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ORIGINAL ARTICLE

A comparative antioxidant profiling of brown, red and green Macroalgae from Kollam coast of Kerala

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ABSTRACT

The increasing demand for natural antioxidants has intensified interest in marine macroalgae, which are rich in bioactive metabolites capable of mitigating oxidative stress and associated degenerative disorders. The present study provides a comparative antioxidant profiling of selected brown, red, and green macroalgae collected from the Kollam coast of Kerala. Six commonly occurring species were evaluated: *Sargassum wightii* and *Padina tetrastromatica* (brown algae), *Gracilaria corticata* and *Hypnea musciformis* (red algae) and *Ulva lactuca* and *Chaetomorpha antennina* (green algae). Methanolic extracts of each species were analyzed for extraction yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities using DPPH, ABTS and FRAP assays. Results revealed significant variation in antioxidant profiles among the three algal groups. Brown macroalgae, particularly *Sargassum wightii*, exhibited the highest TPC, TFC, and overall radical scavenging activity, attributed to the presence of phlorotannins and fucoxanthin. *Gracilaria corticata* and *Hypnea musciformis* demonstrated moderate antioxidant potential, reflecting their pigment composition and sulfated polysaccharide content.

Green algae, especially *Ulva lactuca*, showed comparatively lower but notable antioxidant activity, supported by chlorophyll derived compounds and ulvan.

Overall, brown algae displayed superior antioxidant efficiency, followed by red and green taxa. The findings highlight the Kollam coast as a valuable source of natural antioxidants and support the potential application of these seaweeds in nutraceuticals, functional foods, and marine derived therapeutic formulations.

KEY WORDS

Brown algae; Red algae; Green algae; Antioxidant activity; Phenolics; Flavonoids; Macroalgae; Kollam coast; DPPH; ABTS; FRAP

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Introduction

Marine macroalgae are increasingly recognized as rich sources of bioactive compounds with significant health-promoting properties. Among these, brown (Phaeophyceae), red (Rhodophyceae), and green (Chlorophyceae) macroalgae are known to contain a wide variety of secondary metabolites such as polyphenols, flavonoids, carotenoids, sulfated polysaccharides, and vitamins that exhibit strong antioxidant activity [1-3]. These compounds play a crucial role in neutralizing free radicals and protecting biological systems from oxidative stress, which is implicated in aging, cancer, cardiovascular diseases, and neurodegenerative disorders.

Several studies have demonstrated the variability in antioxidant activity among macroalgal groups from different geographical regions, highlighting the influence of species-specific biochemical compositions and environmental factors on antioxidant potential [4]. Antioxidants derived from natural sources are preferred over synthetic antioxidants due to their safety and additional biological benefits. Seaweeds have been reported to possess higher antioxidant capacity compared to many terrestrial plants, mainly due to their unique marine environment, which exposes them to intense ultraviolet radiation and oxidative stress, thereby stimulating the synthesis of protective antioxidant compounds [5,6]. In particular, brown macroalgae are known to contain phlorotannins, a group of polyphenols that exhibit strong radical scavenging, metal chelating, and reducing properties [3,7].

Synthetic antioxidants like BHT and BHA, though effective, have raised concerns regarding toxicity and carcinogenicity. This has intensified the pursuit of safe, natural, marine-derived antioxidants, particularly from seaweeds that inhabit dynamic intertidal zones and therefore produce protective compounds to counteract intense sunlight, desiccation, and fluctuating environmental stress. Such adaptive biochemistry makes seaweeds promising candidates for nutraceuticals, pharmaceuticals, functional foods, and cosmeceutical applications.

Objectives of the Study

The present study titled “A comparative antioxidant profiling of brown, red and green Macroalgae from the Kollam coast of Kerala”, was undertaken with the following objectives:

- To collect and identify selected brown, red, and green macroalgae from the coastal regions of Kollam, Kerala.
- To evaluate and compare the antioxidant potential of the selected macroalgal species using standard in vitro antioxidant assays.
- To estimate the total phenolic content in the macroalgal extracts and assess its contribution to antioxidant activity.
- To determine free radical scavenging activity of the macroalgae through assays such as DPPH and FRAP.
- To compare inter-group variations (brown, red, and green algae) in antioxidant efficiency.

