Indian J. Fish., 71 (3): 123-131, 2024 doi:10.21077/ijf.2024.71.3.138211-15

Available online at: epubs.icar.org.in/index.php/IJF

Biochemical and nutritional profiling of selected tropical green seaweeds

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Abstract

The nutritional composition and anti-inflammatory properties of six tropical green seaweeds *viz., Ulva lactuca*, *Ulva linza*, *Halimeda macroloba*, *Halimeda gracilis*, *Chaetomorpha antennina* and *Chaetomorpha linum* were evaluated. *U. lactuca* exhibited the highest carbohydrate content (66.1%), while *U. linza* (12.89%) and *U. lactuca* (12.06%) showed the highest protein content, indicating their potential as plant-based protein sources. *H. gracilis* contained the highest ash content (35.12%), highlighting its mineral richness, particularly calcium, magnesium and phosphorus. Lipid content was low across all species, but *U. linza* exhibited the highest polyunsaturated fatty acid content (22.94%), with α-linolenic acid (13.72%) which could support cardiovascular health. Mineral analysis revealed high calcium levels in *C. linum* (18.99 mg 100 g^{-1}), contributing to bone health. Pigment analysis showed *U. linza* contained the highest chlorophyll-a (7.60 µg ml^{-1}) and total carotenoids (0.30 µg ml^{-1}), adding antioxidant potential to its bioactivity. *Chaetomorpha linum* exhibited the strongest anti-inflammatory activity (IC50 = 1.60 mg ml^{-1}), with bioactivity correlating to the favorable n-3/n-6 fatty acid ratio. Amino acid analysis identified *U. linza* as the richest source of essential amino acids, particularly methionine (6.37 mg g^{-1}) and valine (6.30 mg g^{-1}), making it an excellent candidate for dietary supplements. The results of the study suggest that green seaweeds belonging to the family Ulvaceae could be a potential non-conventional source for dietary products and functional food supplements.

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Keywords:
Anti-inflammatory activities, Chlorophyta seaweeds, Essential amino acid, Non-conventional nutritional sources, Polyunsaturated fatty acid

Received : 22.06.2023
Accepted : 06.07.2024

Introduction

Edible seaweeds, or sea vegetables, have long been regarded as essential components of traditional diets in Asian countries such as Japan, Korea, and China. Renowned for their high protein content and low levels of fat, sugars and cholesterol, seaweeds have emerged as ideal candidates for health-conscious diets (Rogel-Castillo *et al.,* 2023). The nutrient composition of green seaweeds, particularly those from the *Chlorophyta* division, is influenced by several factors, including geographical location, climate and seawater composition. Nevertheless, common nutritional elements of these seaweeds include proteins, lipids, fatty acids, trace elements, amino acids and carbohydrates (Kasimala *et al.,* 2015). Given the variability in composition due to species, habitat and environmental conditions, detailed nutritional analyses are necessary to fully understand their health benefits. In addition to their macronutrient content, seaweeds are rich in dietary fibre, often surpassing terrestrial food sources and provide essential minerals, contributing up to 40% of their dry weight (Oucif *et al.,* 2020). Despite their promising nutritional profile, green seaweeds are under-utilised in the nutraceutical and pharmaceutical sectors. However, recent studies have highlighted their value, particularly the favourable sodium-to-potassium ratio (Na/K), which plays a key role in maintaining body fluid balance and cardiovascular health (Oucif *et al.,* 2020). Although green seaweeds generally exhibit lower lipid content, they are an important source of polyunsaturated fatty acids (PUFAs), which are known for their beneficial effects in functional foods and therapeutic applications (Ganesan *et al.,* 2020). Furthermore, seaweeds possess bioactive compounds that exhibit antioxidant,

antimicrobial and anti-inflammatory properties, further enhancing their potential as valuable dietary resources.

Tropical green algae from the *Chlorophyta* division, particularly species from the *Ulva* genus, are gaining attention as unconventional yet promising nutritional resources. Historically consumed species, such as *Ulva pertusa* ("ao-nori"), are notable examples of green seaweeds with significant culinary and nutritional value. Of particular interest, *Ulva lactuca* has been shown to contain a wide range of essential amino acids, positioning it as a valuable source of dietary protein (Unis *et al.,* 2023). Despite the increasing recognition of the nutritional and environmental benefits of green seaweeds, a comprehensive framework for integrating seaweed ecosystems into blue carbon markets, as exists for other marine systems (Emmer *et al.,* 2015), has not yet been established. This highlights the need for further research into the nutritional profiling of seaweeds and their broader implications for food security and sustainability.

Given these considerations, the present study aims to evaluate the nutritional composition of selected tropical green seaweeds *viz., Ulva lactuca, Ulva linza, Halimida macroloba, Halimida gracilis, Chaetomorpha antennina* and *Chaetomorpha linum* focusing on their amino acid, lipid, fatty acid, and mineral profiles. By conducting a detailed analysis of these parameters, this research seeks to demonstrate the potential of these seaweeds as nutrient-dense food sources and their applicability in human diets, particularly regarding protein quality and essential nutrient intake. Additionally, the present study also aims to assess the anti-inflammatory properties of these seaweeds. These findings will contribute to the growing body of knowledge on seaweeds as sustainable food resources and inform the development of functional food products.

Materials and methods

Study location and processing of seaweeds

The green seaweeds *U. linza* (Linnaeus, 1753), *U. lactuca* (Linnaeus, 1753), *H. macroloba* (Decaisne, 1841), *H. gracilis* (Harvey ex J. Agardh, 1887), *C. antennina* (Bory) Kutzing, 1847 and *C. linum* (O.F. Muller) Kutzing, 1845, were collected from the Gulf of Mannar coast (9.2724°N, 79.1287°E) for the present study (Fig. 1). The specimens were thoroughly rinsed with tap water to remove adhered sand, epiphytes and detritus. Subsequently, the seaweeds were shade-dried and finely pulverised using an electric blender. The ground samples were stored in sealed vials, wrapped in aluminum foil and maintained at -20ºC until further use.

