Comparative analysis of proximate compositions, mineral and functional chemical groups of 15 different seaweed species

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Seaweed is a popular edible source and is associated with many foods and pharmaceutical industries around the world. The current research aims to provide information on the chemical composition of 15 seaweed species, consisted of Chlorophyta, Ochrophyta/Phaeophyceae, and Rhodophyta macroalgae, collected from coastal areas of Sri Lanka. Seaweed samples were subjected to the analysis of lipids, proteins, ash and macro, micro, trace and ultra-trace elements. The highest protein content was recorded in the brown algae. Maximum dietary fiber and ash contents were recorded from green algae. The highest predominant fatty acids were observed from green seaweeds (Caulerpa racemosa); however, linoleic acid (C18:2n6) is the dominant fatty acid of all macroalgae. Mineral contents were highest in the red macroalgae; however, copper, zinc and magnesium were also comparatively higher in green alga Ulva lactuca. In conclusion, 15 seaweed species belonging to the three different classes of seaweeds are investigated in details to obtain their biochemical, mineral and fatty acid compositions for the synthesis of novel therapeutic agents. In order to explore biorefinery processes for these seaweeds, as well as how they can potentially be cultivated, more extensive studies are required. Studying and determining the nutritional values of seaweeds will be beneficial with the potentials for future industrial uses and research.

Seaweeds are classified into three taxonomic groups Chlorophyta (green algae), Ochrophyta (Phaeophyceae; brown algae) and Rhodophyta (red algae), mainly based on their pigmentation and morphological features1. Conventionally, seaweeds have been used as medications, valuable food commodities, fertilizer supplements and animal feeds2. They have been used as edible commodities due to their ability to reduce the risks of many non-communicable diseases3. Further, studies by Honkanen4 demonstrated enormous health benefits of fresh or dried seaweeds. Seaweeds have been known to contain bioactive compounds5 that are derived from sulfated polysaccharides, polyphenols, carotenoids, proteins and lipids6. Seaweeds have demonstrated anti-inflammatory7, wound healing8, anti-cancer9, anti-diabetic activities, and anti-degenerative activities6. Isolated compounds of seaweeds have been subjected to clinical trials to investigate their potential drug abilities in the field of oncology11. Green, brown and red algae are known for their antiviral, anthelmintic, antibacterial and antifungal activities12. Moreover, pharmaceutical preparations of seaweeds have been introduced to the market as a result of recent investigations13. Polyunsaturated fatty acids (PUFAs) regulate a wide range of functions, including inflammatory responses14, blood pressure, blood clotting, brain development15, and nervous system regulation16. It has been observed that fish oil is the major source of n-3 and n-6 long-chain PUFAs, such as arachidonic acid, EPA, and DHA. These

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Seaweeds also contain many essential fatty acids. Red and brown algae, for instance, are good potential sources of polysaccharides and dietary fibres, lipids, proteins, essential amino acids and vitamins. A, B, C, and E20. Seaweeds also contain many essential fatty acids. Red and brown algae, for instance, are good potential sources of polysaccharides and dietary fibres, lipids, proteins, essential amino acids and vitamins. A, B, C, and E20. Seaweeds also contain many essential fatty acids. Red and brown algae, for instance, are good potential sources of polysaccharides and dietary fibres, lipids, proteins, essential amino acids and vitamins. A, B, C, and E20.

Materials and method

Table 1. Name of seaweed species, collected location and voucher numbers.