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- To identify macroalgal species with superior antioxidant properties that may serve as potential natural sources of antioxidants.
- To provide baseline scientific data supporting the nutraceutical and pharmaceutical significance of macroalgae from the Kollam coast [8].

Limitations of the Study

Despite providing valuable insights into the antioxidant potential of macroalgae from the Kollam coast of Kerala, the present study has certain limitations:

- **Limited number of species**

  The study focused on selected representative species of brown, red, and green macroalgae, which may not fully reflect the antioxidant diversity of all macroalgal species present along the Kollam coast.

- **Seasonal variation not assessed**

  Samples were collected during a specific period; therefore, seasonal fluctuations in biochemical composition and antioxidant activity were not evaluated.

- **Geographical constraint**

  The study was restricted to the Kollam coastal region, and the results may not be directly extrapolated to macroalgae from other coastal regions of Kerala or India.

- **In vitro analysis only**

  Antioxidant activity was assessed using vitro assays, which may not accurately represent in vivo antioxidant efficacy or bioavailability.

- **Limited range of antioxidant assays**

  Although standard assays such as DPPH and FRAP were employed, inclusion of additional assays (e.g., ABTS, ORAC, metal chelation) could have provided a more comprehensive antioxidant profile.

- **Extraction method constraints**

  The use of a single solvent or extraction protocol may have limited the recovery of certain bioactive compounds with varying polarity.

- **Lack of compound-level characterization**

  The study did not involve isolation or identification of individual antioxidant compounds responsible for the observed activities.

- **Absence of toxicity and safety evaluation**

  Potential cytotoxicity or safety aspects of the macroalgal extracts were not investigated.

- **Environmental factors not analyzed**

  Variations in antioxidant content due to environmental parameters such as salinity, light intensity, and nutrient availability were not considered.

Materials and Methods

Sample collection → Cleaning → Drying → Powdering → Extraction

Study area and sample collection

Macroalgal samples were collected from selected coastal regions of Kollam, Kerala, including near-shore rocky shores and intertidal zones known for rich algal growth. Representative species of brown (Phaeophyta), red (Rhodophyphyta) and green (Chlorophyphyta) macroalgae were hand-picked during low tide. Samples were transported to the laboratory in sterile polythene bags, washed thoroughly with seawater to remove sand, epiphytes, and debris, followed by rinsing with distilled water [9].

Sample preparation

Cleaned algal samples were shade-dried for 48–72 hours and powdered using a mechanical grinder. The powdered material was stored in air-tight containers at room temperature until extraction.

Extraction of antioxidant constituents

Approximately 5 g of dried algal powder was extracted using 80% methanol in a Soxhlet apparatus for 6–8 hours. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The dried extracts were stored at 4°C in amber vials for subsequent biochemical assays.

Phytochemical estimation

Total phenolic content (TPC)

Reaction setup → Incubation → Color development → Absorbance reading

Results were expressed as mg gallic acid equivalents (GAE) per gram of extract. Total phenolics were estimated using the Folin–Ciocalteu method.

- About 1 ml of extract was mixed with 5 ml of Folin–Ciocalteu reagent (diluted 1:10) and incubated for 5 minutes.
- Then, 4 mL of 7% sodium carbonate was added.
- The mixture was incubated for 30 minutes at room temperature and absorbance was measured at 765 nm.
- Results were expressed as mg gallic acid equivalents (GAE) per g of extract.

Total flavonoid content (TFC)

Sample preparation → Reaction setup → Incubation → Color development → Absorbance measurement → Quantification

Preparation of macroalgal extracts

- Dried and powdered macroalgal samples were extracted using methanol (or ethanol).
- The extracts were filtered and concentrated.
- Stock solutions of the extracts were prepared at known concentrations for analysis.

Preparation of reagents

- Aluminium chloride ($AlCl_3$) solution (2% w/v in methanol).
- Potassium acetate solution (1 M).
- Standard flavonoid solution: Quercetin prepared at different concentrations for calibration.
- Methanol used as solvent and blank.

Reaction setup

- To a test tube, a measured volume of macroalgal extract was added.
- Aluminium chloride solution was added to the extract.

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expressed as mean ± standard deviation (SD). Statistical significance among algal groups was determined using one-way ANOVA, followed by post hoc tests ($p < 0.05$). Data analysis was performed using SPSS and Excel.

