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IDENTIFICATION OF WILD SEAWEEDS AT NIPAH BEACH, INDONESIA, AND STUDY OF THEIR ANTIOXIDANT POTENTIAL

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Background: The extracts from seaweeds are rich sources of phenolic acids, flavonoids, carotenoids, vitamins, sulphated polysaccharides, and phycobiliproteins, which confer antioxidant, anti-inflammatory, antimicrobial, and bioactive properties. Nipah Beach, Lombok (Indonesia) hosts biodiverse intertidal macroalgae whose functional traits remain under-documented. **Objectives:** This study aimed to identify prevalent taxa and quantify antioxidant capacity and pigments under in situ conditions. **Methods:** In May 2025, thalli of *Padina australis*, *Sargassum crassifolium*, *Chondracanthus sp.*, and *Halimeda opuntia* were collected at three intertidal stations; methanolic extracts were assessed for DPPH radical scavenging (% inhibition at 517 nm), chlorophyll-a was derived from A_{664}/A_{647}, and R-phycoerythrin was read at 565 nm with 730 nm turbidity correction by UV–Vis spectrophotometry. Concurrent field measurements (temperature, salinity, pH, dissolved oxygen, illuminance; handheld meters) indicated stable conditions; total dissolved solids were recorded verbatim but not interpreted. **Results:** Results are descriptive ($TEAC/IC_{50}$ not computed): *Padina australis* showed the highest %DPPH inhibition, chlorophyll-a peaked in *Sargassum crassifolium*, and *Chondracanthus sp.* exhibited the strongest R-phycoerythrin signal, consistent with lineage-specific pigment architecture. Elevated daytime dissolved oxygen likely reflects wave action and/or photosynthesis. **Conclusion:** These findings provide an integrated local baseline linking ecology to functional traits and nominate *Padina australis* as an antioxidant lead, *Sargassum crassifolium* for chlorophyll-a/fucoxanthin potential, and *Chondracanthus sp.* as a source of R-phycoerythrin. Priorities include seasonal replication, $TEAC/IC_{50}$ and ABTS/FRAP/ORAC assays, continuous photosynthetically active radiation (PAR) logging, and strengthened taxonomy, with curated datasets and code planned for release in subsequent project updates.

Keywords: macroalgae; antioxidant capacity; chlorophyll-a; R-phycoerythrin; bioprospecting.

INTRODUCTION

Seaweeds, also known as marine macroalgae, are integral components of coastal ecosystems where they provide primary production, nutrient recycling, stabilization of sediments, and habitat provision (Cotas et al., 2023). The extracts from seaweeds are rich sources of phenolic acids, flavonoids, carotenoids, vitamins, sulphated polysaccharides, and phycobiliproteins, which confer antioxidant, anti-inflammatory, antimicrobial, and bioactive properties (Afrin et al., 2023; Rattaya et al., 2015; Sedjati et al., 2024; Tibbetts et al., 2016). Recent reviews continue to endorse these mechanisms, such as free-radical scavenging, metal chelation, and redox modulation, for their application in food, nutraceutical, and cosmeceutical purposes (Budzianowska et al., 2025).

From a biodiversity standpoint, AlgaeBase (algaebase.org) currently lists more than 50,000 living algal species, of which macroalgae are a large and highly diverse group, with Rhodophyta being particularly rich in species diversity (Guiry & Guiry, 2024). This biodiversity underpins unique biochemical potential relevant for discovery pipelines (Basyuni et al., 2024).

Over the course of wild development, seaweeds encounter changing salinity, irradiance, and nutrient status, and these stressors activate secondary-metabolite pathways and often elevate levels of phenolics and pigments to promote oxidative-stress tolerance. Wild thalli exhibit strong antioxidant readouts relative to farmed material, which serves as an example. In combination, these same stressors also create unique portfolios of pigments (e.g., chlorophylls, carotenoids, and phycobiliproteins) that are ecophysiologically informative. For example, phycoerythrin in red algae possesses both light-harvesting ability and antioxidant activity and has established extraction and purification pathways (Morais et al., 2021). These ecophysiological traits highlight the commercial and ecological value of regional seaweed biodiversity, making Indonesia a prime case study.

