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# Nutritional Properties, Antioxidant Activity, and Heavy Metal Accumulation in Selected Marine Macro-Algae Species of Sri Lanka

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**Abstract:** In recent years, the emergence of drug resistance and sensitivity in leading diseases has heightened global interest in natural nutraceuticals as primary health supplements. However, comprehensive scientific scrutiny is essential before marketing these as supplements. In this study, we assessed the nutritional composition, antioxidant activities, and trace metal accumulation in eleven selected Sri Lankan coastal seaweed species. *Gracilaria corticata* had the highest ($p < 0.05$) ash and crude fiber content among the species. Protein content ranged from 4.87% to 23.67% (DW), with *Ulva rigida* displaying the highest ($p < 0.05$). Crude fat content ranged from 0.09% to 4.13% (DW), with *Cladophora herpestica* having the highest ($p < 0.05$) crude fat content. *Sargassam cinereum*, *Turbinaria ornata* and *Sargassum crassifolium* had the highest ($p < 0.05$) TPC content (51.32 ± 0.61–28.90 ± 2.68 mg/GAE g) and the highest ($p < 0.05$) radical scavenging antioxidant activity compared to other seaweeds. The study findings indicate that most of the studied metals in seaweeds exceeded the WHO-recommended levels. Aluminum was the highest ($p < 0.05$) accumulated metal in seaweeds compared to other metals. Toxic heavy metals, such as arsenic, cadmium and chromium, levels in all of the studied seaweeds surpassed the WHO limits. While seaweeds displayed acceptable nutritional and antioxidant properties, heavy metal presence poses a potential health risk to consumers. Products using seaweeds with accumulated heavy metals may have lower nutritional quality. Thus, this study underscores the need for comprehensive scientific investigation before developing high-quality natural food products or supplements from seaweeds.

**Keywords:** seaweeds; nutrition; antioxidant activity; heavy metals; trace elements



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## 1. Introduction

The seaweeds, also known as macroalgae, are consumed in many Asian countries, and are gaining increased attention in Western countries due to their functional properties [1]. These multicellular organisms thrive in intertidal and subtidal zones, utilizing light for photosynthesis and converting inorganic resources and minerals into biomass through the harnessing of light energy [2,3]. They are categorized into three main taxonomic groups based on their photosynthetic pigments: green algae, brown algae, and red algae, each characterized by distinct pigments such as chlorophylls, fucoxanthins, and phycobilins [3,4].

Microalgae, despite being low in calories, are rich in essential nutrients, including vitamins, minerals, polyunsaturated fatty acids, bioactive metabolites, proteins, polysaccharides, and dietary fibers [2,5]. However, variations in nutrient composition exist among species and environmental conditions [5]. Seaweeds, with their lower fat content but

adequate levels of other nutrients and bioactive compounds compared to terrestrial plants, offer a convenient source of nutrition [2,5]. Although seaweeds generally contain lower total lipid content, their consisting lipids are mainly of polyunsaturated fatty acids including docosahexaenoic acid and eicosapentaenoic acid (ω-3 fatty acids) [6]. The protein content in seaweeds varies from 5% to 47% of dry mass, with red seaweeds having the highest protein content and green seaweeds the lowest [6]. A significant portion of algal proteins comprises amino acids, particularly aspartic and glutamic acids, with some species like *Palmaria palmata* and *Ulva* spp. containing essential amino acids such as histidine, leucine, isoleucine, and valine [7]. Seaweeds are also rich in dietary fiber, which contributes to a reduced risk of certain chronic diseases [8]. The structural composition of algal fibers differs from terrestrial plant fibers and consists primarily of non-starchy fiber, which helps regulate blood glucose levels [6,9]. For instance, some seaweeds like *Laminaria digitata* have a higher total fiber percentage (6.2%) compared to brown rice (3.8%), resulting in a minimal glycemic load [7].

Beyond their nutritional value, seaweeds are a valuable source of bioactive compounds with various beneficial effects, including antioxidant, antibacterial, antifungal, cytotoxic, anti-inflammatory, and antidiabetic properties [10–14]. These bioactive compounds, such as polyphenols, flavonoids, phlorotannins, sulfated polysaccharides, and carotenoids, contribute to the antioxidant properties of seaweeds [5].

Phenolic acids are structurally characterized by the presence of an aromatic ring and one or more hydroxyl groups. The diversity of phenolic acids range from simpler molecules like hydroxycinnamic acids to more complex polymeric forms with molecular sizes ranging from 126–650 kDa [15]. Phlorotannins are secondary metabolite only found in marine algae. They are a large group of natural polyphenolic compounds consisting polymeric structural units of phloroglucinol (1,3,5-trihydroxybenzene) and can be categorize into six different classes namely, phloroethols, fuhalols, fucophloroethols, fucols eckols, and carmalols. In addition to wide range of bioactivities such as antimicrobial, anticancerous, anti-inflammatory, anti-diabetic and UV radiation protection [16], phlorotannins known to have powerful antioxidant activity due to their ability to act as chelating agents with reactive oxygen species [6,17]. The relative high concentration of phenolic compounds in marine algae contributes for their antioxidant properties [18].

Carotenoids are linear lipid-soluble polymeric pigments which protect photoxidation of algae by inactivating reactive oxygen species formed by exposure to light and air. For instance, the unusual chemical structure of tetraterpinoid carotenoid fucoxanthin can donate an electron to quench reactive oxygen species [6,19]. Likewise the antioxidant activity of the carotenoids may help prevent human diseases [17].

Phlorotannins and fucoxanthin, in particular, are known to mitigate cellular damage caused by free radicals. Clinical studies have also established a correlation between the antioxidant capacity of microalgae and their DPPH assay capacities [6].

Concerning about algal polysaccharides, they are different from terrestrial plant polysaccharides. Especially, sulfated polysaccharides like fucoidans, fucan sulfate, ulvan and carrageenan are investigated for their biological activity and found to possess antioxidant activity. Compared to synthetic antioxidants like BHT and BHA, recent studies have shown that sulfated polysaccharides are more potent in nitric oxide scavenging [1].

In contrast to other organisms, marine algae produce a wide array of bioactive secondary metabolites in response to the extreme environmental conditions occurring in their habitat. The prevalence of such bioactive metabolites in marine algae facilitate the algal survival in extremely competitive environments [18].

Furthermore, seaweeds are a rich source of essential minerals and trace elements essential for human nutrition, with mineral content ranging from 8% to 40%. Unlike edible land plants, seaweeds contain a diverse array of minerals, although the variation in mineral content can be attributed to factors such as the seaweed's phylum, geographical origin, and seasonal, environmental, and physiological variations [20].

However, it is crucial to note that, while seaweeds are generally rich in trace elements, certain metals such as aluminum, cadmium, and lead can be toxic to humans even at lower concentrations [21]. Therefore, caution should be exercised when considering the potential presence of these toxic metals in seaweed samples. The significance of this study extends beyond the exploration of nutritional and antioxidant properties and differs from previously published data by delving into the assessment of seaweeds' safety for human consumption. The thorough elemental analysis, encompassing trace elements and heavy metals, provides crucial data for making well-informed decisions about the suitability of incorporating these seaweeds into the diet.