Biochemical analyses of seaweeds

The moisture content of the seaweed samples was determined *via* the oven-drying method at 105ºC, in accordance with the protocol established by AOAC (1990). Carbohydrate content was measured using the method developed by Miller (1959), which employs di-nitrosalicylic acid (DNSA) as the reagent, with absorbance readings taken at 540 nm. Ash content was quantified following

Fig. 1. (a) Sample collection sites of seaweeds from the south-east coast of India (9.2724° N, 79.1287° E). Representative photographs of the green seaweeds (b) *U. lactuca,* (c) *U. linza* (d) *H. gracilis*, (e) *H. macroloba* (f) *C. linum* (g) *C. antennina*

standard procedures outlined by AOAC (2005). For elemental analysis, approximately 0.1 g of each sample was digested using a di-acid mixture composed of 7 ml HNO_3 and 3 ml HCl. Open digestion was carried out for 1 h, followed by microwave-assisted digestion at 160 and 180°C, with a 30 min hold time and a 10 min ramp period. The digested samples were diluted with ultrapure water and analysed for metal content using inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher, iCAPQ, Waltham, MA), in accordance with Method 3015A (2007).

Pigments, including chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, were extracted and quantified following the methods of Hiscox and Israelstam (1979) and Kirk and Allen (1965). Lipid extraction was performed using the Folch *et al*. (1957) method, utilising a chloroform-methanol ($CHCl_3$-MeOH, 2:1 v/v) solvent mixture, with lipid content expressed as a percentage of dry weight. Fatty acids were analysed from the extracted lipids using gas chromatography (Shimadzu, Tokyo, Japan) equipped with a flame ionization detector (FID).

Protein content, including crude and true protein, was determined using the Lowry *et al*. (1951) method, where samples were treated with 5% trichloroacetic acid (TCA) and Folin-Ciocalteu reagent, with absorbance measured at 660 nm using a UV-VIS spectrophotometer (Agilent Cary® 50 UV-Vis, Santa Clara, CA) and bovine serum albumin (BSA) as the standard. Amino acids were quantified according to the method of Chakraborty and Joseph (2015), involving hydrolysis of samples with 6 M HCl, derivatisation with phenyl isothiocyanate (PITC) and subsequent analysis using reversed-phase high-performance liquid chromatography (HPLC) (Waters PICO.TAG amino acid analysis system), with detection at λ_{max} 254 nm. Amino acid results were expressed in mg g^{-1} of seaweed, based on comparisons with standard amino acid profiles.

In vitro anti-inflammatory assay

The crude extract was prepared using the method developed by Chakraborty and Paulraj (2010). Coarsely ground seaweed was extracted with methanol (95% v/v) under agitation on a rotary shaker at 50°C. Following extraction, the methanolic solution was filtered and concentrated under reduced pressure at 40-50°C. The concentrated extract was then fractionated with ethyl acetate (EtOAc). The resultant crude extract was stored at -20°C until further use. To evaluate its anti-inflammatory activity, the extract was tested for its capacity to inhibit the pro-inflammatory enzyme 5-lipoxygenase (5-LOX), according to the method described by Baylac and Racine (2003). Percentage inhibition was determined using the formula: $\{(A_c-A_s)/A_c\}$ x 100, where A_c represents the absorbance of the control sample and A_s represents the absorbance of the extracted sample. The IC50 values, indicating the concentration of the extract required to achieve 50% inhibition of 5-LOX activity, were subsequently calculated and reported.

Statistical analysis

Statistical analysis of samples was carried out by analysis of variance (ANOVA) using the Statistical programme for Social Sciences (SPSS Inc., ver. 13.0, USA). Analyses of all constituents were performed in triplicate and the final results were represented as mean values ± standard deviation (SD), wherein the significance of variance was represented as $p<0.05$. The principle variance was identified by principal component analysis (PCA) and chosen parameters, such as total saturated fatty acid (ΣSFA), total monounsaturated fatty acid (ΣMUFA), total polyunsaturated fatty acid (ΣPUFA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), total *n*-3 and *n*-6 fatty acids (Σ*n*-3 and Σ*n*-6) of the studied seaweeds were deduced.

Results

Proximate composition levels of the various seaweeds considered in the present study are illustrated in Table 1. Carbohydrate content ranged significantly, with *U. lactuca* exhibiting the highest value at 66.1%, while *H. macroloba* presented the lowest carbohydrate content of 11.7±0.40%. This suggests that *U. lactuca* could serve as a substantial carbohydrate source, beneficial for various applications in food and industrial contexts. Crude protein content was highest in *U. linza* (12.89%), closely followed by *U. lactuca* (12.06%), indicating these species could be a valuable protein source, particularly for vegetarian and vegan diets. In contrast, *H. gracilis* exhibited the lowest protein content. Moisture content analysis showed that *U. lactuca* had the highest value (16.5%), which may reflect its potential use in fresh or minimally processed products, while *C. linum* had the lowest moisture content (8.52%). Ash content, indicative of the mineral composition, was markedly high in *H. gracilis* and *H. macroloba*, with values of 35.12 and 32.35%, respectively, suggesting these species are particularly mineral-rich. Lipid content was generally low across all species, which is consistent with the typically low-fat nature of seaweeds. *U. lactuca* having the highest concentration (1.07%), while *H. macroloba*, *H. gracilis*, and *Caulerpa* species showed minimal values (0.03-0.07%). Regarding pigments, *U. linza* exhibited the highest chlorophyll-a levels (7.60 µg ml^{-1}), while *H. macroloba* had the highest chlorophyll-b (10.13 µg ml^{-1}) and total chlorophyll (15.27 µg ml^{-1}) concentrations. Carotenoids were found to be most abundant in *U. linza* (0.30 µg ml^{-1}), while *U. lactuca* and *C. linum* had the lowest concentrations (0.02 and 0.01 µg ml^{-1}, respectively).