| Type and voucher number | Species name     | Location          | GPS point            |
|-------------------------|------------------|-------------------|----------------------|
| **Chlorophyta**         |                  |                   |                      |
| G1                      | Ulva lactuca     | Thalpe            | N 05°59.792'E 080°16.898' |
| G2                      | Caulerpa racemose| Ahangama          | N 05°58.006'E 080°22.482' |
| G3                      | Halimeda opuntia | Kankasanthurai    | N 09°48.592'E 080°02.546' |
| G4                      | Caulerpa racemose| Point Pedro       | N 09°49.501'E 080°15.119' |
| G5                      | Caulerpa sertularioides | Kankasanthurai | N 09°48.592'E 080°02.546' |
| G6                      | Ulva lactuca     | Negombo           | N 07°12.170'E 079°48.570' |
| G7                      | Chaetomorpha antennina | Chilaw       | N 07°36.220'E 079°47.120' |
| G8                      | Chaetomorpha crassa | Chilaw        | N 07°36.220'E 079°47.120' |
| **Phaeophyta**          |                  |                   |                      |
| B1                      | Padina antillarum| Ahangama          | N 05°58.006'E 080°22.482' |
| B2                      | Sargassum ilicifolium| Thalpe       | N 05°59.792'E 080°16.898' |
| B3                      | Sargassum polycystum| Point Pedro     | N 09°49.501'E 080°15.119' |
| B4                      | Turbinaria ornate | Kankasanthurai    | N 09°48.592'E 080°02.546' |
| B5                      | Stochoespernum polyposoides | Point Pedro | N 09°49.401'E 080°14.593' |
| B6                      | Sargassum ilicifolium| Kankasanthurai | N 09°48.592'E 080°02.546' |
| B7                      | Sargassum ilicifolium| Negombo         | N 07°12.170'E 079°48.570' |
| B8                      | Padina antillarum| Negombo           | N 07°12.170'E 079°48.570' |
| B9                      | Sargassum ilicifolium| Ahangama      | N 05°58.006'E 080°22.482' |
| **Rhodophyta**          |                  |                   |                      |
| R1                      | Gracilaria corticata | Ahangama        | N 05°58.006'E 080°22.482' |
| R2                      | Gracilaria corticata | Negombo        | N 07°12.170'E 079°48.570' |
| R3                      | Acanthophora spicifera| Negombo        | N 07°12.170'E 079°48.570' |
| R4                      | Gelidiopsis variabilis | Chilaw       | N 07°36.220'E 079°47.120' |
| R5                      | Gracilaria corticata | Chilaw          | N 07°36.220'E 079°47.120' |
| R6                      | Jania adhaerens   | Chilaw            | N 07°36.220'E 079°47.120' |

There is a knowledge gap on nutritional properties and chemical composition of seaweeds originating from the coastal waters of Sri Lanka. Therefore, this study was aimed to gather such information to favour potential development of seaweed farming in the area. Furthermore, our previous study was able to establish data on the diversity and distribution of various seaweed species in Sri Lanka23. In addition, few numbers of surveys have recently been conducted to identify and map their bathometric division in the sea of Sri Lanka. The dominant seaweeds found in Sri Lanka belong to Phaeophyceae (Sargassum spp., Padina spp., Turbinaria sp., and Stochoespernum spp.), Chlorophyta (Caulerpa spp., Chaetomorpha spp., Ulva spp. and Halimeda spp.) and Rhodophyta (Gracilaria spp., Acanthophora spp., Gelidiopsis spp., and Jania spp.)33. Given the tremendous potential for the extensive culture of native seaweeds along Sri Lanka’s coastal waters, a first and crucial step would be to determine the chemical composition of them in order to ascertain their potential usage as a food and feed sources as well as their potential industrial applications based on agar, alginate, and carrageenans. Another study conducted in Sri Lanka using twenty-three seaweed species demonstrated a high rate of cell proliferation, migration, toxicity and wound healing properties when studied in the in-vitro and in-vivo experimental models34. According to the authors’ knowledge, no information regarding the chemical composition of seaweeds from Sri Lanka has been published. Based on these preliminary findings, we investigated the functional chemical groups, proximate composition, and fatty acid and mineral content of representative green, brown, and red seaweeds collected from the Sri Lankan shore and we emphasized on their prospective therapeutic uses.

Materials and method

Samples collection and preparation. Seaweed species samples, belonging to Ochrophyta/Phaeophyceae (Brown), Chlorophyta (Green) and Rhodophyta (Red), were collected from Northern, Southern and Northwestern coastal sites of Sri Lanka (Table 1, Fig. 1). The fresh seaweed samples were then washed thoroughly with...
tap water to remove all sand particles and epiphytes. Then these seaweed samples were air dried at 40 °C until consistent weights were obtained. Next, each sample was ground using an electrical grinder to prepare <0.5 mm particle size powder. After that the prepared powder samples were freeze-dried for four days and later, they were milled till they become fine powder (using water-cooled mill) and samples were stored at −20 °C until further use.