## 2. Materials and Methods

### *2.1. Seaweed Sample Collection*

Eleven seaweed species were collected from coastal areas in Sri Lanka at depths ranging from 0.2 to 1 m. The seaweed specimens were identified using the identification guide [22] and authenticated by Prof. K.M.G.G Jayasuriya, Professor in Botany, Department of Botany, Faculty of Science, University of Peradeniya. The specimens were deposited at the National Institute of Fundamental Studies for future references. Detailed information and voucher numbers of the seaweeds can be found in Table 1, and their photographs are available in Figure 1.

**Table 1.** Seaweed species, voucher number, type of algae, collected location and collected date. Abbreviations: R, red algae (Rhodophyceae); G, green algae (Chlorophyceae); B, brown algae (Phaeophyaceae).

| Seaweed Species | Voucher No. | Type | Collected Location | Location Coordinates | Collected Date |
|---|---|---|---|---|---|
| *Gelidiopsis variabilis* | SW1 | R | South Bar, Mannar | N 8°97′13″, E 79°88′06″ | 31 August 2016 |
| *Pterocladiella caerulescens* | SW2 | R | Madiha, Matara | N 5°56′11″, E 80°30′56″ | 25 September 2016 |
| *Gracilaria corticata* | SW3 | R | Wellamadama, Matara | N 5°94′26″, E 80°56′74″ | 26 September 2016 |
| *Caulerpa racemosa* | SW4 | G | | | |
| *Ulva rigida* | SW5 | G | Madiha, Matara | N 5°56′11″, E 80°30′56″ | 25 September 2016 |
| *Codium tomentosum* | SW6 | G | Madiha, Matara | N 5°56′11″, E 80°30′56″ | 25 September 2016 |
| *Cladophora herpestica* | SW7 | G | Madiha, Matara | N 5°56′11″, E 80°30′56″ | 25 September 2016 |
| *Sargassum crassifolium* | SW8 | B | Madiha, Matara | N 5°56′11″, E 80°30′56″ | 25 September 2016 |
| *Sargassum cinereum* | SW9 | B | Erukkalampiddy, Mannar | N 9°2′27″, E 79°52′43″ | 30 August 2016 |
| *Turbinaria ornata* | SW10 | B | South Bar, Mannar | N 8°97′13″, E 79°88′06″ | 31 August 2016 |
| *Padina antillarum* | SW11 | B | Wellamadama, Matara | N 5°94′26″, E 80°56′74″ | 25 September 2016 |

After collection, the samples were thoroughly rinsed with seawater at the collection site and then placed in polythene bags. Subsequently, the samples were transported to the laboratory and washed once more with distilled water to remove any epiphytes, adhered sand particles, and debris.

To facilitate drying, all samples were air-dried at room temperature for 5–7 days, followed by further drying at 60 °C in a hot air oven until a constant weight was achieved. Afterward, the dried samples were ground to a particle size of less than 2 mm and stored in airtight polythene bags at −20 °C for future analysis. Voucher specimens of the seaweeds were stored in a −80 °C freezer at the National Institute of Fundamental Studies in Kandy, Sri Lanka.

### *2.2. Proximate Analysis*

Moisture, dry matter, ash, crude protein, crude fat, and crude fiber content in seaweed samples were analyzed in replicates according to the AOAC method [23].

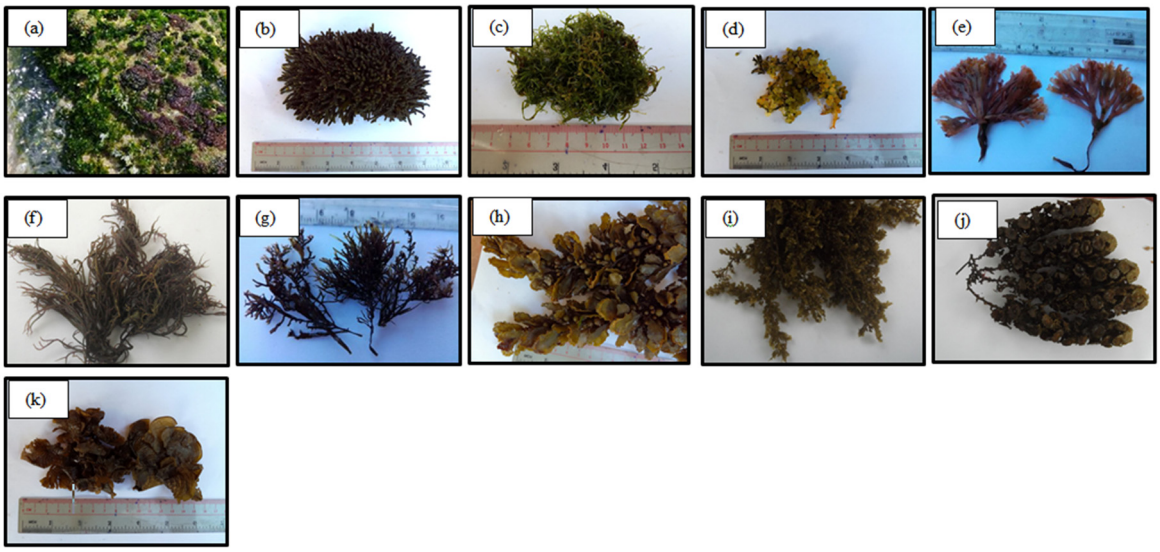


**Figure 1.** Seaweed species, sample number; (**a**) *Ulva rigida*, R1, (**b**) *Codium tomentosum*, G3, (**c**) *Cladophora herpestica*, G4, (**d**) *Caulerpa racemosa*, G1, (**e**) *Gracilaria corticata*, R3, (**f**) *Gelidiopsis variabilis*, R1, (**g**) *Pterocladiella caerulescens*, R2, (**h**) *Sargassum crassifolium*, B1, (**i**) *Sargassum cinereum*, B2, (**j**) *Turbinaria ornata*, B3, (**k**) *Padina antillarum*, B4.

#### 2.2.1. Determination of Moisture Content

The moisture content of seaweeds was determined following the oven drying method described by AOAC [22]. Two grams of macroalgal samples were placed in crucibles and dried in the YAMATO IC600 drying oven (Yamato Scientific Co., Ltd., Tokyo, Japan) overnight at 105 °C until a constant weight was achieved.

$$\text{Moisture } (\%) = \frac{\text{Sample dry weight with crucible} - \text{Crucible}}{\text{Sample fresh weight with crucible} - \text{Crucible weight}} \times 100$$

#### 2.2.2. Determination Dry Matter and Ash

First, 0.5–1.0 g of dried sample was weighed into previously dried and weighed porcelain crucibles. These crucibles were then placed in the YAMATO IC600 drying oven (Yamato Scientific Co., Ltd., Tokyo, Japan) overnight at 105 °C. After drying, the samples were removed and allowed to cool in desiccators.