Mineral composition analysis further supported the nutritional value of these seaweeds. Calcium (Ca) content was highest in *C. antennina* (18.97 mg 100 g^{-1}) and *C. linum* (18.99 mg 100 g^{-1}), with moderate levels observed in *U. linza* and *U. lactuca*. Magnesium (Mg) was present in highest concentration in *C. linum* (25.03 mg 100 g^{-1}), followed by *C. antennina* (20.71 mg 100 g^{-1}), while *H. macroloba* and *H. gracilis* showed much lower values (0.47 and 0.73 mg 100 g^{-1}, respectively). Phosphorus (P) levels were most concentrated in *C. antennina* (2.01 mg 100 g^{-1}), while potassium (K) was highest in *U. lactuca* (0.71 mg 100 g^{-1}). Among the micronutrients, manganese (Mn) was found in significant amounts in *U. linza* (3.67 mg 100 g^{-1}) and *U. lactuca* (4.80 mg 100 g^{-1}). Iron (Fe) content ranged from 0.20 mg 100 g^{-1} in *C. linum* to 2.40 mg 100 g^{-1} in *C. antennina*. Copper (Cu) was detected in small amounts in *U. lactuca* and *H. gracilis* (0.14 and 0.10 mg 100 g^{-1}, respectively), while selenium (Se) was found in trace amounts across species, except for *U. lactuca*, where it was not detected.

The fatty acid profiles showed distinct variations in their saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid contents (Table. 2). Among the saturated fatty acids, C16:0 (palmitic acid) was the predominant component across all species, ranging from 20.2% in *U. linza* to 38.37% in *H. gracilis*. Notably,

Table 1. Nutritional and biochemical composition of the seaweeds

Nutrients	*U. linza*	*U. lactuca*	*H. macroloba*	*H. gracilis*	*C. antennina*	*C. linum*
Crude protein (%)	12.89^{a} ± 0.01	12.06^{a} ± 0.19	8.81^{c} ± 0.04	6.81^{d} ± 0.17	10.76^{b} ± 0.32	11.65^{b} ± 0.05
True protein (mg g^{-1})	10.52^{a} ± 0.02	9.34^{b} ± 0.03	6.51^{c} ± 0.01	4.06^{d} ± 0.04	8.46^{b} ± 0.02	8.12^{b} ± 0.01
Carbohydrates (%)	56.2^{b} ± 0.86	66.1^{a} ± 0.14	11.7^{d} ± 0.40	14.65^{d} ± 0.08	25.12^{c} ± 0.09	20.78^{c} ± 0.13
Lipids (%)	0.39^{b} ± 0.02	1.07^{a} ± 0.03	0.03^{c} ± 0.04	0.05^{c} ± 0.01	0.04^{c} ± 0.03	0.07^{c} ± 0.04
Ash (%)	27.1^{b} ± 0.43	22.13^{b} ± 0.01	32.35^{a} ± 0.25	35.12^{a} ± 0.6	14.2^{c} ± 0.35	19.9^{c} ± 0.30
Moisture (%)	15.52^{a} ± 0.05	16.5^{a} ± 0.02	10.62^{b} ± 0.10	11.2^{b} ± 0.03	9.63^{c} ± 0.07	8.52^{c} ± 0.04
Pigments (µg ml^{-1})						
Chl-a	7.60^{a} ± 0.35	0.15^{e} ± 0.02	5.31^{b} ± 0.27	2.56^{c} ± 0.04	1.93^{d} ± 0.09	0.25^{e} ± 0.06
Chl-b	2.97^{c} ± 0.12	0.22^{d} ± 0.03	10.13^{a} ± 0.15	3.92^{b} ± 0.03	4.29^{b} ± 0.14	0.39^{d} ± 0.05
Total chlorophyll	10.58^{b} ± 0.07	0.38^{d} ± 0.05	15.27^{a} ± 0.30	6.47^{c} ± 0.04	6.24^{c} ± 0.14	0.64^{d} ± 0.11
Carotenoids	0.30^{a} ± 0.15	0.02^{d} ± 0.04	0.14^{b} ± 0.03	0.21^{a} ± 0.05	0.10^{c} ± 0.04	0.01^{d} ± 0.00
Macronutrient (mg 100 g^{-1})						
Ca	13.4^{d} ± 0.25	12.45^{c} ± 0.16	0.41^{a} ± 0.04	0.64^{b} ± 0.07	18.97^{e} ± 0.11	18.99^{e} ± 1.59
Na	0.18^{c} ± 0.02	0.45^{d} ± 0.07	0.02^{a} ± 0.01	0.05^{b} ± 0.01	0.04^{a} ± 0.03	0.02^{a} ± 0.00
Mg	12.65^{d} ± 0.04	10.04^{c} ± 0.08	0.47^{a} ± 0.03	0.73^{b} ± 0.05	20.71^{e} ± 0.07	25.03^{f} ± 0.11
P	0.12^{a} ± 0.01	0.17^{a} ± 0.05	0.51^{b} ± 0.06	0.55^{b} ± 0.04	2.01^{d} ± 0.05	1.04^{c} ± 0.12
K	0.12^{b} ± 0.02	0.71^{e} ± 0.09	0.06^{a} ± 0.07	0.65^{e} ± 0.04	0.21^{c} ± 0.04	0.27^{d} ± 0.16
Micronutrient (mg 100 g^{-1})						
B	0.55^{b} ± 0.04	0.88^{c} ± 0.03	ND	1.02^{d} ± 0.4	ND	0.22^{a} ± 0.24
Mn	3.67^{e}± 0.12	4.80^{f} ± 0.26	0.07^{a} ± 0.03	1.14^{d} ± 0.03	0.45^{c} ± 0.09	0.39^{b} ± 0.23
Fe	0.37^{b} ± 0.01	0.43^{c} ± 0.04	1.24^{d} ± 0.03	1.32^{d} ± 0.02	2.40^{e} ± 0.03	0.20^{a} ± 0.01
Ni	0.05^{b} ± 0.04	0.68^{c} ± 0.01	0.02^{a} ± 0.01	0.04^{a} ± 0.03	0.01^{a} ± 0.02	0.06^{b} ± 0.02
Zn	0.27^{c} ± 0.01	0.23^{c} ± 0.01	0.02^{a} ± 0.01	0.04^{a} ± 0.02	0.02^{a} ± 0.03	0.06^{b} ± 0.02
Cu	ND	0.14^{c} ± 0.06	0.04^{a} ± 0.02	0.10^{b} ± 0.01	0.09^{b} ± 0.02	0.04^{a} ± 0.03
Se	0.01^{a} ± 0.02	ND	0.01^{a} ± 0.02	0.03^{a} ± 0.01	0.02^{a} ± 0.03	0.01^{a} ± 0.02

Data are expressed as mean (n = 3) ± SD. Different superscripts (a-e) indicates significant difference (p< 0.05) within the rows. ND, Non-detectable.