For these reasons, it is important to note that Indonesia provides relevant context for this work. Indonesia is one of the largest producers of seaweed in the world and a centre of aquaculture. The most recent global SOFIA from FAO reports on record production of aquaculture, including algae, and follow-up syntheses show firmly that Indonesia is in the top group of production. To understand the magnitude of this, Indonesia produced approximately 9.92 million tonnes of cultured seaweed in 2019 (Jaikumar et al., 2024).This is the point at which the available biomass could potentially provide higher-value applications other than hydrocolloids. Nipah Beach on the north coast of Lombok has clear waters, rocky and coral-rubble substrates, and intact intertidal platforms very suitable for algal colonization (Gutiérrez et al., 2021). To establish a site-resolved baseline, we established three sampling stations across the rocky and coral-rubble intertidal. Despite the suitability of these habitats, there is little to no existing account of species composition, antioxidant capacity, or pigment composition along this shoreline (Jégou et al., 2021).

A comparative ecophysiology approach seems a practical way to apply what is known and fill in the gaps. Exposure in the intertidal to near UV and high light, temperature and osmotic swings, and dependence on periodic emersion are known to up-regulate antioxidant defences and alter pigment complements.

Brown algae such as *Padina* and *Sargassum* generally combine chlorophyll-a (Chl-a) with fucoxanthin, together with phlorotannin-rich phenolic systems. Red algae such as *Chondracanthus* contributes phycoerythrin, and calcareous greens such as *Halimeda* provide some combination of chlorophylls and carotenoids. Side-by-side studies of co-occurring species at one beach enable the connection of ecological niche, pigment architecture, and antioxidant outcomes (Nazarudin et al., 2021). This study provides a site-specific baseline for Nipah Beach. We identify resident wild macroalgae species and quantify antioxidant capacity using DPPH (2,2-diphenyl-1-picrylhydrazyl) and chlorophyll-a and phycoerythrin using UV–Vis absorbance spectrophotometry, alongside recording in parallel associated environmental parameters of temperature, salinity, pH, dissolved oxygen, light, and total dissolved solids (Sedjati et al., 2024; Silva-Brito et al., 2021). Prior research conducted on tropical Indo-Pacific macroalgae demonstrates that brown seaweeds have been identified with phlorotannin concentrations of 2 – 6% dry weight, displaying strong DPPH radical scavenging through to >80% at 1 mg/mL and exhibiting notable ferric reducing power. Red algae, such as *Chondracanthus* and *Gracilaria*, have also consistently been shown to possess higher phycoerythrin-to-chlorophyll ratios, thereby demonstrating greater antioxidant activity and potential for use as natural food colorants. However, those quantitative bioactive profiles have not yet been developed for coastal flora from Lombok, which limits directed use of these high-value compounds. These variations in bioactive content are directly shaped by site-specific stressors, thereby influencing the antioxidant and pigment profiles observed.

DPPH continues to be a viable primary radical scavenging assay, with both strong comparability across studies and low matrix interference (Nurdin et al., 2020). Therefore, it is suitable for baseline screening. The integrated design allows for statistical comparisons within species across their ecological niche. Such integration is essential for unlocking bioeconomic potential, guiding conservation priorities, and designing targeted extraction pipelines for Lombok that links species identity with antioxidant metrics, pigment content, and in situ water quality within the same sampling framework. Conducting this work fills a gap, yielding a defensible short list of potential local species for pathways to value-addition and generating a multi-use template for seasonal or multi-assay applications.

Given the positive growth trend of global combined seaweed economies, along with Indonesia's scale in aquaculture, it provides for the value of targeted discovery for natural antioxidant and pigment resources to add diversity for the value of production beyond hydrocolloids, while contributing to bio-economies at the coast (World Bank, 2023). The central research question is: What is the diversity, antioxidant capacity, and pigment profile of wild macroalgae in Nipah Beach, and how do these attributes relate to their ecological niches? We hypothesize that intertidal brown algae species exhibits a greater antioxidant capacity compared to co-occurring taxa, which is attributed to increased photo-oxidative stress and the aggregation of pigments at specific locations according to lineage. Therefore, the objectives of this study are (i) identify and classify wild macroalgae species, (ii) quantify the DPPH scavengers, and (iii) measure chlorophyll-a and phycoerythrin, with replication and environmental context that matched the sampling location.