**Analytical methods.** Experiments were carried out to measure the contents of moisture, dry matter, ash, dietary fiber, lipid and protein present in the prepared samples. Each chemical assay experiment for an individual seaweed sample was performed in triplicates.

**Analysis of moisture content.** The seaweed samples were dried at 40 °C in air dryer machine until persistent weight was obtained. The moisture content was determined by oven (Victor, England) drying method at 105 °C.

**Analysis of ash content.** Dried samples that were prepared for measuring the moisture content was used for determining the total ash content. The method described by Pomeranz and Meloan was applied to quantify the crude ash content of seaweed samples. Consequently, the samples were burnt according to the protocol and ashes were kept in a muffle furnace (Gallenkamp, England) at 550 °C for 6 h until a constant weight is gained.

**Analysis of protein content.** We used the method described by Smith et al. to measure protein content of the seaweed samples. The standards were prepared using Bovine Serum Albumin (BSA) in the following steps: 1 mL of BSA stock (2 mg mL⁻¹ in water) was prepared and serial dilutions (five–eight) were made with concentrations ranging from 20 to 2000 μg mL⁻¹. Bicinchoninic Acid (BCA) working reagent (WR) was prepared by calculating the total volume of WR needed. To make WR, 50 parts of BCA reagent A was mixed with 1 part of BCA reagent B (50:1, reagent A:B) (the mixture appeared bright green). It was necessary to prepare a sample to WR

![Figure 1](https://www.esri.com/en-us/arcgis/products/arcgis-desktop/overview).
phyceae, Chlorophyta, and Rhodophyta, were undertaken to determine their potential for nutritional (feed, fatty acids by gas chromatography (GC). Free fatty acids were obtained by the fatty acids methyl esters (FAMEs) Preparation of fatty acid methyl esters. The fatty acid composition was determined as the methyl esters of extraction tube. The mixture of chloroform and methanol was then allowed to evaporate. The residue inside the tube was measured for their weights. Preparation of fatty acid methyl esters. The fatty acid composition was determined as the methyl esters of fatty acids by gas chromatography (GC). Free fatty acids were obtained by the fatty acids methyl esters (FAMEs) extraction method described by Levy et al.27 and the method modified by Premaratha et al.28. Firstly, approximately 100 mg of seaweed oil/extract was weighed into a separate glass tube and added 3 mL of 5% H₂SO₄ in methanol. Then the mixture was heated at 50 °C for 1 h in an oven and it was gently shaken for 30 s after every 15 min intervals. Once the one-hour cycle was completed, the sample was placed on a cold water/crushed ice layer. The distilled water (2 mL) and hexane (3 mL) was added to the tube, then the sample was shaken thoroughly and allowed to separate into two layers. Thereafter, 2 mL of the upper layer was drawn into a clean glass tube and was allowed to evaporate using N₂ gas. As the final step in this procedure, 500 μL of hexane was added to the tube. The tube with the solution was labelled and stored in the freezer (~20 °C) until subjected to GC analysis. Analysis of fatty acid composition. A Thermo Scientific TRACE 1300 Gas chromatography equipped with a Flame Ionization Detector (FID) and a fused silica capillary column omega (30 m × 0.25 mm internal diameter and 0.20 μm film thickness) with temperature limits in the range of 40 to 240 °C was used to analyse the FAME samples. Helium was used as carrier gas with a flow rate of 3 cm/s. The temperature was set at 250–280 °C for both injector and detector (Carrier mode. Flow control, Split mode, Splitless, column flow: 1.000 mL/min, purge flow: 5.000 mL/min, Split flow: 10.0 mL/min). Injection was performed in splitless mode with a volume of 1 μL. The values were always averaged over at least three injections of each duplicate extract. The individual fatty acids’ concentrations were calculated and expressed as mass percentages of total identified fatty acids. Peaks of the gas chromatogram are proportional to fatty acid quantities and total fatty acids. Finally, to identify and quantify fatty acids, we used FAME Mix (PUFA-2Animal source, Catalog No: 47015-U) as standards. Determination of macro, trace and ultra-trace elements. Total of seventeen minerals (Co, Mg, Cr, Ni, Cd, Cu, Mn, Fe, Zn, Pb, Mo, Li, Se, Sr, Na, K and Ca) were determined in the seaweed samples using ICE 3000 series atomic absorption spectrometers (AAS), graphite furnace technique. Exactly 0.1 g of dry ash sample was weighed into a glass tube, and 10 mL concentrated nitric acid (HNO₃) was added. The mixture was allowed to stand for few hours until the content become colourless. The digested material was then filtered through Whatman (No. 40) filter paper. The collected 2 mL filtrate was serially diluted with deionized distilled water to reach 5 mL and the minerals were detected by spectrometry (iCETM 3000, Thermo Scientific). FTIR spectra acquisition. The FTIR spectra of seaweed materials were recorded using the iS50 (Nicolet) Fourier infrared spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) after milling dried samples by cryomilling. The spectra were scanned at room temperature in absorption mode at the wavelength of 400–4000 cm⁻¹, with the Omnic software version 9.2. Statistical analysis. Statistical analyses was conducted using GraphPad Prism 6 software and Data management and descriptive statistics were done using Microsoft Excel (Microsoft, Redmond WA, USA). Multiple groups were compared using either one-way or two-way analysis of variance depending on the number of variables, followed by Tukey’s multiple comparison test. Statistical significance was achieved when P < 0.05. All data were presented as mean ± standard error of mean (SEM).