Results

The Kollam coast of Kerala, situated along the southwest coast of India, is renowned for its rich macroalgal diversity due to rocky substrata, nutrient-rich waters, and favourable monsoonal patterns. Despite its ecological richness, systematic comparative antioxidant profiling of the abundant brown, red, and green macroalgae from this region remains limited. Understanding species-wise antioxidant variations is essential for identifying high-value resources with industrial and therapeutic potential.

In the present study, six representative macroalgal species commonly found along the Kollam coastline were selected:

Brown algae (Phaeophyta)

- ***Sargassum wightii*:** A dominant brown seaweed known for high levels of fucoxanthin, phlorotannins, and polysaccharides such as alginates. It exhibits potent antioxidant and anti-inflammatory activities.
- ***Padina tetrastromatica*:** Characterized by calcified zones and layered thalli, this species contains phenolics, flavonoids, terpenoids, and bioactive sulfated polysaccharides contributing to strong antioxidant capacity [10].

Red algae (Rhodophyta)

- ***Gracilaria corticata*:** An agar-yielding red seaweed rich in phycoerythrin, galactans, and phenolic compounds with known antioxidant and antimicrobial properties.
- ***Hypnea musciformis*:** A commercially significant species producing carrageenan-like sulfated polysaccharides, along with pigments and polyphenols responsible for free radical scavenging activity [11].

Green algae (Chlorophyta)

- ***Ulva lactuca*:** Commonly known as sea lettuce, this species is abundant in chlorophylls, carotenoids, proteins, and ulvan-type sulfated polysaccharides with established antioxidant and immunomodulatory effects.
- ***Chaetomorpha antennina*:** A filamentous green alga containing terpenoids, phenolics, vitamins, and minerals that contribute to its antioxidant and metabolic health benefits.

These species were selected based on their abundance, ecological importance, and previous reports indicating significant biochemical diversity. The comparative evaluation includes extraction yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant assays such as DPPH, ABTS, and FRAP. This study aims to provide a comprehensive understanding of antioxidant variations across brown, red, and green macroalgae from the Kollam coast (Figure 1).

Figure 1. Selected brown, red, and green macroalgae from the Kollam coast used for antioxidant profiling.

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Extraction yield

The extraction yield varied among the three groups of macroalgae. Brown algae showed the highest yield, followed by red and green algae. The extraction yield is shown in Table 1 and Figure 2.

Table 1. Extraction yield of selected Macroalgae.

Species	Group	Extraction yield (%)
Sargassum wightii	Brown	18.42 ± 0.56
Padina tetrastromatica	Brown	16.87 ± 0.44
Gracilaria corticata	Red	13.52 ± 0.38
Hypnea musciformis	Red	12.14 ± 0.29
Ulva lactuca	Green	10.26 ± 0.31
Chaetomorpha antennina	Green	9.48 ± 0.27

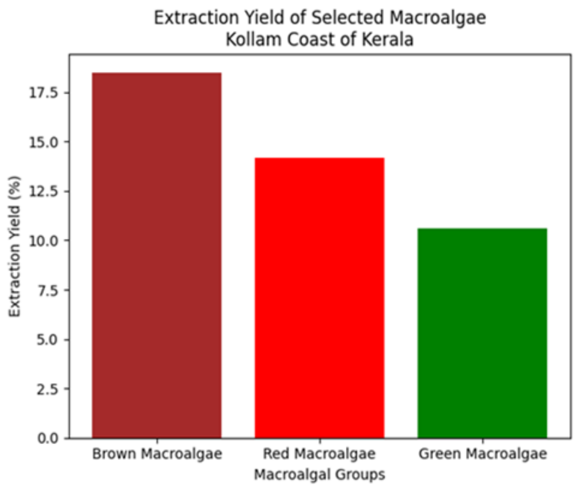

Figure 2. Extraction yield of selected Macroalgae.

Total Phenolic Content (TPC)

Brown macroalgae exhibited significantly higher total phenolic content compared to red and green macroalgae (Table 2). Among the brown algae, Sargassum wightii recorded the highest TPC value, which correlates strongly with its antioxidant capacity. Similar trends have been reported in tropical seaweed by earlier researchers [1,2]. TPC was highest in brown algae, with Sargassum wightii recording the maximum phenolic concentration (Table 2) [12].

Table 1. Total Phenolic content (mg GAE/g extract).