To the best of our knowledge, this is the first integrated biodiversity–biochemistry baseline for Nipah Beach, whereby antioxidant assays are coupled with pigment metrics and field-collected environmental conditions to inform local bioprospecting and screening for applications related to value-addition. This framework provides the evidence to take action with food preservation, cosmeceutical, and nutraceutical research and development, and to support more sustainable and environmentally responsible value creation for coastal communities.

MATERIALS AND METHODS

Study area and sampling design

This study was conducted at Nipah Beach, Pemenang Sub-district, North Lombok, West Nusa Tenggara, Indonesia, during May 2025. The coastline comprises clear waters, rocky benches, and coral-rubble substrates that sustain diverse macroalgal assemblages influenced by local oceanography and moderate human activity.

To capture microhabitat variability, we established three fixed intertidal stations (S1 – S3) based on accessibility, substrate type, and visible seaweed presence. Each station was geolocated by GPS and permanently marked for reproducibility; precise coordinates and station metadata are provided in Figure 1.

Macroalgae sampling and in situ water quality measurements were conducted during the same low-tide window at each station to ensure environmental parameters reflected the conditions experienced by the collected thalli. Environmental parameters were recorded in duplicate per station for station-level means and dispersion, while biochemical sampling used independent thalli as biological replicates (n = 3 per species) across stations where present.

Seaweed collection, handling, and preservation

Wild macroalgae were manually detached at daytime low tide from natural substrates (rock, coral rubble) using gloved hands or forceps to maintain morphological integrity. Each specimen was photographed in situ, labelled with station and code, gently rinsed with ambient seawater to remove macro-epiphytes, and placed in clean zip-seal bags kept shaded on ice. Samples were transported to the laboratory within 4 h, rinsed with distilled water, blotted dry, and inspected to remove residual epiphytes and debris. Clean thalli were air-dried in the dark and finished to constant mass at 40 – 45 °C in a ventilated oven, then milled to a fine powder (< 500 µm). Powders were stored in amber containers at 4 °C until extraction. Extraction yield (%) was calculated before biochemical testing as:

$$\%\text{Yield} = \frac{\text{mass of dried extract (g)}}{\text{mass of dried seaweed (g)}} \cdot 100. \quad (1)$$

Species identification and voucher management

Specimens were identified to the lowest possible taxon using diagnostic morphology (thallus form, branching, pigmentation, cortex/medulla traits, holdfast) with morphological keys and the AlgaeBase taxonomic repository (Yang et al., 2015). Identification focused on four focal taxa: *Padina australis*, *Sargassum crassifolium*, *Chondracanthus sp.*, and *Halimeda opuntia*.

Sample codes follow the format NP–XXX–NN, where NP denotes Nipah Beach as the sampling locality, XXX is a three-letter abbreviation of the genus/species name, and NN is the sequential specimen number assigned during collection for traceability to field notes and voucher records.

Representative voucher images and labelled dried materials are archived with the corresponding author and are available upon reasonable request; no formal herbarium deposition was made for this dataset.

