pinifolia* extract may interact more efficiently with the DPPH system than with the ABTS system.

The higher DPPH value should therefore not be interpreted simply as evidence that the extract has greater antioxidant capacity overall. Rather, it indicates stronger radical-scavenging activity under the specific conditions of the DPPH assay. Differences in solvent compatibility, extract composition, reaction time, radical accessibility, and the chemical structure of antioxidant constituents can influence the response obtained from each method. The use of both assays provides a broader assessment of the extract's radical-scavenging potential.

The observed antioxidant activity is also consistent with the detection of phenolics and flavonoids. These compounds may contribute to radical neutralization through electron donation and hydrogen-atom transfer. Nevertheless, the relatively modest TPC and TFC values suggest that other constituents may also contribute to the measured activity. Tannins, alkaloids, saponins, and other unidentified metabolites may participate in the overall antioxidant response. Similar antioxidant activity has been reported in tropical seagrasses, including *E. acoroides*, *T. hemprichii*, and *H. pinifolia* (Tangon et al., 2020a, b; Cordova et al., 2022; Santoso et al., 2023).

Overall, the results demonstrate that *H. pinifolia* from Pasiagan contains diverse phytochemical constituents, appreciable carbohydrates and protein, a relatively high ash fraction, low cytotoxicity in the brine shrimp assay, and measurable radical-scavenging activity. These findings provide preliminary evidence of its biochemical and bioactive potential. Nevertheless, further studies involving mineral profiling, compound identification, cellular antioxidant assays, and more comprehensive toxicological evaluation are necessary to establish the specific compounds responsible for these activities and to assess their suitability for nutraceutical applications.

Conclusions. This study provides a preliminary biochemical characterization of *H. pinifolia* collected from Pasiagan, Bongao, Tawi-Tawi, Philippines. The species exhibited a diverse phytochemical profile, with alkaloids, tannins, flavonoids, phenolic compounds, saponins, steroids, and triterpenoids detected, together with appreciable carbohydrate and protein contents and a relatively high ash fraction.

The extract showed low cytotoxicity, with an LC_{50} of $867.32 \pm 0.28 \mu\text{g mL}^{-1}$, and demonstrated measurable radical-scavenging activity in both DPPH and ABTS assays. The higher DPPH response compared with ABTS suggests that the extract may contain constituents that interact more efficiently with the DPPH radical system under the conditions tested. These findings support the potential of *H. pinifolia* as a source of naturally occurring bioactive compounds and provide baseline information for further investigation of seagrasses from Tawi-Tawi. However, additional studies are needed to identify the specific bioactive constituents, determine their mechanisms of action, establish mineral composition, and evaluate their safety and efficacy before potential nutraceutical or functional-food applications can be substantiated.

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Authors Contributions. Conceptualization and Methodology: ET; Formal analysis: JAP, AHG, MSS; Investigation: JAP, NSE, SAK, AIH; Data curation: HDM and MSS; Writing - original draft: ET; Writing - review & editing: ET, MSS, HDM; Supervision: ET.

Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author PC upon reasonable request.

Ethical Standards. All procedures conducted in this study complied with institutional and national guidelines for research involving marine plant resources. Seagrass samples of *H. pinifolia* were collected responsibly from Pasiagan, Bongao, Tawi-Tawi, ensuring minimal disturbance to the surrounding ecosystem. No endangered or protected species were harmed during the process, and collection was performed in accordance with local environmental regulations. The study did not involve human participants or vertebrate animals; therefore, ethical approval from an institutional review board was not required. Nevertheless, the research adhered to the principles of responsible scientific conduct, including transparency, reproducibility, and respect for biodiversity.

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