Table 2. Fatty acid composition and anti-inflammatory activities (5-lipoxygenase attenuation) of the seaweeds

Fatty acids†	*U. linza*	*U. lactuca*	*H. macroloba*	*H. gracilis*	*C. antennina*	*C. linum*
Saturated fatty acids						
C14:0	1.16^{c} ± 0.01	1.34^{c} ± 0.02	3.20^{b} ± 0.20	1.34^{c} ± 0.02	12.11^{a} ± 0.06	13.22^{a} ± 0.12
C15:0	0.85^{a} ± 0.06	0.72^{b} ± 0.03	-	-	-	-
C16:0	20.20^{b} ± 0.15	22.68^{b} ± 0.02	32.57^{a} ± 0.01	38.37^{a} ± 0.01	23.65^{b} ± 0.22	25.63^{b} ± 0.15
C18:0	2.86^{c} ± 0.01	2.64^{c} ± 0.05	2.69^{c} ± 0.03	4.41^{a} ± 0.01	3.30^{b} ± 0.12	2.03^{d} ± 0.01
C24:0	0.96^{c} ± 0.06	0.89^{c} ± 0.03	16.36^{a} ± 0.02	15.45^{a} ± 0.02	6.40^{b} ± 0.02	6.66^{b} ± 0.01
C22:0	5.81^{a} ± 0.02	1.77^{b} ± 0.05	-	-	-	-
C17:0	0.23^{a} ± 0.01	0.33^{a} ± 0.00	0.12^{b} ± 0.01	0.22^{a} ± 0.01	-	-
Σ SFA	32.07^{c} ± 0.24	30.37^{c} ± 0.42	54.94^{a} ± 0.26	59.79^{a} ± 0.07	45.46^{b} ± 0.36	47.54^{b} ± 0.96
Monounsaturated fatty acid						
C16:1n-7	1.18^{c} ± 0.01	1.15^{c} ± 0.02	1.58^{c} ± 0.02	4.90^{a} ± 0.20	2.12^{b} ± 0.01	2.45^{b} ± 0.03
C18:1n-7	10.1^{c} ± 0.02	14.7^{b} ± 0.20	7.63^{c} ± 0.06	12.11^{b} ± 0.13	20.52^{a} ± 0.32	21.03^{a} ± 0.13
Σ MUFA	11.28^{c} ± 0.01	15.85^{b} ± 0.03	9.21^{c} ± 0.02	17.01^{b} ± 0.12	22.64^{a} ± 0.61	23.48^{a} ± 0.15
Polyunsaturated fatty acid						
C20:5n-3 (EPA)	0.12^{a} ± 0.02	0.15^{a} ± 0.02	0.06^{b} ± 0.03	0.05^{b} ± 0.01	0.01^{b} ± 0.00	0.03^{b} ± 0.02
C22:6n-3 (DHA)	0.15^{a} ± 0.02	0.13^{b} ± 0.01	0.04^{c} ± 0.02	0.02^{c} ± 0.02	0.01^{c} ± 0.02	0.03^{c} ± 0.01
C18:3n-3	13.72^{a} ± 0.12	12.75^{b} ± 0.04	2.17^{d} ± 0.03	-	3.23^{c} ± 0.15	2.10^{d} ± 0.20
C18:2n-6	7.75^{a} ± 0.06	6.00^{a} ± 0.30	1.83^{b} ± 0.15	0.10^{cd} ± 0.00	1.65^{b} ± 0.02	1.99^{b} ± 0.01
C 20:4n-6	1.20^{a} ± 0.02	0.08^{cd} ± 0.04	-	-	0.22^{b} ± 0.01	0.13^{c} ± 0.01
Σ PUFA	22.94^{a} ± 0.01	19.11^{b} ± 0.01	4.10^{c} ± 0.01	0.17^{d} ± 0.02	5.12^{c} ± 0.01	4.28^{c} ± 0.22
Σ n-3	13.99^{a} ± 0.02	13.03^{a} ± 0.01	2.27^{b} ± 0.01	0.14^{d} ± 0.02	3.25^{b} ± 0.01	2.16^{c} ± 0.22
Σ n-6	8.95^{a} ± 0.04	6.08^{b} ± 0.02	1.83^{c} ± 0.01	0.10^{d} ± 0.00	1.87^{c} ± 0.01	2.12^{c} ± 0.02
Σ n-3/ Σ n-6	1.56^{b} ± 0.00	2.14^{a} ± 0.22	1.24^{bc} ± 0.27	1.40^{b} ± 0.10	1.73^{a} ± 0.10	1.01^{d} ± 0.10
5-Lipoxygenase (5- LOX) activity‡	2.56^{a}±0.10	3.10^{b}±0.02	1.51^{c}±0.10	1.80^{c}±0.10	2.08^{a}±0.03	1.60^{c}±0.10

†Fatty acid compositions are presented as % total fatty acid. Different superscripts (a-d) indicate significant difference within the same row (p < 0.05). Data are expressed as mean values of three samples (mean ± SD). ND, Non-detectable.

‡5-Lipoxygenase attenuation (anti-inflammatory) activities of the organic extracts of the seaweeds, expressed as IC_{50} values (mg ml^{-1}).