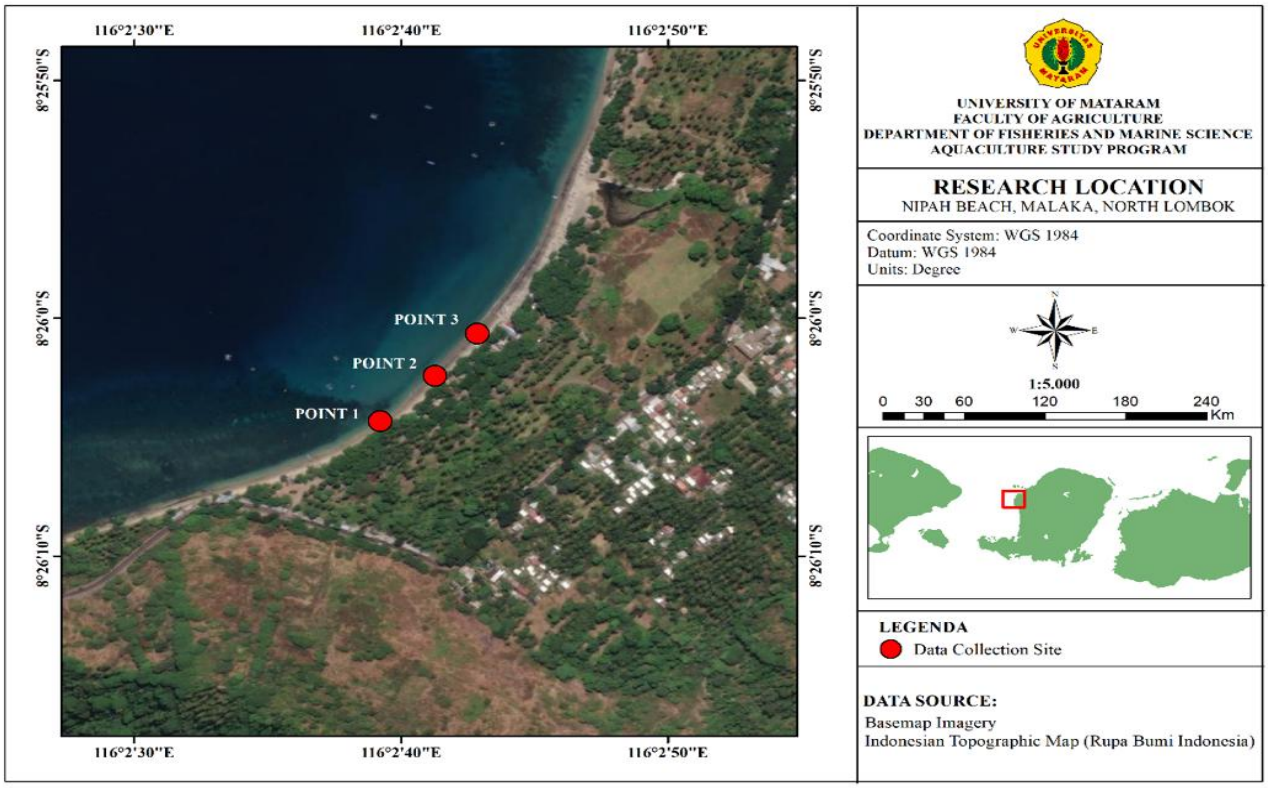

Figure 1. Map of Nipah Beach showing three intertidal sampling stations (S1 – S3) surveyed in May 2025; red dots mark station locations

Table 1. Morphological characteristics and taxonomic assignment of seaweeds collected from Nipah Beach

Sample code	Station	Phylum	Genus/Species	Thallus form	Dominant pigment cue	Substrate	Notes on diagnosis
NP-Pau-01	S1	Phaeophyceae	*Padina australis*	Fan-like blades with concentric bands	Fucoxanthin, chlorophyll-a	Rock	Calcified marginal banding
NP-Scr-11	S2	Phaeophyceae	*Sargassum crassifolium*	Leafy with vesicles and holdfast	Fucoxanthin, chlorophyll-a	Coral rubble	Receptacles present
NP-Cho-07	S2	Rhodophyta	*Chondracanthus sp.*	Flattened, branched blades	Phycoerythrin	Coral	Gelatinous texture
NP-Hop-03	S3	Chlorophyta	*Halimeda opuntia*	Calcareous, segmented fronds	Chlorophylls, carotenoids	Rock	Jointed calcified segments

Extract preparation

For antioxidant testing, 1.00 g of dried powder was combined with 9.0 mL methanol (analytical grade) in amber tubes (1 : 9 w/v), vortexed, and macerated for 24 h at room temperature in the dark. Extracts were clarified by Whatman No. 1 filtration and centrifugation (5,000 × g, 10 min); supernatants were filtered through 0.45 µm PTFE membranes and stored at 4 °C for ≤ 48 h prior to assays.