Species	Group	TPC (mg GAE/g)
Sargassum wightii	Brown	78.63 ± 1.24
Padina tetrastromatica	Brown	71.48 ± 1.11
Gracilaria corticata	Red	52.17 ± 0.96
Hypnea musciformis	Red	48.56 ± 0.74
Ulva lactuca	Green	39.83 ± 0.58
Chaetomorpha antennina	Green	36.24 ± 0.49

Total Flavonoid Content (TFC)

Flavonoid concentration exhibited a pattern similar to phenolics, with brown algae dominating. Flavonoid concentration is shown in Table 3.

Table 3. Total flavonoid content (mg QE/g extract).

Species	Group	TFC (mg QE/g)
Sargassum wightii	Brown	41.72 ± 0.67
Padina tetrastromatica	Brown	38.94 ± 0.51
Gracilaria corticata	Red	29.52 ± 0.46
Hypnea musciformis	Red	27.11 ± 0.39
Ulva lactuca	Green	22.34 ± 0.35
Chaetomorpha antennina	Green	20.87 ± 0.33

DPPH Free Radical Scavenging Assay

DPPH activity revealed strong antioxidant potential, particularly among brown algae. Lower IC_{50} indicates higher activity. The DPPH radical scavenging activity of the macroalgal extracts increased with concentration, as illustrated in Figure 3. Brown algae demonstrated superior scavenging ability, with IC_{50} values significantly lower than those of red and green algae (Table 4). These results are consistent with previous reports on the strong antioxidant potential of phlorotannin-rich brown algae [5,6].

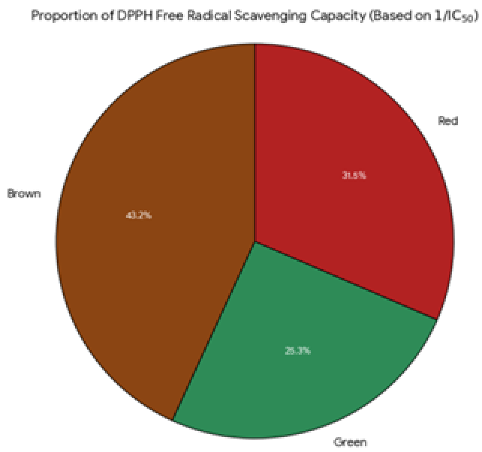

Figure 3. Comparative DPPH radical scavenging activity (IC_{50} values) of selected brown, red, and green macroalgae.

Table 4. DPPH Radical scavenging activity (IC_{50} values, μg/mL).

Species	Group	IC_{50} (μg/mL)
Sargassum wightii	Brown	48.16 ± 0.89
Padina tetrastromatica	Brown	55.23 ± 0.77
Gracilaria corticata	Red	68.92 ± 1.02
Hypnea musciformis	Red	72.15 ± 1.14
Ulva lactuca	Green	84.27 ± 1.26
Chaetomorpha antennina	Green	91.64 ± 1.38

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Ferric reducing antioxidant power (FRAP)

FRAP values followed the trend: Brown > Red > Green. The strong reducing ability of brown algae may be attributed to their high content of phlorotannin's and other polyphenolic compounds [3]. The FRAP values for all macroalgal extracts are presented in Table 5 and Figure 4.

Table 5. FRAP Activity (µmol Fe^{2+}/g extract).

Species	Group	FRAP Value
Sargassum wightii	Brown	512.46 ± 7.21
Padina tetrastromatica	Brown	478.33 ± 6.84
Gracilaria corticata	Red	362.18 ± 5.39
Hypnea musciformis	Red	337.42 ± 5.08
Ulva lactuca	Green	281.94 ± 4.63
Chaetomorpha antennina	Green	265.72 ± 3.98

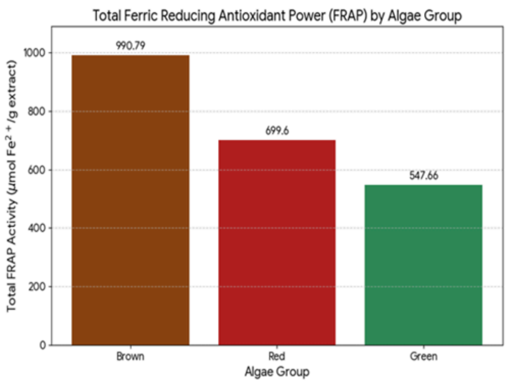

Figure 4. Ferric reducing antioxidant power (FRAP).