Following the drying step, the sample weights were measured and then placed in the CARBOLITE Muffle Furnace at 600 °C for 4 h. Finally, the samples were cooled in desiccators and accurately weighed [21].

$$\text{Dry matter}(\%) = \frac{\text{Sample dry weight with crucible} - \text{Crucible weight}}{\text{Sample weight with crucible} - \text{Crucible weight}} \times 100$$

$$\text{Ash}(\%) = \frac{\text{Sample ash weight with crucible} - \text{Crucible weight}}{\text{Sample dry weight} - \text{Crucible weight}} \times 100$$

#### 2.2.3. Determination of Crude Protein

First, 0.25 g of dried samples were weighed and placed in Kjeldahl digestion flasks. To each flask, 10 g of a digestion mixture (powdered $K_2SO_4$ and small crystals of $CuSO_4$ mixed in a 5:1 ratio) and 5 mL of 98% concentrated $H_2SO_4$ acid were added. The flasks were then put into a digestion apparatus (Perstop Analytical Company, Tokyo, Japan). They were allowed to boil for one hour until the solutions became clear. After digestion was completed, the flasks were cooled, and 5 mL of distilled water was added.

Next, 25 mL of 4% boric acid was added to each of the 250 mL conical flasks, and they were placed in a preheated distillation apparatus (Automatic Kjeldahl Apparatus Company,

Tokyo, Japan). The Kjeldahl flasks were then connected to the distillation apparatus and 20 mL of 40% NaOH was slowly added until the solution turned blackish brown. Distillation continued until 75 mL was collected in the boric acid trap. After that, the flask containing boric acid and the distillate were titrated with 0.1N $H_2SO_4$ until the color changed to pink, and the burette reading was recorded [23].

$$\text{Percentage of crude protein} = \frac{\text{Burette reading} \times \text{Normality of } H_2SO_4 \times 8.75 \times 10}{\text{Weight of sample} \times \text{Dry matter percentage}}$$

#### 2.2.4. Determination of Crude Fat

Initially, cleaned fat extraction beakers were placed in a drying oven for one hour at 100 °C and then transferred to desiccators until they reached room temperature. The dried fat extraction beakers were weighed after cooling. One gram of the dried and ground sample was weighed onto a filter paper, wrapped, and placed into a pre-dried asbestos thimble. After adding 310 mL of acetone to the fat-extracting beaker, it was secured with the sample tube connected to the Soxhlet extraction unit. Fat extraction was carried out for a duration of approximately 4 h.

Finally, the beaker was transferred into a Heraeus 208 Vacuum Oven (Thomas Scientific, Swedesboro, NJ, USA) set at a temperature of 80 °C for 12 h (overnight), and the weight of the beaker with fat was measured [23]. The percentage of crude fat was calculated using the formula below [23].

$$\text{Percentage of crude fat} = \frac{\text{Weight of beaker and fat} - \text{Weight of empty beaker}}{\text{Weight of dry sample} - \text{Dry matter percentage}} \times 100$$

### *2.3. Determination of Crude Fiber*

Crude fiber content was determined using 1 g of dried seaweed samples. The samples were subjected to hydrolysis with 50 mL of 0.3 N $H_2SO_4$ for 30 min, followed by treatment with 25 mL of 1.5 N NaOH for an additional 30 min. Subsequently, 0.5 g of EDTA was added, and the mixture was boiled for an additional 5 min. The resulting mixture was then filtered using suction filtration. The remaining crude residue was washed with distilled water and dried overnight at 100 °C in a drying oven (Yamato Scientific Co., Ltd., Tokyo, Japan). Afterward, the samples were allowed to cool in desiccators and were weighed precisely. These weighed samples were then converted to ash using the CARBOLITE Muffle Furnace (Carbolite Gero Ltd., Hope Valley, UK), following the procedure described by Kirk and Sawyer (1991), which involved heating them at 520 °C for 4 h. Finally, the ash samples were cooled in desiccators and accurately weighed [23].

$$\text{Crude Fiber}(\%) = \frac{\text{Dry weight of the sample} - \text{Ash weight of the sample}}{\text{Weight of dry sample} \times \text{Dry matter percentage}} \times 100$$

### *2.4. Preparation of Crude Ethanol Extract of the Samples*

First, each ground sample was mixed with absolute ethanol. Subsequently, the mixture underwent sonication at a temperature of 30 °C for a duration of 30 min. After the sonication process, the resulting extract was filtered using cotton wool to remove macro particles. The filtered extract was then concentrated using a rotary evaporator. To ensure accuracy and reliability, this entire procedure was conducted in triplicate for each sample. The collected crude ethanol extracts obtained through this process were subsequently used for assays.

### *2.5. Antioxidant Assays*

#### 2.5.1. Total Phenolic Content

The total polyphenolic content was determined using the Folin–Ciocalteu method with slight modifications adapted from Singleton and Rossi [24]. Sample stocks at a concentration of 0.2 mg/mL were prepared using crude ethanol extracts of the samples, with gallic acid used as the standard.

In a 96-well plate, 40 µL of each sample was added, followed by 25 µL of distilled water and 105 µL of Folin reagent. After 3 min, 7.5% (*w*/*v*) $Na_2CO_3$ was added, and the mixture was incubated for 30 min at room temperature. The absorbance was measured at 760 nm against the blank sample using a microplate spectrophotometer (Thermo Fisher Scientific™ Multiskan™ GO, Vantaa, Finland). Three replicates were used for each sample. Blank samples were prepared using 40 µL from the sample stocks and 210 µL of distilled water.

#### 2.5.2. DPPH Assay

The ability of the extracts to neutralize the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) was investigated using the method described by Williams et al. in 1995 [25]. Different sample volumes (50 µL, 75 µL, 100 µL, 125 µL, and 150 µL) of 5 mg/mL sample stocks were added to 96-well plates, followed by 100 µL (100 µM) DPPH and various volumes of distilled water. The reaction mixture was incubated for 30 min at room temperature, and the absorbance was recorded at 517 nm using a microplate spectrophotometer. The percentage of radical scavenging activity at each concentration of the sample was calculated (five different concentrations with three replicates). Control samples of DPPH were prepared with 150 µL of methanol and 100 µL of DPPH. A blank sample was used for each seaweed sample. A scatter graph plotting concentration against the percentage of radical scavenging activity was created. The equation Y = mx + c (where x = concentration and Y = percentage of radical scavenging activity) was fitted, and the x value at Y = 50 was calculated.

#### 2.5.3. ABTS Assay

The ABTS free radical-scavenging activity of each sample was determined according to the method described by Arnao et al. in 2001 [26]. A mixture of ABTS (2 mM) and potassium persulfate (70 mM) was allowed to stand overnight at room temperature in the dark to generate the ABTS radical cation, 16 h prior its use. The $ABTS^+$ solution was then diluted with 80% methanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. Next, 25 µL of each sample stock was added to a 96-well plate, followed by the addition of 200 µL of ABTS+ solution. The absorbance was recorded at 734 nm after 6 min of incubation at room temperature. A standard curve was generated using a Trolox standard solution at various concentrations (6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid).