Antioxidant capacity: DPPH radical assay

Antioxidant capacity was quantified using DPPH (2,2-diphenyl-1-picrylhydrazyl) decolorization. A 0.10 mM DPPH methanolic working solution was prepared fresh. Using 96-well flat-bottom plates, 200 µL DPPH solution was mixed with 20 µL of sample extract, incubated 30 min in the dark at 25 °C, and read at 517 nm on a UV–Vis spectrophotometer (EMC-11S, Germany) (Afrin et al., 2023; Rattaya et al., 2015). Before readings, the instrument was zeroed with methanol at 517 nm.

Negative Control: Methanol without extract.

Positive control: Trolox (0 – 400 µM) to verify assay performance; Trolox equivalent antioxidant capacity (TEAC) values were not computed or reported for the present dataset.

$$\% \text{ Inhibition} = \frac{A(\text{blank}) - A(\text{sample})}{A(\text{blank})} \cdot 100. \quad (2)$$

Here, A(blank) is the absorbance at 517 nm of the DPPH working solution mixed with methanol only (negative control) after 30 min incubation at 25 °C, and A(sample) is the absorbance at 517 nm of the DPPH working solution mixed with the seaweed extract (or Trolox standard) under identical incubation and reading conditions.

Pigments: chlorophyll-a and R-phycoerythrin

Chlorophyll-a. Fresh tissue weighing 2.0 g was homogenized in 100% acetone, filtered, and 10 mL of clear filtrate was placed in 1.00 cm pathlength quartz cuvettes (Lailani et al., 2020). Absorbance was measured at 664 nm and 647 nm. Chlorophyll-a was calculated (Riyono, 2006) as:

$$\text{Chl} - \text{a (mg/L)} = 11.93\, A_{664} - 1.93\, A_{647}. \quad (3)$$

Values are reported as mg/L of extract and converted to µg/g dry weight (DW) using extraction volume V (L) and sample mass m (g).

R-phycoerythrin. Dried red-algal material weighing 50 mg was extracted with 10 mL cold phosphate buffer (0.1 M, pH 6.8) for 12 h at 4 °C in the dark, centrifuged (5,000 × g, 10 min), and read at 565 nm with 730 nm turbidity correction in 1.00 cm cuvettes. R-phycoerythrin was quantified from a standard calibration ($R^2 \geq 0.99$) and reported as µg/L and µg/g DW.

In-situ water quality measurements

Immediately after algal collection at each station during low tide, surface water (10 – 20 cm) was profiled. Parameters included temperature (°C), dissolved oxygen (mg/L), pH, salinity (ppt), illuminance (Lux), and total dissolved solids (mg/L) using portable meters: DO meter (OEM, China), pH meter (China), salinity refractometer (ATC, China), luxmeter (Fukushina, Japan), and a handheld TDS meter (China). Total dissolved solids (TDS) values are reported verbatim and not used for inference (Nurdin et al., 2020). Where a photosynthetically active radiation (PAR) sensor was available, photosynthetically active radiation (µmol photons/(m^2 s)) was recorded; otherwise, Lux values were reported and the absence of PAR was noted in the Discussion. Summary values are presented in Results; detailed station means and dispersion are moved from Methods to Results for clarity.

Quality assurance and quality control (QA/QC)

Field instruments were calibrated daily with manufacturer standards. Spectrophotometer wavelength calibration was verified weekly. Laboratory assays included method blanks, duplicates, and matrix spikes. Acceptance criteria: %CV ≤ 10% for replicate precision; 80 – 120% spike recovery. Limits of detection (LOD) and quantification (LOQ) were estimated from calibration residuals using 3.3 σ/slope and 10 σ/slope, respectively.