Correlation analysis

A comparative evaluation of antioxidant efficiency among the three macroalgal groups. Brown macroalgae consistently outperformed red and green algae across all antioxidant assays. This trend supports earlier findings that brown seaweeds possess superior antioxidant properties due to their unique polyphenolic composition [7,8].

A strong positive correlation was observed between:

- TPC and TFC(r = 0.89)
- TPC and DPPH activity (r = −0.86; lower IC_{50} = stronger activity)
- TPC and FRAP(r = 0.92)

This suggests phenolic compounds contribute significantly to antioxidant potential.

Discussion

In the present study, the antioxidant capacities of selected brown, red and green macroalgae from the Kollam coast of Kerala were comparatively evaluated using multiple in vitro assays (e.g., DPPH, FRAP, TAC). Consistent with global trends in seaweed antioxidant research, brown macroalgae generally exhibited higher antioxidant activity compared to red and green counterparts. This pattern has been widely attributed to the distinct bioactive compounds found in brown seaweed, particularly phlorotannins and carotenoids such as fucoxanthin, which are potent free radical scavengers [4] and exhibit stronger electron or hydrogen donation capacity than many terrestrial polyphenols

Brown algae biochemical basis and concordant studies

Our finding that *Sargassum/Padina* taxa showed the highest TPC/TFC and lowest DPPH IC_{50} aligns with multiple Indian and international reports (including targeted studies on *Sargassum wightii* and *Padina spp.*) that document high polyphenolic yields and robust antioxidant metrics in brown algae. These works also emphasize phlorotannin's and sulfated polysaccharides (fucoidans) as major contributors to antioxidant activity and note practical extraction approaches used in recent years.

Red algae- intermediate activity, distinct molecules

Red macroalgae (e.g., *Gracilaria spp.*) in our dataset showed moderate antioxidant values, consistent with the literature showing Rhodophyta occupy an intermediate biochemical niche. Several recent papers demonstrate that while total phenolic numbers in red algae are often lower than in brown algae, red-specific compounds (brominated phenols, Florido side, sulfated phenolics) can produce significant activity in particular assays and applications, especially when solvent and fractionation methods are optimized. This helps explain why red taxa sometimes perform strongly in ABTS/ORAC or targeted bioassays despite lower bulk TPC.

Green algae - ulvans, carotenoids and assay dependence

Green algae such as *Ulva* typically returned the lowest bulk phenolics and the weakest DPPH/FRAP values in our study, which mirrors systematic reviews and experimental work showing Chlorophyta are richer in ulvans (sulfated polysaccharides), pigments (carotenoids/chlorophyll derivatives) and oligosaccharides with antioxidant functions distinct from phlorotannin's. Recent meta-analyses and reviews highlight that ulvans and ulvan-derived oligosaccharides display measurable antioxidant and functional bioactivities, but these often require different extraction/fractionation and may be better captured by specific assays (e.g., ORAC, cellular assays) rather than by total phenolic metrics alone.

Correlation patterns and assay interpretation

The strong positive correlation we observed between TPC and FRAP and the inverse correlation between TPC and DPPH IC_{50} are in line with experimental syntheses showing that total phenolics frequently explain a large share of variance in electron transfer and hydrogen-atom transfer-based assays across macroalgae. However, assay chemistry matters: bromophenols, sulfated polysaccharides and carotenoids interact differently with DPPH, FRAP, ABTS and ORAC, so a multi-assay panel and targeted compound profiling (HPLC-MS/MS, NMR) are necessary to generate a complete antioxidant fingerprint.

Environmental & seasonal drivers relevance to kollam results

Recent field and physiological studies emphasize that light

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exposure, tidal regime, nutrient fluxes and seasonal stressors strongly influence secondary metabolite production in seaweeds (notably phlorotannin induction in brown algae under high irradiance/wave exposure). Therefore, local environmental variability along the Kollam coast likely modulates the absolute levels we measured; seasonal replication and microhabitat stratification (intertidal vs. subtidal) would be required to separate taxonomic from environmental effects as recommended by several regional seasonal studies.