H. macroloba and *H. gracilis* exhibited significantly higher total SFA content, with values of 54.94 and 59.79%, respectively, compared to other species. In contrast, *C. antennina* and *C. linum* displayed moderate SFA levels (45.46 and 47.54%). Monounsaturated fatty acids (MUFAs) were more abundant in *C. linum* and *C. antennina*, with total MUFA contents of 23.48 and 22.64%, respectively. The C18:1n-7 (oleic acid) content was particularly high in these species, with *C. linum* containing 21.03% and *C. antennina* 20.52%, while *U. linza* and *H. gracilis* showed lower levels (10.1 and 12.11%, respectively). Polyunsaturated fatty acids (PUFAs) were most prominent in *U. linza* (22.94%) and *U. lactuca* (19.11%), with the n-3 PUFAs, especially C18:3n-3 (α-linolenic acid), contributing significantly (13.72 and 12.75%, respectively). In contrast, *H. macroloba* and *H. gracilis* exhibited much lower PUFA levels (4.10 and 0.17%, respectively). The n-6 PUFA content was highest in *U. linza* (8.95%) and lowest in *H. gracilis* (0.10%). The n-3/n-6 ratio was highest in *U. lactuca* (2.14) and *C. antennina* (1.73), indicating a favourable balance between these essential fatty acids. However, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two critical omega-3 fatty acids, were present in lower amounts. The amino acid composition revealed notable variations in both essential amino acids (EAA) and non-essential amino acids (NEAA). Among the essential amino acids, valine content was significantly higher in *U. linza* (6.30 mg g^{-1}), whereas *H. gracilis* exhibited the lowest concentration (2.96±0.01 mg g^{-1}). Leucine levels were prominent in *U. lactuca* (5.01 mg g^{-1}), while *U. linza* had the least amount (0.50 ± 0.02 mg g^{-1}). Isoleucine was most abundant in *U. linza* (9.86 mg g^{-1}), followed by *U. lactuca* (7.44±0.01 mg g^{-1}), with lower concentrations observed in *C. antennina* and *C. linum* (1.97 and 2.36 mg g^{-1}, respectively). Threonine content varied across species, with *U. lactuca* (6.52 mg g^{-1}) and *C. antennina* (6.43 mg g^{-1}) having the highest concentrations, while *H. gracilis* (2.86 mg g^{-1}) showed the lowest. *H. gracilis* also demonstrated higher levels of lysine (8.70±0.02 mg g^{-1}), comparable to *H. macroloba* (8.49 mg g^{-1}). The methionine concentration was highest in *U. linza* (6.37 mg g^{-1}) and lowest in *H. gracilis* (2.90 mg g^{-1}). Phenylalanine was most abundant in *U. lactuca* (6.30 mg g^{-1}) and least in *H. macroloba* (4.18 mg g^{-1}). In terms of non-essential amino acids, alanine content was highest in *U. linza* (4.30 mg g^{-1}) and lowest in *H. macroloba* (1.37 mg g^{-1}). Glycine concentrations followed a similar trend, with *C. linum* showing the highest value (4.07 mg g^{-1}) and *H. macroloba* the lowest (2.01 mg g^{-1}). Proline levels were elevated in *H. gracilis* (4.65 mg g^{-1}), while *C. antennina* exhibited the lowest (1.71 mg g^{-1}). Serine concentrations were highest in *C. linum* (5.28 mg g^{-1}), with *H. macroloba* having the lowest value (2.12 mg g^{-1}). Glutamic acid content was significantly higher in *C. linum* (5.72 mg g^{-1}) and lowest in *H. gracilis* (1.28 mg g^{-1}). Similarly, asparagine was most abundant in *U. linza* (5.71 mg g^{-1}) and least in *H. macroloba* (2.53 mg g^{-1}). Hydroxyproline levels were remarkably higher in *U. linza* (4.54 mg g^{-1}), whereas *U. lactuca* showed a marked decrease (1.22 mg g^{-1}). Cysteine content varied among species, with the highest values recorded in *H. macroloba* (2.84 mg g^{-1}) and the lowest in *C. antennina* (1.27 mg g^{-1}).

The contents of lipids and fatty acids in the studied seaweeds were statistically analysed using PCA (Fig. 2). The stacked data of the first-order principal component constituted PC1 (51.26%), whereas the second-order component was represented by PC2 (48.73%). Greater positive loadings of PC1 were implicated by the lipids of *H. gracilis* and *C. antennina* (LDHG and LDCA, respectively), along with PUFA of *H. macroloba* (PFHM) and EPA of *U. linza, H. macroloba*, *H. gracilis* and *C. antennina* (EPUl, EPHM, EPHG and EPCA, respectively). Further, DHA of *U. lactuca* and *C. antennina*, *n*-3 PUFAs of *H. gracilis* (N3HG), *n*-6 PUFAs of *H. macroloba* (N6HM) and *n*-6 PUFAs of *C. linum* displayed positive loadings of PC1. Significant

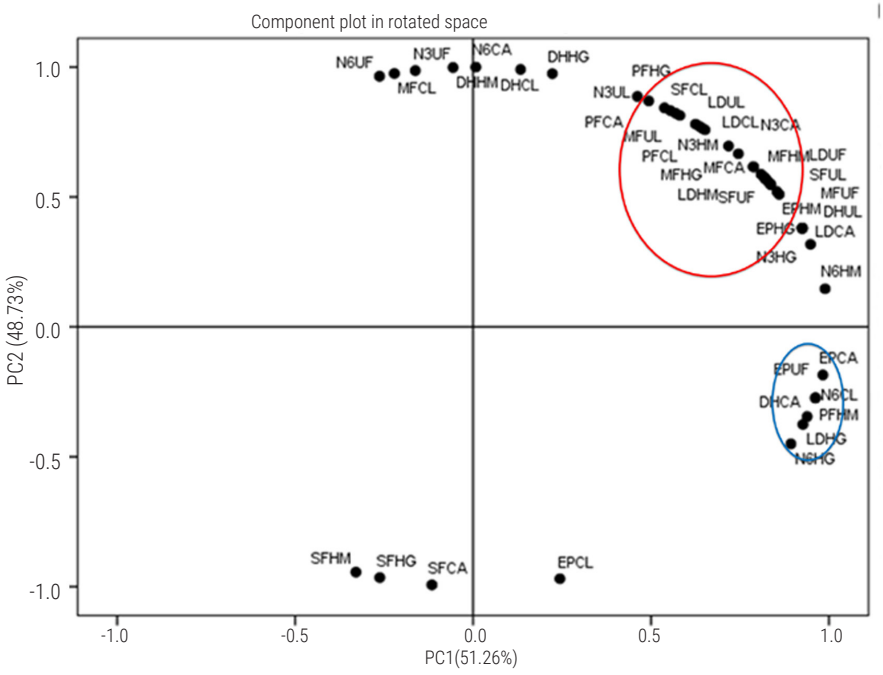

Fig. 2. Loading plot diagram showing correlation of lipid content, SFA, MUFA, PUFA, DHA, EPA, *n*-3, and *n*-6 fatty acids of *U. linza* (Ul), *U. lactuca* (UL), *H. macroloba* (HM), *H. gracilis*(HG), *C. antennina* (CA) and *C. linum* (CL)

positive loadings of PC2 axis were MUFA of *C. linum* (MFCL), DHA of *H. macroloba* (DHHM), DHA of *H. gracilis* (DHHG) and *n*-6 PUFAs of *U. linza* (N6UF).