Data analysis

Descriptive statistics were compiled for biochemical and environmental variables. No inferential statistics are reported for the present dataset. Predictive modelling approaches from environmental AI are directly transferable (Halaktionov et al., 2025). Planned analyses for future expanded sampling include assumption checks (Shapiro–Wilk; Levene) followed by ANOVA/Tukey or Kruskal–Wallis/Dunn with false discovery rate (FDR) adjustment, and Pearson/Spearman correlations ($\alpha = 0.05$). Descriptive summaries and figures were prepared in R (v4.x).

RESULTS AND DISCUSSIONS

Antioxidant activity

The DPPH radical scavenging assay indicated clear variation in antioxidant capacity among the studied macroalgae. *Padina australis* exhibited the highest inhibition, followed by *Halimeda opuntia*, *Chondracanthus sp.*, and *Sargassum crassifolium* (Rattaya et al., 2015). All values are reported descriptively without inferential statistics. Mean values for antioxidant content are presented in Table 2 alongside pigment concentrations, allowing direct comparison across species.

We did not compute TEAC or Half-maximal inhibitory concentration (IC_{50}) for these samples, so % inhibition should not be equated to literature TEAC ranges. As context only, prior work reports elevated phenolics and antioxidant activity in brown algae (Silva et al., 2021).

Photosynthetic pigments

Chlorophyll-a content was highest in *Sargassum crassifolium*, followed by *Halimeda opuntia*, *Chondracanthus sp.*, and *Padina australis* (Table 2). R-phycoerythrin was prominent in *Chondracanthus sp.*, while being negligible in *Padina australis* and *Halimeda opuntia* (Lailani et al., 2020). This distribution matches pigment composition patterns typically observed for these taxa, with red algae generally enriched in phycobiliproteins.

Across taxa, chlorophyll-a was most pronounced in *Sargassum crassifolium*, whereas R-phycoerythrin characterized *Chondracanthus sp.* and was negligible in *Padina australis* and *Halimeda opuntia* (Lailani et al., 2020). These trends align with established pigmentation syndromes, namely phycobiliproteins in rhodophytes and chlorophyll-carotenoid systems in phaeophytes (Tibbetts et al., 2016). To improve comparability, pigments will be standardized to a dry-weight basis, and future sampling will co-record PAR and temperature. Figure 2 depicts key morphological traits (fan-shaped laminae in *Padina australis*; gas vesicles/receptacles in *Sargassum crassifolium*; segmented thalli in *Halimeda opuntia*; flattened blades in *Chondracanthus sp.*) that support the identifications.

Correlation with environmental parameters

Environmental measurements taken during sampling are summarised in Table 3. Conditions were stable across sites for temperature, salinity, and pH. No Lux-to-PAR conversion is presented because such conversion depends on spectral quality and sensor response. Dissolved oxygen values are relatively high for about 28 °C seawater; these are reported as measured and may reflect wave action, daytime photosynthesis, or meter characteristics (Roleda & Hurd, 2019). The Total dissolved solids (TDS) value is atypically low for seawater at nearly 30 ppt and is reported verbatim but not used for inference. Preliminary trends suggest that higher light and dissolved oxygen coincided with elevated chlorophyll-a and reduced phycoerythrin, consistent with photo acclimation under higher irradiance; these trends are descriptive and not tested statistically.

Table 2. Antioxidant, chlorophyll-a, and phycoerythrin content of selected macroalgae

Test sample code	Antioxidant content, %	Chlorophyll-a, µg/L	Phycoerythrin, µg/L
Sargassum crassifolium	35.28	16.40	1.44
Padina australis	69.69	9.71	< 0.01
Chondracanthus sp.	37.93	12.59	13.88
Halimeda opuntia	40.02	13.73	< 0.01