Results
A comprehensive analysis of 15 different Sri Lankan seaweed species (Table 1), belonging to Ochrophyta/Phaeophyceae, Chlorophyta, and Rhodophyta, were undertaken to determine their potential for nutritional (feed, etc.) and medicinal purposes. The mean dry matter, ash, total protein, lipid and total dietary fiber percentage of seaweeds are shown in Table 2. In the present study, the maximum protein content was recorded in the brown algae while, the minimum protein content was reported from green algae (Table 3). In this study, protein content varied between 15.64 ± 2.11% in green algae, 26.69 ± 2.21% in red algae, and 24.13 ± 6.30% in brown
algae. Protein content ranged from 0.69 to 62.04%, where brown seaweeds contained more proteins, followed by the red and green seaweeds. Indeed, the protein content of brown algae species, *Sargassum ilicifolium* (B2) 28.02 ± 0.68%, and *S. ilicifolium* (B9) 30.31 ± 0.58 showed variable amount of nutritional properties according to different oceanographic terrain in Sri Lanka. High proportions of total dietary fiber were reported from all the 15 seaweed species, with the highest content variation observed in green algae, *Caulerpa racemosa*. The ash content was higher in green algae than in red algae.

### Table 2. Proximate composition values and the respective seaweed species (mean ± SE).

| Seaweed sample no. | Seaweeds species         | Moisture  | Dry matter | Ash     | Lipid   | Protein    | Total dietary fiber |
|--------------------|--------------------------|-----------|------------|---------|---------|------------|---------------------|
| Chlorophyta        |                          |           |            |         |         |            |                     |
| G1                 | Ulva lactuca             | 89.67 ± 0.64 | 9.51 ± 0.06 | 0.14 ± 0.04 | 1.37 ± 0.04 | 0.69 ± 0.23 | 81.59 ± 0.40        |
| G2                 | Caulerpa racemosa        | 98.08 ± 0.35 | 2.25 ± 0.03 | 0.41 ± 0.35 | 3.73 ± 0.19 | 0.77 ± 0.34 | 81.70 ± 0.91        |
| G3                 | Halimeda opuntina        | 98.92 ± 0.34 | 0.73 ± 0.02 | 47.36 ± 0.57 | 0.03 ± 0.001 | 16.72 ± 0.40 | 35.72 ± 0.55        |