The total amino acid content (ΣAA) was significantly higher in *U. linza* (92.55 mg g^{-1}), followed by *U. lactuca* (78.32 mg g^{-1}) and *C. linum* (77.16 mg g^{-1}) (Table 3). The lowest values were recorded in *H. gracilis* (58.5 mg g^{-1}). The sum of essential amino acids (ΣEAA) was notably higher in *U. linza* (47.11 mg g^{-1}) and *C. antennina* (41.46 mg g^{-1}), while *H. gracilis* showed the lowest concentration (31.74 mg g^{-1}). Similarly, the total non-essential amino acids (ΣNEAA) were highest in *U. linza* (45.44 mg g^{-1}) and *U. lactuca* (37.17 mg g^{-1}), with *H. gracilis* recording the lowest concentration (29.76 mg g^{-1}). The ratio of essential to non-essential amino acids (ΣEAA/ΣNEAA) was highest in *C. antennina* (1.33), suggesting a balanced amino acid profile that may contribute to the nutritional value of the species.

Apart from nutritional profile of these seaweeds, the organic extracts of these seaweeds were also assessed for 5-lipoxygenase (5-LOX) activity, which is an indicator of anti-inflammatory potential and was highest in the organic extract of *U. lactuca* (3.10) and *U. linza* (2.56), while extract of *H. macroloba*, *H. gracilis* and *C. linum* demonstrated lower activity (1.51, 1.80 and 1.60, respectively).

Discussion

Green seaweeds are recognised for their high protein content, offering a diverse range of essential amino acids crucial for human health. Beyond protein, they are also rich in dietary fibre and essential minerals such as iron, magnesium, and calcium. The presence of omega-3 fatty acids in these seaweeds enhances their nutritional value, with potential health benefits that include improved cardiovascular function and pharmacological properties. Additionally, the antioxidants found in green seaweeds contribute to their ability to combat oxidative stress, further amplifying their health-promoting effects. Given these attributes, green seaweeds have emerged as a significant focus of research in the quest for sustainable, nutrient-dense food sources. The results from the proximate composition analysis emphasise the potential of green seaweeds as nutritious and sustainable food sources. *U. lactuca*, with its high carbohydrate content (66.1%), emerges as a promising candidate for carbohydrate-rich food applications, including industrial uses as a thickening agent or energy source. This finding is consistent with previous research by El-Beltagi *et al*. (2022), which demonstrated the significant capacity of seaweeds to accumulate carbohydrates. Additionally, the notable crude protein content found in *U. linza* (12.89%) and *U. lactuca* (12.06%) underscores their potential to serve as protein supplements, particularly in