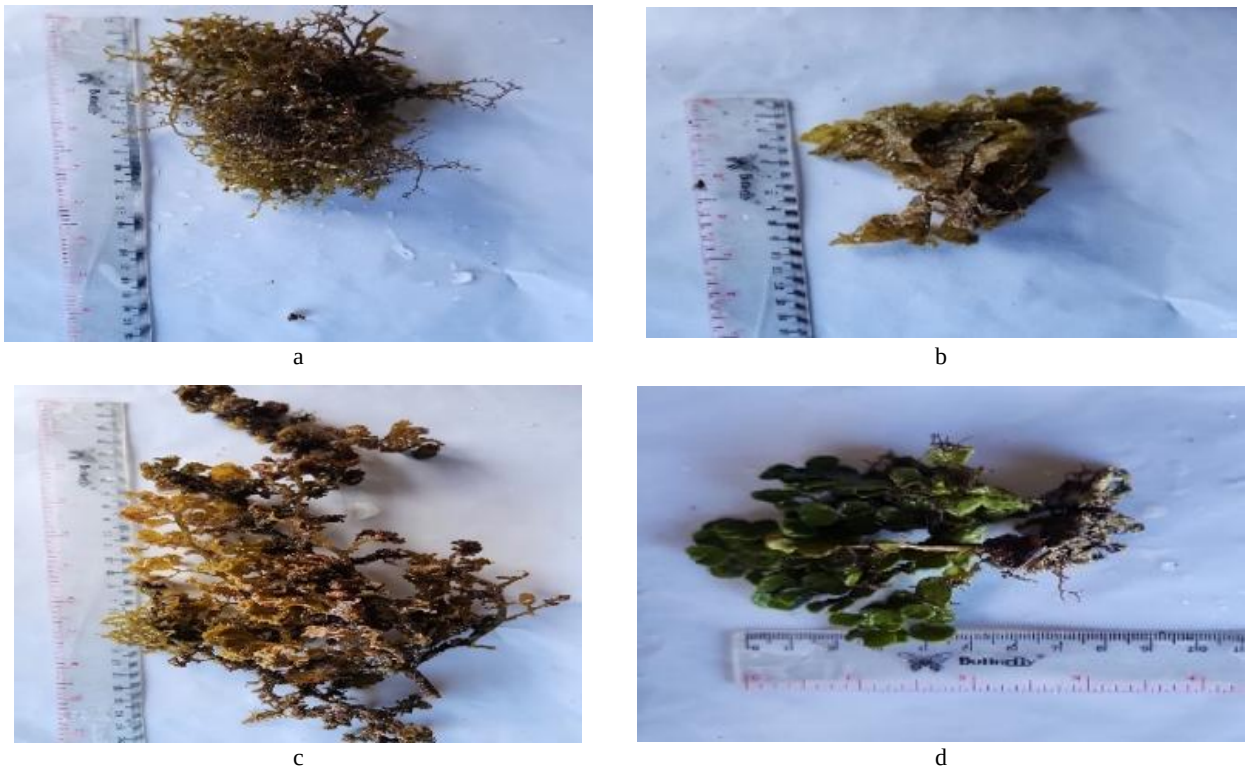

Figure 2. Representative specimens collected at Nipah Beach: a – *Chondracanthus sp.*, showing flattened, branched blades; b – *Padina australis* with fan-shaped lamina and concentric calcified bands; c – *Sargassum crassifolium* with a branched thallus bearing gas vesicles/receptacles; d – *Halimeda opuntia* with articulated, calcified segments; rulers provide a visible scale for morphological comparison

Table 2. Environmental parameters recorded during sampling (as read from hand-held meters)

Parameter	Value range
Temperature, °C	27.7 – 27.9
Salinity, ppt	30
pH	7.68 – 7.72
Dissolved oxygen, mg/L	11.15 – 12.60
Light intensity, Lux	39,000 – 40,000
Total dissolved solids, mg/L	57.70 – 57.80**

*Note: **TDS value is unexpectedly low for marine salinity and is not interpreted further*

Broader scientific context and applications

The strong antioxidant signal in *Padina australis* and the pigment profiles of *Sargassum crassifolium* and *Chondracanthus sp.* are consistent with prior reports on bio-actives in tropical macroalgae and may have relevance for functional foods, nutraceuticals, cosmetics, and natural colorants. These avenues fit circular-economy design for marine biomaterials (Sreenath et al., 2025). This framing aligns with upstream waste-governance that reduces land-based inputs to coastal waters (Bredun et al., 2024). Any translational use would require confirmatory studies with replicate variability, TEAC and IC_{50} determinations, seasonal replication, and validated optical measurements (Chowdhury et al., 2025; Sedjati et al., 2024; Tibbetts et al., 2016). Limitations include the absence of molecular profiling, lack of seasonal sampling, and reliance on descriptive rather than inferential statistics in the present report.