The scavenging activity of different concentrations of extracts and fractions against the ABTS radical was also measured to calculate $IC_{50}$, following a procedure similar to the DPPH scavenging method described above.

#### 2.5.4. FRAP Assay

The FRAP assay was conducted following the method described by Al-Farsi et al. in 2005 [27]. The FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6), a 10.0 mM solution of tripyridyl triazine (TPTZ), and a 20.0 mM solution of $FeCl_3 \cdot 6H_2O$ in a 10:1:1 volume ratio. Then, fifty microliters of each sample at a concentration of 5 mg/mL were added to a 96-well plate, followed by the addition of 2 mL of the FRAP reagent. The reaction mixture was incubated at room temperature for 4 min, and the increase in absorbance at 593 nm was measured. A standard curve was generated using different concentrations of $FeSO_4$ (1.5 mM).

The antioxidant capacity, based on the ability to reduce ferric ions in the sample, was calculated from the linear calibration curve and expressed as mM $FeSO_4$ equivalents per gram of sample (DW).

### *2.6. Trace Elements and Heavy Metal Analysis*

Trace elements and heavy metals analysis were performed on eight seaweed species (R1, R3, G1, G2, G3, B1, B2, and B3) using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) [28]. Each freeze-dried seaweed sample, weighing 0.3 g, was digested with 6 mL of 69% nitric acid in a microwave digester (MARS, CEM Corporation, Matthews, NC, USA) for a duration of 40 min.

After digestion, the samples were diluted with 25 mL of distilled deionized water and filtered through a 0.45 µm filter paper. The resulting filtrate was then utilized for analysis by ICP-OES (iCPA 7000 series, Thermo Scientific, Waltham, MA, USA). A multi-element standard solution (5 for ICP) was employed as the standard reference material for calibration.

### *2.7. Statistical Analysis*

SAS version 9.1, statistical software was used to analyze data. All the data were presented as mean ± standard deviation.

## 3. Results and Discussion

### *3.1. Proximate Analysis*

The proximate compositions of the seaweeds are presented in Table 2. The results of the proximate analysis are reported based on dry weight (DW), except for moisture content. The moisture content of the samples ranged from 80.84% to 93.76% based on fresh weight. Among the seaweed species, *C. herpestica* (G4) exhibited the highest ($p < 0.05$) moisture content, while *U. rigida* (G2) had the lowest ($p < 0.05$) moisture content. The ash content of the red algae ranged from 16.69% ± 0.00% to 35.20% ± 0.17%. As for the green algae and brown algae, their ash values ranged from 26.78% ± 0.04% to 28.18% ± 0.61% and 31.18% ± 0.05% to 33.39% ± 0.11%, respectively. Among the seaweeds investigated, *Gracilaria corticata* (R3) had the highest ash content of 35.20% ± 0.17% ($p < 0.005$), while *S. cinereum* (B2) exhibited the lowest content at 12.96% ± 0.37% (Table 2). The ash values were compared with similar seaweed species reported by Premarathna et al. (2022) [27] from various locations in Sri Lanka. The ash content of R3 was higher compared to *G. corticata* samples collected from Ahangama and Negombo in Sri Lanka, which were 08.17% ± 0.49% and 21.98% ± 0.23%, respectively. The ash content in *Gelidiopsis variabilis* (R1) was 16.69% ± 0.00%, which was lower than the reported value for *Geldiopsis variabilis* (21.64% ± 0.03%) collected from Chilaw, Sri Lanka [29].

**Table 2.** Proximate composition of seaweeds.

| Types of Seaweed | Sample No. | MC% (FW) | Ash% (DW) | Crude Protein% (DW) | Crude Fat% (DW) | Crude Fiber% (DW) |
|---|---|---|---|---|---|---|
| *Gelidiopsis variabilis* | R1 | 91.31 | 16.69 ± 0.00 $^{g}$ | 13.66 ± 0.04 $^{d}$ | 1.46 ± 0.09 $^{cd}$ | 9.94 ± 0.13 $^{c}$ |
| *Pterocladiella caerulescens* | R2 | 87.36 | 22.58 ± 0.19 $^{fc}$ | 22.58 ± 0.39 $^{ab}$ | 2.09 ± 0.14 $^{bc}$ | 10.49 ± 0.04 $^{d}$ |
| *Gracilaria corticata* | R3 | 85.68 | 35.20 ± 0.17 $^{a}$ | 19.43 ± 0.27 $^{b}$ | 2.68 ± 0.09 $^{b}$ | 18.73 ± 0.39 $^{a}$ |
| *Caulerpa racemosa* | G1 | 92.89 | 26.78 ± 0.04 $^{dc}$ | 18.61 ± 0.82 $^{bc}$ | 0.54 ± 0.66 $^{c}$ | 12.33 ± 0.08 $^{c}$ |
| *Ulva rigida* | G2 | 80.84 | 20.26 ± 0.11 $^{cf}$ | 23.67 ± 0.73 $^{a}$ | 0.09 ± 0.02 $^{c}$ | 11.64 ± 0.31 $^{cd}$ |
| *Codium tomentosum* | G3 | 91.80 | 21.43 ± 0.73 $^{c}$ | 13.85 ± 0.48 $^{d}$ | 1.31 ± 0.23 $^{cd}$ | 10.66 ± 0.39 $^{d}$ |
| *Cladophora herpestica* | G4 | 93.76 | 28.18 ± 0.61 $^{d}$ | 17.53 ± 1.90 $^{bc}$ | 3.89 ± 0.40 $^{a}$ | 15.42 ± 0.26 $^{b}$ |
| *Sargassum crassifolium* | B1 | 89.02 | 31.18 ± 0.05 $^{bc}$ | 10.44 ± 0.51 $^{c}$ | 2.65 ± 0.01 $^{b}$ | 15.78 ± 0.11 $^{b}$ |
| *Sargassum cinereum* | B2 | 88.02 | 12.96 ± 0.37 $^{h}$ | 8.30 ± 0.24 $^{f}$ | 2.47 ± 0.22 $^{b}$ | 17.46 ± 0.38 $^{a}$ |
| *Turbinaria ornata* | B3 | 87.02 | 21.11 ± 0.12 $^{c}$ | 4.87 ± 0.85 $^{g}$ | 4.13 ± 0.12 $^{a}$ | 15.33 ± 0.56 $^{b}$ |
| *Padina antillarum* | B4 | 87.97 | 33.39 ± 0.11 $^{bc}$ | 18.44 ± 0.51 $^{bc}$ | 2.33 ± 0.00 $^{b}$ | 16.32 ± 0.04 $^{b}$ |

Values are expressed as mean ± S.D (Standard deviation); Values with different alphabet letters are significantly different ($p < 0.05$). MC, moisture content; FW, fresh weight; DW, dry weight.

The ash content typically found in edible seaweed is generally higher compared to that found in terrestrial plants, with the exception of spinach. Most vegetables from land have an ash content ranging from 5% to 10% of their dry weight, while spinach has a mineral content of 20.4% [20,30]. The high ash content in the seaweeds can be attributed to the elevated levels of salt and minerals present in their habitat. Furthermore, various

factors such as seaweed species, location, geography, season, environment, physiology, and mineralization processes contribute to variations in ash content [31]. The consumption of seaweed ash is known to have preventive effects against diseases such as arthritis, fever, gout, fluid retention, bladder problems, and constipation [27].