Translational context (extraction → formulation)

Literature has shifted strongly toward translation:optimized extraction/fractionation, stability and bioavailability enhancement (e.g., encapsulation), and formulation for nutraceutical or cosmeceutical use. High-yielding brown-algal extracts such as those we observed in Kollam are therefore promising feedstocks but moving beyond screening requires targeted isolation, structure elucidation of active molecules (phlorotannins, bromophenols, *ulvans*) and bioaccessibility/bioavailability testing. Recent application-focused Indian and international studies illustrate feasible value-chain steps from seaweed to product [13].

Limitations and recommended next steps

- **Assay breadth**

  Complement in vitro DPPH/FRAP with ABTS, ORAC and cell-based oxidative stress assays to capture the full antioxidant potential.

- **Compound identification**

  Undertake targeted LC-MS/MS and NMR to identify and quantify dominant phlorotannins, bromophenols and *ulvans* responsible for activity.

- **Seasonal & spatial replication**

  Sample across monsoon, pre-monsoon and post-monsoon periods and include multiple Kollam micro-sites to partition seasonal/enviromental effects.

- **Fractionation & formulation**

  Test fractionation (polar/nonpolar/oligosaccharide fractions), stability, and simple bioavailability models (in vitro digestion, encapsulation trials) to assess translational potential. These steps follow the integrated workflows described in recent translational studies.

Summary

This study presents a comparative evaluation of antioxidant properties in six selected macroalgae - *Sargassum wightii*, *Padina tetrastromatica*, *Gracilaria corticata*, *Hypnea musciformis*, *Ulva lactuca*, and *Chaetomorpha antennina* collected from the Kollam coast of Kerala. Representing brown, red, and green algal groups, these species were examined for their extraction yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities using standard DPPH, ABTS and FRAP assays.

The findings revealed distinct interspecific and intergroup variations in antioxidant potential. Among the studied seaweeds, the brown algae (*Sargassum wightii* and *Padina tetrastromatica*) exhibited the highest levels of phenolic and flavonoid compounds, along with superior radical scavenging and ferric reducing power. *Sargassum wightii* emerged as the most potent antioxidant source. The red algae, *Gracilaria corticata* and *Hypnea musciformis*, showed moderate antioxidant activity, influenced by their sulfated polysaccharides and pigment composition. Meanwhile, the green algae, *Ulva lactuca* and *Chaetomorpha antennina*, demonstrated comparatively lower but noteworthy antioxidant activities, attributed to chlorophylls, carotenoids, and ulvan-type polysaccharides.

Overall, the study highlights significant antioxidant diversity among seaweeds of the Kollam region, with brown algae ranking highest, followed by red and green algae. These results underscore the potential application of Kollam coast macroalgae as natural antioxidant sources for nutraceutical, pharmaceutical, and functional food industries, while also contributing valuable baseline data for future marine bioresource utilization.

Conclusion

The present comparative study on *Sargassum wightii*, *Padina tetrastromatica*, *Gracilaria corticata*, *Hypnea musciformis*, *Ulva lactuca* and *Chaetomorpha antennina* from the Kollam coast of Kerala provides valuable insights into the antioxidant diversity among brown, red, and green macroalgae. The results demonstrate marked variations in extraction yield, phenolic and flavonoid content, and overall antioxidant performance across the three algal groups.

Among the species studied, the brown algae - *Sargassum wightii* and *Padina tetrastromatica* showed the highest antioxidant capacity, reflecting their elevated levels of phlorotannins, fucoxanthin, and other bioactive metabolites. *Sargassum wightii* emerged as the most potent species, indicating its strong potential as a natural antioxidant source. The red algae, *Gracilaria corticata* and *Hypnea musciformis*, displayed moderate yet significant antioxidant activity, supported by their pigment composition and sulfated polysaccharides. In contrast, the green algae, *Ulva lactuca* and *Chaetomorpha antennina*, exhibited comparatively lower antioxidant activity; however, their chlorophylls, carotenoids, and ulvan-type polysaccharides contributed noteworthy bioactivity [14].

The findings confirm that brown macroalgae possess superior antioxidant properties, followed by red and green species. The study highlights the Kollam coastal region as an important reservoir of bioactive seaweed and underscores the significant potential of these species for applications in nutraceuticals, pharmaceuticals, functional foods, and marine-derived antioxidant formulations. Furthermore, future research focuses on the isolation of active compounds, their structural characterization, and in vivo evaluations will strengthen the industrial and therapeutic applicability of these macroalgal resources.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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