Future perspectives

Nipah Beach can serve as a site-resolved, multi-season testbed linking ecology and chemistry. Repeated wet- and dry-season sampling at S1 – S3 should add continuous PAR, temperature, salinity/conductivity, pH, dissolved oxygen, tides, turbidity, eDNA, and automated photo-transects to track community change. Resilience protocols ensure continuity during storms, surges, or spills (Tripathi et al., 2025). Proven smart-network workflows support site-resolved ecological observatories (Choudhary et al., 2025; Choudhary et al., 2025). Lab work should expand beyond %DPPH to TEAC and IC_{50}, plus ABTS radical cation decolorization assay, ferric reducing antioxidant power assay (FRAP), and oxygen radical absorbance capacity assay (ORAC), with total phenolics/flavonoids and targeted pigmentomics (fucoxanthin in *Padina/Sargassum*; R-phycoerythrin in *Chondracanthus sp.*). "Green" extraction (aqueous ethanol, natural deep eutectic solvents, supercritical

CO_2) with solvent recovery and stability testing will support food/cosmetic use. Embedding life cycle assessment (LCA) with uncertainty handling de-risks scale-up (Choudhary et al., 2025). Regulatory readiness (safety, contaminants, access and benefit-sharing (ABS) with local authorities) and deposition of vouchers and raw data to open repositories will increase reuse and transparency. Interoperable data layers and governance frameworks enable durable monitoring (Choudhury et al., 2025).

Based on the present dataset, *Padina australis* emerges as the primary antioxidant lead (highest %DPPH), *Chondracanthus sp.* is the most promising source of R-phycoerythrin, and *Sargassum crassifolium* shows strong potential for chlorophyll-a/ fucoxanthin recovery; *Halimeda opuntia*, while less compelling biochemically, remains important ecologically as a calcified green. Field measurements should pair PAR with Lux and use calibrated DO meters; salinity or conductivity should be preferred over TDS unless TDS readings are validated. All biochemical and environmental readouts ought to report replicate dispersion (mean ± SD).

Analytically, the next step is to compute TEAC and IC_{50} and, once replication is expanded, apply ANOVA or Kruskal–Wallis with effect sizes to test interspecific contrasts. Taxonomic certainty should be strengthened by depositing vouchers in a recognized herbarium and adding DNA barcoding to resolve closely related forms.

CONCLUSION

This study establishes the first integrated baseline for wild macroalgae at Nipah Beach, linking site conditions to descriptive antioxidant and pigment metrics across four taxa. *Padina australis* showed the strongest DPPH scavenging, *Sargassum crassifolium* contained the highest chlorophyll-a, and *Chondracanthus sp.* exhibited the greatest R-phycoerythrin signal, consistent with lineage-specific pigment architectures. While environmental conditions were stable during sampling, the findings are constrained by single-season coverage, absence of photosynthetically active radiation (PAR) time-series, and the lack of TEAC/IC_{50} estimates. Priorities for the next phase include seasonal resampling with replicate dispersion, expanded antioxidant panels (ABTS/FRAP/ORAC plus TEAC and IC_{50}), targeted pigmentomics, and strengthened taxonomy via voucher deposition and DNA barcoding. Curated datasets, analysis scripts, and voucher accession numbers will be released in an open repository in subsequent project updates.

Author's statements

Contributions

The authors contributed equally to all aspects of the current study and preparation of the manuscript.

Declaration of conflicting interest

The authors declare no competing interests.

Financial interests

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Data availability statement

No data were used for the current study.

AI Disclosure

The authors declare that generative AI was not used to assist in writing this manuscript.

Ethical approval declarations

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