| G4                 | Caulerpa racemosa        | 95.19 ± 0.25 | 5.14 ± 0.06 | 20.72 ± 0.67 | 6.70 ± 0.11 | 23.78 ± 0.28 | 44.63 ± 0.61        |
| G5                 | Caulerpa sertularioides  | 97.06 ± 0.40 | 3.40 ± 0.12 | 24.57 ± 0.50 | 1.63 ± 0.21 | 22.05 ± 0.55 | 48.46 ± 0.41        |
| G6                 | Ulva lactuca             | 96.64 ± 0.02 | 3.37 ± 0.02 | 10.25 ± 0.17 | 1.48 ± 0.05 | 16.30 ± 0.80 | 68.63 ± 0.41        |
| G7                 | Chaetomorpha antennina   | 94.08 ± 0.02 | 5.86 ± 0.06 | 42.29 ± 0.69 | 1.58 ± 0.17 | 18.14 ± 0.70 | 32.62 ± 0.69        |
| G8                 | Chaetomorpha crassa      | 96.06 ± 0.53 | 3.03 ± 0.02 | 32.50 ± 0.14 | 1.27 ± 0.04 | 12.49 ± 0.18 | 52.70 ± 0.67        |

| Pheophyta          |                          |           |            |         |         |            |                     |
| R1                 | Padina antillarum        | 96.97 ± 0.34 | 3.25 ± 0.06 | 0.26 ± 0.16 | 4.25 ± 0.10 | 19.66 ± 0.30 | 67.59 ± 0.48        |
| R2                 | Sargassum ilicifolium    | 95.92 ± 0.37 | 4.34 ± 0.05 | 13.15 ± 0.41 | 4.45 ± 0.12 | 28.02 ± 0.68 | 51.46 ± 0.53        |
| R3                 | Sargassum polycystum     | 92.58 ± 0.32 | 7.58 ± 0.11 | 18.48 ± 0.21 | 4.50 ± 0.21 | 16.15 ± 0.33 | 54.49 ± 0.95        |
| R4                 | Turbinaria ornate        | 95.74 ± 0.41 | 3.79 ± 0.07 | 0.85 ± 0.20 | 3.33 ± 0.08 | 23.54 ± 0.53 | 62.04 ± 0.58        |
| R5                 | Stockospermum polypodioides | 92.21 ± 0.01 | 7.22 ± 0.03 | 10.21 ± 0.47 | 5.63 ± 0.16 | 08.02 ± 0.26 | 68.63 ± 0.61        |
| R6                 | Sargassum ilicifolium    | 96.17 ± 0.59 | 3.76 ± 0.06 | 0.33 ± 0.28 | 2.51 ± 0.04 | 43.87 ± 0.37 | 45.32 ± 0.42        |
| R7                 | Sargassum ilicifolium    | 94.40 ± 0.64 | 6.18 ± 0.02 | 11.06 ± 0.96 | 1.54 ± 0.04 | 22.89 ± 0.33 | 58.92 ± 0.95        |
| R8                 | Padina antillarum        | 96.04 ± 0.58 | 5.07 ± 0.04 | 41.83 ± 0.26 | 2.35 ± 0.03 | 24.83 ± 0.40 | 66.64 ± 0.49        |
| R9                 | Sargassum ilicifolium    | 96.07 ± 0.54 | 4.45 ± 0.05 | 08.43 ± 0.13 | 3.30 ± 0.09 | 30.31 ± 0.58 | 55.29 ± 0.59        |