In this study, the highest ($p < 0.05$) content of crude protein was found in green algae, *Ulva rigida* (G2), with 23.67% ± 0.73%, while the lowest protein content was observed in brown algae, *Turbinaria ornata* (B3), with 4.87% ± 0.85%. These findings align with those of Ibañez and Cifuentes in 2013, which reported that brown algae typically contain lower protein content (5% to 15% of the dry weight), whereas green and red algae contain the highest protein content (10% to 47% of the dry weight) [32]. A previous study on *U. rigida* collected from the northwest Iberian coast found a protein content of 17.8% [33], which is comparatively lower than that of G2. Brown algae, *T. ornata*, collected from Kankasanthurai, Sri Lanka, reported a protein content of 23.54% ± 0.53% [29], which is higher than that of B3.

Rajapakse and Kim in 2011 mentioned that the protein content of several red seaweeds is quantitatively comparable to that of legumes, where 30% to 40% of dry matter consists of proteins [34]. However, the protein content of the red algae investigated in this research was found to be lower than that of legumes. Variations in seaweed protein content are reported in similar species harvested at different locations and during different seasons [29,34,35]. Additionally, variations in protein content may depend on the method employed for protein estimation [36].

Highest ($p < 0.05$) fat content was found in brown algae, *T. ornate* (B3) with 4.13 ± 0.12% and *C. herpestica* (G4) while green algae, *U. rigida* (G2) accounts for the lowest with 0.09 ± 0.02% (Table 2). From the fat content range, it is evident that the least available component of all the investigated seaweeds is fat. It was mentioned by Rajapakse and Kim (2011) [34] that the lipid content of the seaweeds ranges from 1% to 5% of dry matter and the content and the composition of fat can greatly vary depending on the type of seaweed [34]. *T. ornata*, collected from Kankasanthurai, Sri Lanka exhibited a lower fat content of 3.3% ± 0.08% compared to that of B3 [29]. When comparing G2 with *U. rigida* samples collected from the Northwest Iberian coast and Varvara, Bulgaria, they were reported to have fat contents of 0.9% [33] and 0.79%, respectively [37]. In contrast, a comparison of the fat content of R3 with *G. corticata* found in the Thondi coast of Southeast India showed a higher fat content of 7.07% ± 0.33%.

Crude fiber indicates the amount of fiber that can be digested and affects energy digestibility. Generally, there is a significantly lower amount of crude fiber found in seaweeds compared to land plants [31]. The crude fiber content of the analyzed seaweeds ranged from 9.94% ± 0.13% to 18.73% ± 0.39%. Significantly high ($p < 0.05$) fiber contents were found in *G. corticata* (R3) and *S. cinereum* (B2), while the lowest ($p < 0.05$) crude fiber content was observed in *G. variabilis* (R1), both of which are red algae. The crude fiber content of green algae ranged from 10.66% ± 0.39% to 15.42% ± 0.26%, while in brown algae, it ranged from 17.46% ± 0.38% to 15.33% ± 0.56%.

The proximate analysis and comparison of results with previously published data reveal variations. As noted by Wong and Cheung in 2000, the chemical composition of seaweeds varies depending on factors such as species, habitats, maturity, and environmental conditions [9].

### *3.2. Antioxidant Activity*

Phenolic compounds are commonly found in plants, reportedly having several biological activities including antioxidant properties. Previous reports have revealed that marine seaweed extracts, especially polyphenols, have antioxidant activity [36]. Phenolic compounds are regarded for their important dietary roles as antioxidants and chemo preventive agents [37]. The total phenolic contents of the seaweeds are shown in Figure 2. Highest ($p < 0.05$) total phenolic content was recorded in *Sargassum cinereum* (B2) (55.38 ± 0.61 mg/GAE g) and the lowest in *Codium tomentosum* species (G3) (2.26 ± 0.28 mg/GAE g). The total phenol content

and antioxidant activity of the examined seaweeds may be higher than the observed data suggests, as the drying temperature of 60 °C could have removed some volatile compounds responsible for the TPC and antioxidant properties of seaweeds [38].

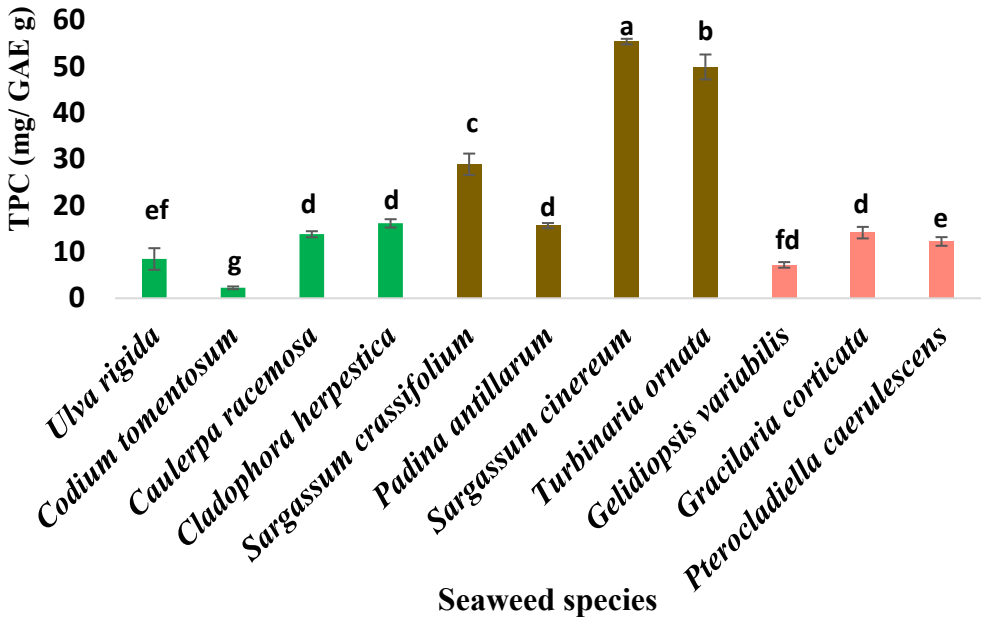


**Figure 2.** Total phenolic content in the studied seaweed extracts. Mean values with different superscript letters were significantly different ($p < 0.05$).

The FRAP values of the selected seaweeds are presented in Figure 3. Although the green algae *C. racemosa* had a lower total phenolic content (TPC) compared to some other seaweeds, it exhibited the highest ferric-reducing antioxidant power (FRAP) ($p < 0.05$) among them and agreed with previous observations [39–41]. The value was 420.19 ± 6.78 mM $Fe^{2+}$/g of crude ethanol extract for *C. racemosa* (G1), followed by *S. cinereum* (B2) with 212.52 ± 6.54 mM $Fe^{2+}$/g of crude ethanol extract, and *T. ornata* (B3) with 200.34 ± 8.39 mM $Fe^{2+}$/g of crude ethanol extract. In contrast, *Ulva rigida* (G2) displayed the lowest ($p < 0.05$) FRAP value at 19.59 ± 0.17 mM $Fe^{2+}$/g of crude ethanol extract. The findings support previous observations that oil cakes extracted from some seeds showed a negative correlation between TPC and the antioxidant efficiencies of the corresponding extracts, suggesting that phenolic compounds are not the sole contributors to the antioxidant activities [39].