Table 3. Amino acid composition in green seaweeds

Amino acids†	*U. linza*	*U. lactuca*	*H. macroloba*	*H. gracilis*	*C. antennina*	*C. linum*
Essential amino acids						
Valine	$6.30^{a} \pm 0.01$	$3.89^{b} \pm 0.02$	$4.85^{b} \pm 0.02$	$2.96^{c} \pm 0.01$	$3.56^{b} \pm 0.03$	$3.52^{b} \pm 0.01$
Leucine	$0.50^{d} \pm 0.02$	$5.01^{a} \pm 0.03$	$3.45^{b} \pm 0.01$	$2.37^{b} \pm 0.02$	$1.91^{c} \pm 0.02$	$2.70^{b} \pm 0.02$
Isoleucine	$9.86^{a} \pm 0.02$	$7.44^{b} \pm 0.01$	$3.55^{c} \pm 0.01$	$2.39^{d} \pm 0.01$	$1.97^{e} \pm 0.01$	$2.36^{d} \pm 0.01$
Threonine	$4.14^{b} \pm 0.01$	$6.52^{a} \pm 0.02$	$3.28^{c} \pm 0.03$	$2.86^{cd} \pm 0.02$	$6.43^{a} \pm 0.02$	$4.77^{b} \pm 0.02$
Lysine	$8.70^{a} \pm 0.04$	$5.15^{cd} \pm 0.01$	$8.49^{a} \pm 0.02$	$6.57^{c} \pm 0.01$	$8.48^{a} \pm 0.01$	$7.91^{b} \pm 0.01$
Histidine	$6.62^{b} \pm 0.01$	$3.31^{d} \pm 0.01$	$4.43^{c} \pm 0.01$	$4.08^{c} \pm 0.01$	$8.70^{a} \pm 0.02$	$8.50^{a} \pm 0.02$
Methionine	$6.37^{a} \pm 0.02$	$3.53^{c} \pm 0.02$	$4.93^{c} \pm 0.01$	$2.90^{d} \pm 0.01$	$5.57^{b} \pm 0.01$	$5.70^{b} \pm 0.01$
Phenylalanine	$4.62^{b} \pm 0.01$	$6.30^{a} \pm 0.02$	$4.18^{c} \pm 0.02$	$4.61^{b} \pm 0.02$	$4.84^{b} \pm 0.01$	$4.50^{b} \pm 0.02$
Non-essential amino acids						
Alanine	$4.30^{a} \pm 0.01$	$3.66^{b} \pm 0.01$	$1.37^{d} \pm 0.02$	$2.87^{c} \pm 0.01$	$3.15^{b} \pm 0.01$	$2.80^{c} \pm 0.02$
Glycine	$3.46^{b} \pm 0.02$	$3.15^{b} \pm 0.02$	$2.01^{c} \pm 0.01$	$2.61^{c} \pm 0.02$	$2.47^{c} \pm 0.03$	$4.07^{a} \pm 0.01$
Proline	$3.40^{b} \pm 0.01$	$3.69^{b} \pm 0.02$	$2.20^{c} \pm 0.04$	$4.65^{a} \pm 0.02$	$1.71^{d} \pm 0.02$	$1.93^{d} \pm 0.01$
Serine	$2.70^{b} \pm 0.03$	$3.01^{b} \pm 0.04$	$2.12^{c} \pm 0.01$	$2.88^{b} \pm 0.01$	$4.50^{a} \pm 0.01$	$5.28^{a} \pm 0.02$
Glutamic acid	$4.51^{b} \pm 0.02$	$4.19^{b} \pm 0.01$	$3.42^{c} \pm 0.02$	$1.28^{d} \pm 0.02$	$3.41^{c} \pm 0.01$	$5.72^{a} \pm 0.01$
Asparagine	$5.71^{a} \pm 0.01$	$3.13^{c} \pm 0.02$	$2.53^{bc} \pm 0.01$	$4.33^{b} \pm 0.02$	$4.42^{b} \pm 0.02$	$4.32^{b} \pm 0.01$
Aspartic acid	$5.32^{a} \pm 0.02$	$2.83^{c} \pm 0.03$	$3.72^{b} \pm 0.02$	$2.33^{c} \pm 0.02$	$3.41^{b} \pm 0.02$	$4.32^{a} \pm 0.02$
Hydroxyproline	$4.54^{a} \pm 0.05$	$1.22^{d} \pm 0.01$	$3.65^{b} \pm 0.02$	$1.33^{d} \pm 0.01$	$1.75^{d} \pm 0.03$	$2.61^{c} \pm 0.02$
Cysteine	$1.39^{b} \pm 0.04$	$2.56^{a} \pm 0.02$	$2.84^{a} \pm 0.02$	$2.11^{a} \pm 0.01$	$1.27^{b} \pm 0.02$	$1.30^{b} \pm 0.03$
Glutamine	$2.38^{b} \pm 0.01$	$2.23^{b} \pm 0.01$	$4.10^{a} \pm 0.03$	$1.11^{d} \pm 0.02$	$1.79^{c} \pm 0.03$	$1.93^{c} \pm 0.02$
Ornithine	$1.42^{c} \pm 0.02$	$3.51^{a} \pm 0.02$	$4.53^{a} \pm 0.01$	$2.25^{b} \pm 0.01$	$1.41^{c} \pm 0.02$	$1.40^{c} \pm 0.01$
Tyrosine	$6.35^{a} \pm 0.01$	$4.00^{b} \pm 0.01$	$3.41^{c} \pm 0.02$	$2.01^{d} \pm 0.01$	$1.72^{e} \pm 0.03$	$1.57^{e} \pm 0.04$
Σ AA	$92.55^{a} \pm 0.12$	$78.32^{b} \pm 0.21$	$73.06^{b} \pm 0.62$	$58.5^{d} \pm 0.23$	$69.27^{c} \pm 0.74$	$77.16^{b} \pm 0.12$
Σ EAA	$47.11^{a} \pm 0.61$	$41.15^{a} \pm 0.18$	$37.16^{b} \pm 0.38$	$31.74^{c} \pm 0.61$	$41.46^{a} \pm 0.21$	$39.66^{a} \pm 0.31$
Σ NEAA	$45.44^{a} \pm 0.43$	$37.17^{b} \pm 0.07$	$35.9^{b} \pm 0.52$	$29.76^{c} \pm 0.25$	$30.96^{c} \pm 0.08$	$37.2^{b} \pm 0.14$
Σ EA/ Σ AA	$0.50^{b} \pm 0.02$	$0.52^{a} \pm 0.03$	$0.51^{ab} \pm 0.02$	$0.54^{a} \pm 0.01$	$0.60^{a} \pm 0.02$	$0.51^{ab} \pm 0.01$
Σ NEA/ Σ AA	$0.49^{a} \pm 0.01$	$0.47^{a} \pm 0.02$	$0.50^{a} \pm 0.03$	$0.50^{a} \pm 0.02$	$0.45^{a} \pm 0.03$	$0.48^{a} \pm 0.02$
Σ EAA/ Σ NEAA	$1.02^{c} \pm 0.03$	$1.11^{b} \pm 0.01$	$1.20^{a} \pm 0.02$	$1.06^{c} \pm 0.03$	$1.33^{a} \pm 0.01$	$1.06^{c} \pm 0.02$

†Amino acid composition was expressed in mg g^{-1}. The samples were measured in triplicate±SD ($p<0.05$).
AA-amino acid, EAA-essential amino acid, NEAA-non essential amino acid. Different superscripts (a-e) within the same row showed significant difference.

plant-based diets. These protein levels are comparable to conventional plant sources and other seaweeds, reaffirming their nutritional significance (Hofmann *et al.,* 2024). The low lipid content observed across all species, with *U. lactuca* having the highest concentration at 1.07%, further supports their suitability for low-fat dietary applications.

Moisture and ash content analysis revealed that these species retain substantial levels of essential minerals. The high moisture content in *U. lactuca* suggests its potential for commercialisation in fresh food markets, while the elevated ash content in *H. gracilis* and *H. macroloba* (35.12 and 32.35%, respectively) indicates their richness in essential minerals according to previous literature (Alghazeer *et al.,* 2022). The mineral composition analysis further reinforces the health-promoting properties of these seaweeds. The high manganese (Mn) content (4.8 mg 100 g^{-1}) of *U. lactuca* is particularly noteworthy, given the crucial role of manganese in bone formation and enzymatic functions (Garcia et al., 2016). Similarly, the significant calcium (Ca) content in *C. linum* (18.99 mg 100 g^{-1}) emphasises its potential as a calcium supplement, particularly for individuals at risk of developing osteoporosis (Saragih *et al.,* 2024). Furthermore, the consistent magnesium (Mg) concentrations in *C. antennina* and the stable sodium (Na) and phosphorus (P) levels across species highlight their contribution to overall cellular health and metabolic function (Holdt and Kraan, 2011). The elevated zinc (Zn) and iron (Fe) levels in *H. gracilis* and *C. linum* further illustrate the potential of these seaweeds in addressing mineral deficiencies, given the essential roles of Zn and Fe in immune function and oxygen transport (Holdt and Kraan, 2011; Garcia *et al.,* 2016).