DPPH is a free-radical compound widely employed to assess the free radical scavenging ability of samples [42,43]. This method allows for the determination of the anti-radical activity of an antioxidant by measuring the decrease in absorbance of the DPPH radical resulting from the scavenging of hydroxyl radicals through hydrogen donation. The DPPH radical scavenging activities of seaweeds are depicted in Figure 4. In this study, highest ($p < 0.05$) DPPH radical scavenging activity was exhibited by *S. crassifolium* (B1). The concentration of B1 required to scavenge 50% of the DPPH radical was 437.16 ppm ($p < 0.05$). Conversely, *Gelidiopsis variabilis* (R1) displayed the lowest DPPH radical scavenging activity, with an $IC_{50}$ value of 4421.67 ± 94.80 ppm.

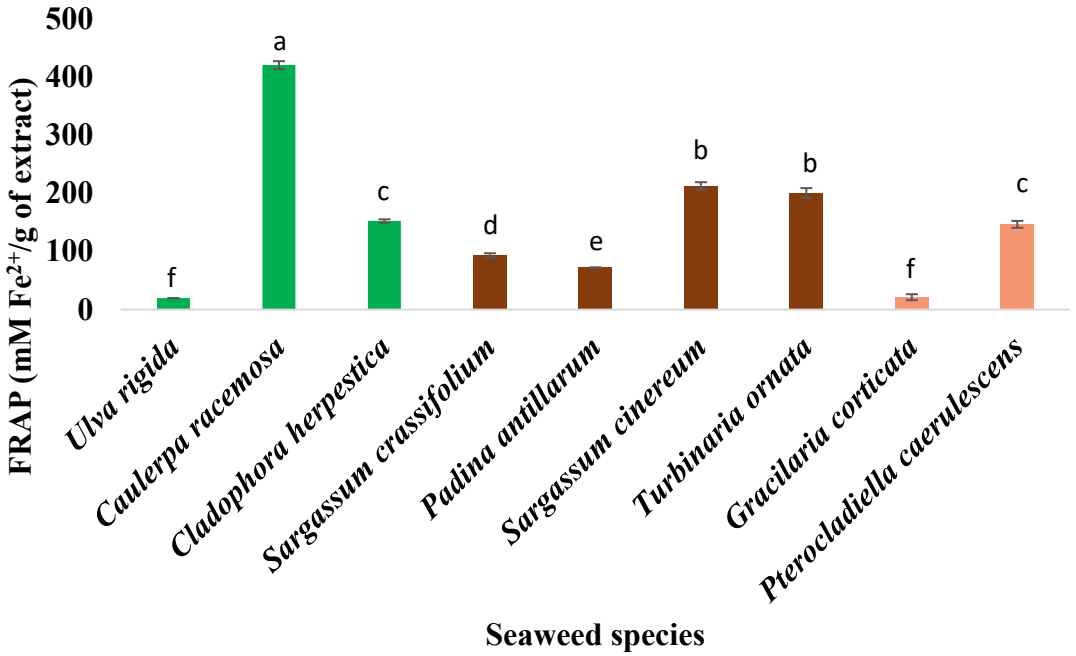


**Figure 3.** Ferric-reducing antioxidant power (FRAP) of some selected seaweed extracts. Mean values with different superscript letters were significantly different ($p < 0.05$).

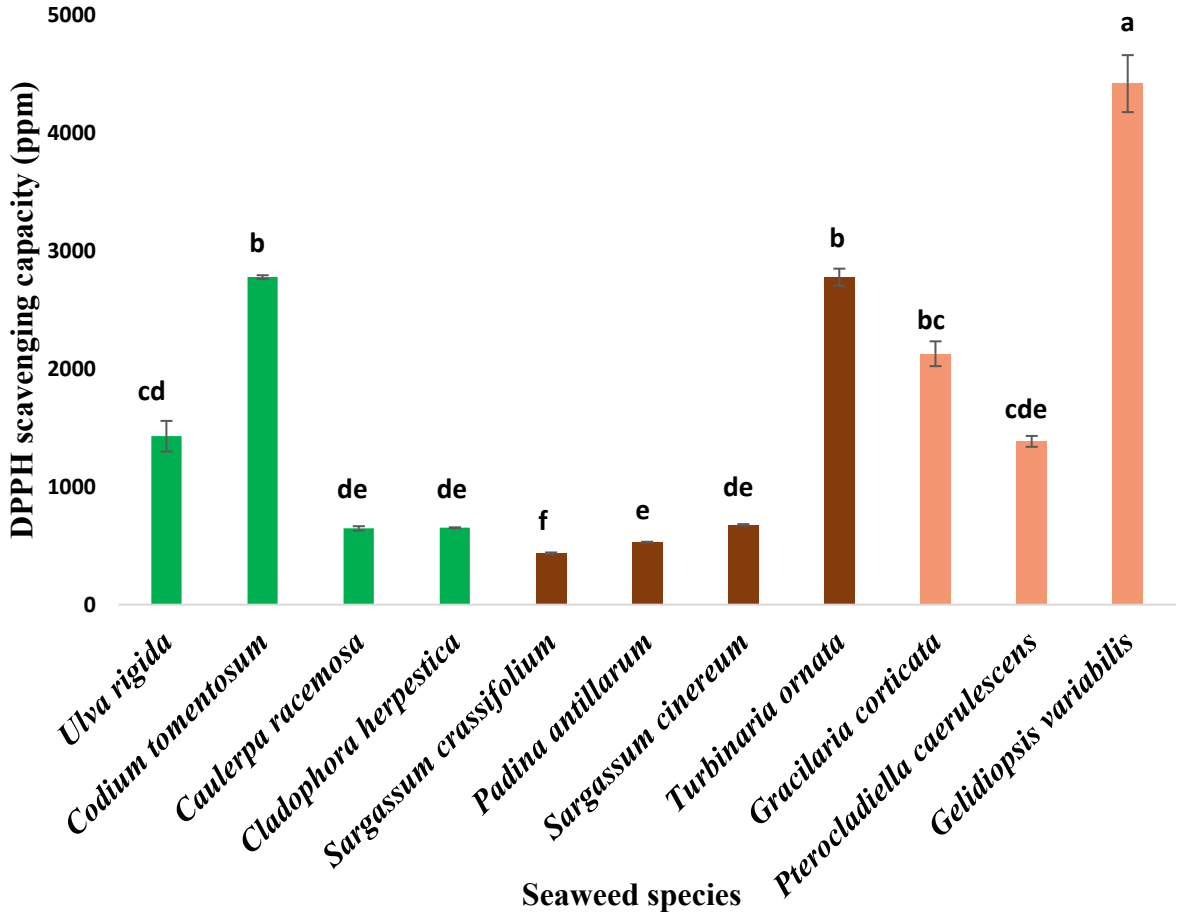


**Figure 4.** DPPH scavenging capacity of the studied seaweed extracts. Mean values with different superscript letters were significantly different ($p < 0.05$).

The ABTS radical cation is reactive towards most antioxidants, including phenolics, thiols, and vitamin C. Figure 5 displays the ABTS radical scavenging activity of selected seaweed species. *Gracilaria corticata* (R3) exhibited the highest ABTS radical scavenging activity at 1016.50 ± 2.74 mM TE/g of the crude ethanol extract ($p < 0.05$). Conversely, the lowest scavenging activity was observed in the brown algae, *T. ornate* (B3), with a value of 657.40 ± 48.97 mM TE/g of the crude ethanol extract.

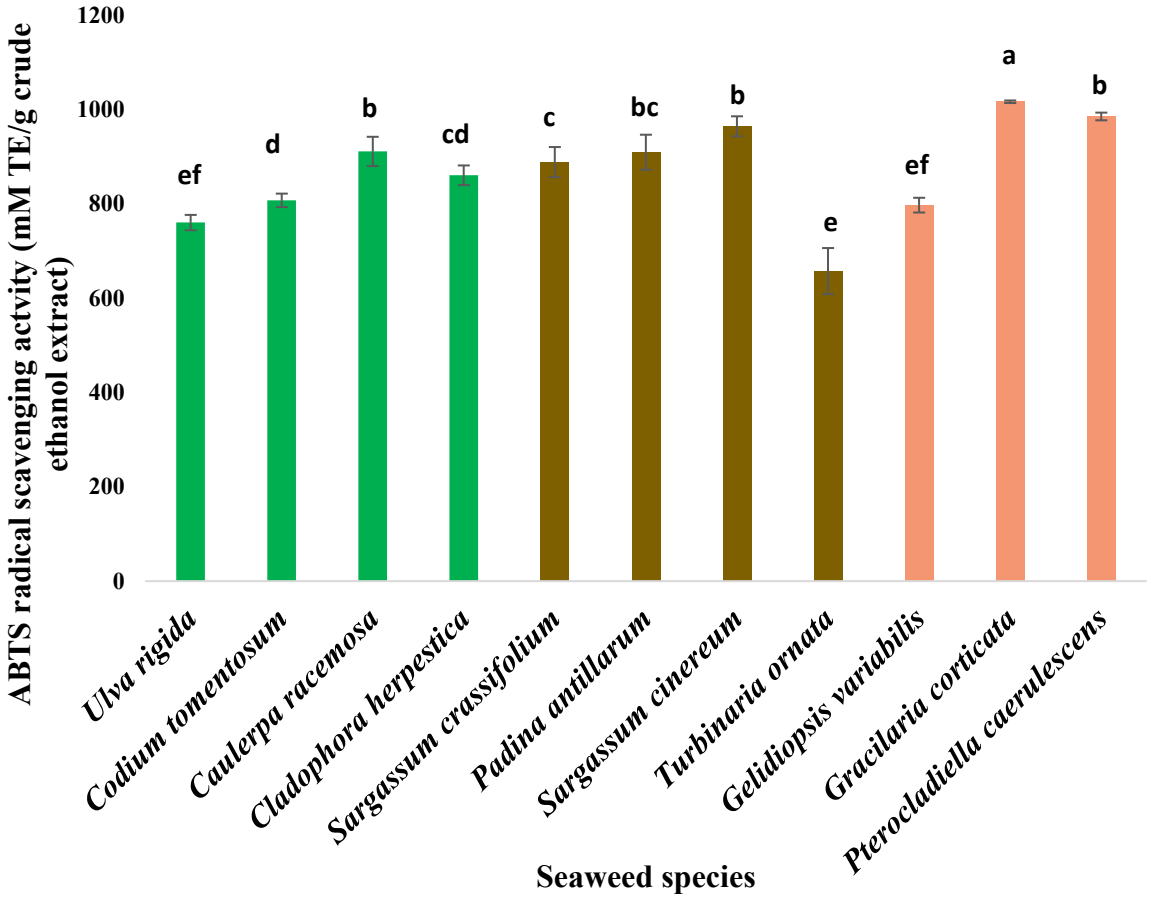


**Figure 5.** ABTS radical scavenging activity of the studied seaweed extracts. Mean values with different superscript letters were significantly different ($p < 0.05$).

### *3.3. Trace Elements and Heavy Metal Analysis*

The different types of seaweed have the capability to accumulate minerals and essential elements from their surrounding environment, including the effective accumulation of arsenic and other heavy metals. The type and concentration of metals that accumulate vary depending on factors such as the seaweed species, collection time, growth phase, and location [44]. Furthermore, metal accumulation in seaweeds is influenced by environmental factors such as wave exposure, temperature, salinity, light, pH, and nitrogen availability [21]. Among these factors, two of the most significant ones in metal accumulation are the bioavailability of metals in the surrounding water and the algae's capacity to uptake these metals [3]. The concentrations of trace elements, including Copper (Cu), Cobalt (Co), Nickel (Ni), Manganese (Mn), Chromium (Cr), Aluminium (Al), Lead (Pb), Arsenic (As), and Cadmium (Cd), were analyzed in eight seaweed species, and the results are presented in Table 3. Essential elements such as Cu, Co, Ni, Mn, and Cr are known to play roles in maintaining biochemical and physiological functions in living organisms. In contrast, Al, Pb, As, and Cd are considered non-essential elements or heavy metals and are recognized for their toxicity, even at low concentrations [45].