The fatty acid profile analysis revealed that *C. linum* is particularly rich in myristic acid (C14:0), known for its antimicrobial properties. The predominance of palmitic acid (C16:0) across all species is consistent with previous studies on the fatty acid composition of seaweeds (Gressler *et al.,* 2010). Although the levels of EPA and DHA were relatively low, the favourable n-3/n-6 ratio observed in *U. lactuca* (2.14) suggests its potential in promoting cardiovascular health and reducing inflammation. On the other hand, the high polyunsaturated fatty acid (PUFA) content found in *U. linza* (22.94%) supports its role in cardiovascular health, in line with studies indicating the cardioprotective effects of PUFAs. The amino acid composition of these seaweeds further underscores their value as protein sources. *U. linza* exhibited the highest total amino acid content (92.55 mg g^{-1}), which is significant given the FAO/WHO/UNU (2007) standards that emphasise the importance of amino acid-rich diets. The abundance of essential amino acids, including lysine and methionine in *U. linza* and the high histidine content in *C. antennina* indicate that these seaweeds could serve as effective dietary supplements for populations with higher protein requirements, such as growing children and athletes (Diniz *et al.,* 2011). Organic extract of *H. macroloba* showed greater anti-inflammatory potential as evidenced by the lower IC_{50} value (1.51±0.10 mg ml^{-1}) when compared to other seaweeds considered in this study (Table 2).

Anti-inflammatory activities were highly correlated with the ratio of *n*-3/*n*-6 polyunsaturated fatty acid of green seaweeds (R^2 = 0.803) (Fig. 3). This can be explained by the resolvins (resolution-phase interaction products) signaling pathway which are bioactive lipid moieties synthesised during the process of inflammation (Abdolmaleki *et al*., 2020). These lipid molecules possess a dual property of possessing anti-inflammatory and pro-inflammatory actions. They counter-regulate inflammation and restore tissue integrity. The *n*-3/*n*-6 proportion is recognised as a useful biomedical marker for evaluating the comparative nutritional value of the marine lipids (Marques *et al*., 2021), wherein an increment in *n*-3/*n*-6 fatty ratio was necessary to preclude coronary heart disorder by lessening plasma lipids and decrease cancer risks (Chakraborty *et al.*, 2014). These tropical green seaweed species are potential storehouses of nutritional and bioactive compounds to be used as high-health food, consequently opening new avenues to develop novel functional food compositions. The elevated 5-LOX activity in *U. lactuca* and *U. linza* highlights their strong potential as anti-inflammatory agents. 5-LOX is a key enzyme in the leukotriene biosynthesis pathway, which contributes to

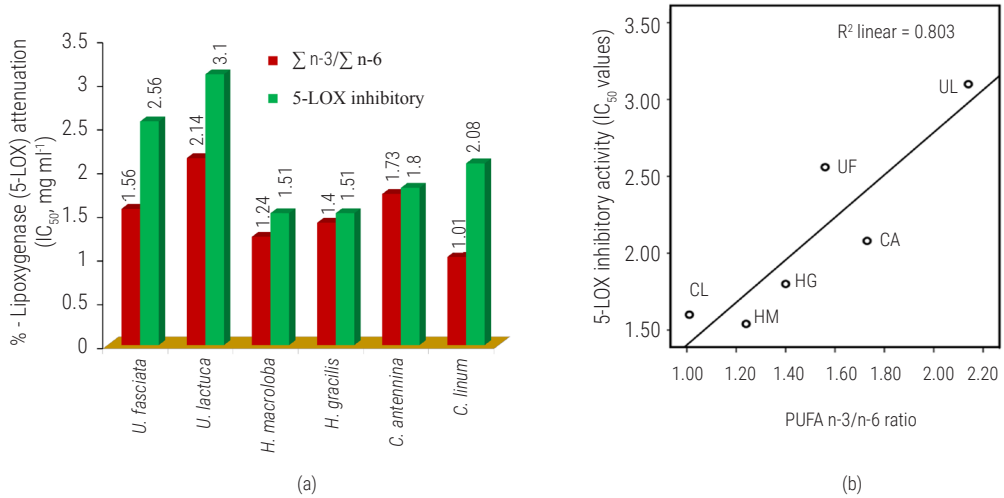

Fig. 3. (a) Comparative *n*-3/*n*-6 fatty acid proportion and 5-LOX attenuation (anti-inflammatory) activities of organic extracts of green seaweeds; (b) Correlation plot between anti-inflammatory activities (as determined by the attenuation properties of the seaweed organic extracts against pro-inflammatory 5-LOX) and *n*-3/*n*-6 PUFA ratio of seaweeds. UI, *U. linza*; UL, *U. lactuca*; HG, *H. gracilis*; HM, *H. macroloba*; CA, *C. antennina*; CL, *C. linum*

inflammatory responses in diseases such as asthma, arthritis and various other inflammatory disorders. The higher inhibition levels in these species suggest that their organic extracts contain bioactive compounds capable of effectively suppressing the 5-LOX enzyme, thereby potentially reducing leukotriene production and alleviating inflammation. In contrast, the lower 5-LOX activity observed in *H. macroloba, H. gracilis* and *C. linum* indicates that these species may possess less potent anti-inflammatory properties. This difference could be attributed to variations in the chemical composition of their bioactive compounds, particularly in their ability to interact with and inhibit the 5-LOX enzyme.

Based on the analysed data, it can be concluded that green seaweeds possess significant nutritional properties, offering potential health benefits that could support the development of pharmaceutical and nutraceutical products. Their rich composition of proteins, essential minerals, and bioactive compounds, coupled with favourable fatty acid profiles, accentuates their value as both functional food sources and therapeutic agents.

Acknowledgments

This work was supported by "Innovation in Science Pursuit for Inspired Research (INSPIRE)" programme funded by the Department of Science and Technology of the Government of India (INSPIRE Registration No. IF210337), DST-SERB funded project (CRG/2021/000338/IBS), and Kerala State Council for Science Technology and Environment [Grant No. 1119/DIR/2016-17/KSCSTE]. The authors thank the Director, ICAR-Central Marine Fisheries Research Institute, Kochi and Head, Marine Biotechnology Fish Nutrition and Health Division, ICAR-CMFRI, Kochi, for guidance and support.

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