**Table 3.** Trace element and heavy metal content in the studied seaweeds (mg/kg wet weight).

| Seaweed Type | Trace Metals | | | | | | Heavy Metals | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cu | Co | Ni | Mn | Cr | Al | Pb | As | Cd |
| *P. caerulescens* | $0.48 \pm 0.15^{c}$ | $0.30 \pm 0.00^{e}$ | $0.95 \pm 0.02^{c,d}$ | $14.29 \pm 0.1^{d}$ | $0.55 \pm 0.02^{e}$ | $123.74 \pm 3.87^{h}$ | $0.19 \pm 0.03^{d}$ | $6.08 \pm 0.04^{f}$ | $2.76 \pm 0.03^{b}$ |
| *G. corticata* | $1.85 \pm 0.19^{c}$ | $0.37 \pm 0.02^{d}$ | $0.95 \pm 0.05^{c,d}$ | $53.41 \pm 1.32^{a}$ | $1.17 \pm 0.12^{d}$ | $246.69 \pm 5.74^{d}$ | $0.32 \pm 0.04^{c}$ | $7.33 \pm 0.2^{e}$ | $3.67 \pm 0.08^{a}$ |
| *C. racemosa* | $1.95 \pm 0.07^{c}$ | $0.66 \pm 0.01^{b}$ | $5.88 \pm 0.79^{a}$ | $34.58 \pm 0.18^{c}$ | $4.17 \pm 0.12^{a}$ | $453.29 \pm 9.15^{c}$ | $0.58 \pm 0.03^{b}$ | $5.90 \pm 0.01^{f}$ | $3.66 \pm 0.02^{a}$ |
| *U. rigida* | $1.574 \pm 0.05^{c}$ | $0.23 \pm 0.01^{f}$ | $2.76 \pm 0.048^{b}$ | $9.74 \pm 0.176^{e}$ | $2.23 \pm 0.159^{b}$ | $180.88 \pm 2.28^{f}$ | $0.374 \pm 0.025^{c}$ | $2.35 \pm 0.027^{g}$ | $0.99 \pm 0.006^{e}$ |
| *C. tomentosum* | $0.50 \pm 0.08^{c}$ | $0.16 \pm 0.00^{g}$ | $1.43 \pm 0.02^{c,d}$ | $9.70 \pm 0.20^{e}$ | $1.93 \pm 0.09^{c}$ | $214.59 \pm 7.06^{e}$ | $0.35 \pm 0.02^{c}$ | $18.50 \pm 0.34^{d}$ | $0.59 \pm 0.01^{f}$ |
| *S. crassifolium* | $0.25 \pm 0.04^{c}$ | $0.25 \pm 0.00^{f}$ | $0.70 \pm 0.01^{d}$ | $7.54 \pm 0.07^{f}$ | $0.68 \pm 0.02^{e}$ | $152.93 \pm 2.98^{g}$ | $0.14 \pm 0.01^{d}$ | $36.91 \pm 0.13^{a}$ | $2.13 \pm 0.01^{c}$ |
| *S. cinereum* | $442.34 \pm 20.94^{a}$ | $1.57 \pm 0.04^{a}$ | $1.6 \pm 0.03^{c}$ | $49.3 \pm 0.58^{b}$ | $1.9 \pm 0.04^{c}$ | $562.13 \pm 6.47^{a}$ | $1.21 \pm 0.07^{a}$ | $30.99 \pm 0.3^{c}$ | $1.34 \pm 0.02^{d}$ |
| *T. ornata* | $37.77 \pm 2.47^{b}$ | $0.54 \pm 0.01^{c}$ | $0.99 \pm 0.06^{c,d}$ | $9.14 \pm 0.09^{e}$ | $1.68 \pm 0.13^{c}$ | $477.43 \pm 4.49^{b}$ | $0.33 \pm 0.01^{c}$ | $33.66 \pm 0.07^{b}$ | $1.04 \pm 0.01^{e}$ |

Values are expressed as mean ± S.D (Standard deviation); Values with different alphabet letters are significantly different ($p < 0.05$).

Among the analyzed seaweeds, significantly elevated concentrations ($p < 0.05$) of Cu, Co, Al, and Pb were observed in brown algae, specifically *Sargassum cinereum* (B2). Among the elements examined, Al demonstrated the highest accumulation, with concentrations ranging from 123.74 ± 3.87 to 562.13 ± 2.98 mg/kg and agreed with the findings of previous studies done in Italy and India. Brown algae, *Sargassum cinereum* (B2), exhibited the highest ($p < 0.05$) concentration of Al, while red algae, *Pterocladiella caerulescens* (R2), had the lowest ($p < 0.05$). It has been reported in previous studies that brown seaweed samples collected from urban industrialized areas tend to contain high levels of aluminum [46]. Likewise, it can be inferred that pollution in Sri Lankan coastal areas may have contributed to the accumulation of elevated levels of Al.

The results reveal that the highest ($p < 0.05$) amount of Pb was accumulated in brown algae, specifically *S. cinereum* (B2) (1.21 ± 0.07 mg/kg), while the lowest amount of Pb was found in another brown algae, *S. crassifolium* (B1) (0.14 ± 0.01 mg/kg). In terms of As content, the highest ($p < 0.05$) amount was detected in brown algae *S. crassifolium* (B1) (36.91 ± 0.13 mg/kg), while the lowest ($p < 0.05$) amount was observed in green algae, specifically *U. rigida* (G2) (2.349 ± 0.027 mg/kg). The study also indicated that higher ($p < 0.05$) levels of As were accumulated in brown algae compared to other seaweed classes. Regarding Cd, the highest levels ($p < 0.05$) were found in red algae, *G. corticata* (R3) (3.67 ± 0.08 mg/kg) and green algae, *Caulerpa racemosa*(G1) (3.66 ± 0.02 mg/kg), while the lowest levels were recorded in green algae, *Codium tomentosum* (G3) (0.59 ± 0.01 mg/kg).

According to WHO standards, the maximum allowable levels of heavy metals, including Pb, As, and Cd, in food and drugs are 10 mg/kg, 1.0 mg/kg, and 0.3 mg/kg, respectively [29]. In comparison with WHO data, the Pb levels in the analyzed seaweeds are within safe limits for human consumption. However, the amounts of As and Cd are notably higher than the WHO-recommended levels. These results suggest that the consumption of seaweeds may pose a health risk to humans [47]. Furthermore, the average concentration of Cr, Mn, Ni, and Zn in all investigated seaweed species exceeded WHO standard levels [48].

Another significant observation from the study is that brown seaweed tends to contain higher concentrations of metals such as Cu, Co, Al, Pb, and As compared to other seaweed classes. Similar observations have been reported by several other studies [45,47]. The reason for such accumulation in brown algae is attributed to the algal polysaccharide called fucoidan, which has the capacity to concentrate metals from the surrounding environment due to its metal-binding properties. Additionally, alginic acid in the cell walls of brown seaweed plays a role in trace metal uptake through non-regulated ion-exchange processes [47].

## 4. Conclusions

This study investigated the nutritional properties, antioxidant activities, and accumulation of trace elements in selected seaweed species from Sri Lanka. Among the species examined, *Gracilaria corticata* exhibited the highest ash and crude fiber content. *Ulva rigida*, a green alga, had the highest protein content. Crude fat content was generally low in all the investigated seaweed species, with *Cladophora herpestica* displaying the highest fat content. *Gracilaria corticata*, *Sargassum crassifolium*, *Caulerpa racemosa*, and *Sargassum cinereum* demon-

strated the highest ABTS and DPPH radical scavenging activity, FRAP and TPC content, respectively.

The findings of the study indicate that the average concentrations of metals present in the seaweeds exceeded the recommended levels set by the World Health Organization (WHO). Specifically, the accumulation of aluminum was significantly higher compared to other trace metals. The concentrations of arsenic and cadmium, both heavy metals, also exceeded the recommended limits set by the WHO. While the analyzed seaweeds displayed acceptable nutritional content and antioxidant activity, the presence of heavy metals poses a potential risk to consumer health. Furthermore, any food products developed using seaweeds with accumulated heavy metals may have lower nutritional quality. Further studies are recommended to explore additional nutritional aspects such as fatty acid composition, amino acids, and vitamins. Additionally, the development of processing methods to remove harmful metals, extract nutritional and bioactive compounds, and produce high-quality food products from seaweeds is encouraged.

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