| Rhodophyta         |                          |           |            |         |         |            |                     |
| R1                 | Gracilaria corticata     | 95.30 ± 1.22 | 4.10 ± 0.04 | 0.17 ± 0.49 | 1.80 ± 0.04 | 26.08 ± 0.64 | 61.59 ± 0.72        |
| R2                 | Gracilaria corticata     | 94.82 ± 0.89 | 5.51 ± 0.02 | 21.98 ± 0.23 | 2.34 ± 0.17 | 16.09 ± 0.30 | 54.65 ± 0.58        |
| R3                 | Acanthophora spicifera   | 95.02 ± 0.54 | 4.87 ± 0.03 | 13.32 ± 0.01 | 3.49 ± 0.13 | 28.89 ± 0.35 | 48.83 ± 0.91        |
| R4                 | Gelidiosps variabilis    | 94.52 ± 0.72 | 5.09 ± 0.05 | 21.64 ± 0.03 | 2.13 ± 0.04 | 30.90 ± 0.23 | 40.42 ± 0.60        |
| R5                 | Gracilaria corticata     | 96.32 ± 0.02 | 3.57 ± 0.05 | 07.15 ± 0.01 | 1.66 ± 0.18 | 28.70 ± 0.46 | 59.15 ± 0.76        |
| R6                 | Jania adhaerens         | 92.96 ± 0.27 | 7.21 ± 0.02 | 05.01 ± 0.01 | 1.52 ± 0.08 | 29.47 ± 0.15 | 56.81 ± 0.38        |

### Table 3. Proximate composition values and the respective seaweed groups (mean ± SE). Data are expressed as values: mean ± SE of three replicates and analysed by one-way analysis of variance. a = when compared with green algae group, b = when compared with brown algae group, c = when compared with red algae group, (*) indicates statistically significant difference from respective group using ANOVA, followed by Tukey comparisons test (p > 0.05). (†) indicates statistically no significant difference from respective group using ANOVA, followed by Tukey comparisons test (p > 0.05).

| Chemical composition % | Green seaweed | Brown seaweed | Red seaweed |
|-------------------------|--------------|---------------|-------------|
| Moisture                | 95.71 ± 1.02 | 95.12 ± 0.56** | 94.82 ± 0.45 |
| Dry matter              | 0.16 ± 0.95  | 0.05 ± 0.55    | 0.06 ± 0.51  |
| Ash                     | 23.06 ± 5.98** | 13.59 ± 3.78  | 12.88 ± 3.04 |
| Lipid                   | 0.22 ± 0.73  | 3.54 ± 0.43    | 0.21 ± 0.29  |
| Protein                 | 15.64 ± 2.11** | 24.13 ± 6.30  | 26.69 ± 2.21 |
| Total dietary fiber     | 56.13 ± 6.05 | 54.48 ± 6.58  | 53.57 ± 3.18 |
In this study, lipids were recorded to be the least available component from majority of the seaweed species. The highest and lowest lipid contents were recorded from the species of Chlorophyta: Caulerpa racemosa and Halimeda opuntia, respectively (Table 2). A total of 17 identifiable fatty acids were recorded in this study (Table 4). Palmitic acid (C16:0) was the dominant saturated fatty acid that was detected in this study. While palmitoleic acid (C16:1n7) was the major monounsaturated fatty acid, linoleic acid (C18:2n6) and homo-gamma-linolenic acid (C20:3n6) were the major polyunsaturated fatty acid (PUFA) present in Rhodophyta species. Among the PUFA, docosahexaenoic acid (DHA) (C22:6n3) was present in lowest concentration (Table 5).

Table 4. The percentage as a mean value of fatty acid composition for each seaweed species. (G1) Ulva lactuca, (G2) Caulerpa racemosa, (G3) Halimeda opuntia, (G4) Caulerpa serrulatoides, (G6) Ulva lactuca, (G7) Chaetomorpha antennina, (G8) Chaetomorpha crassa, (B1) Padina antillarum, (B2) Sargassum ilicifolium, (B3) Sargassum polycystum, (B4) Turbinaria ornata, (B5) Stoechospermum polyphytoides, (B6) Sargassum ilicifolium, (B7) Sargassum ilicifolium, (B8) Padina antillarum, (R1) Sargassum ilicifolium, (R2) Gracilaria corticata, (R3) Gracilaria corticata, (R4) Acanthophora spicifera, (R5) Gelidiopsis variabilis, (R5) Gelidiopsis corticata, (R6) Jania adhaereus.

### Table 4

| Fatty acid | Chlorophyta | Phaeophyta | Rhodophyta |
|-----------|-------------|------------|------------|
| C14:0     | 0.13        | 0.01       | 0.06       |
| C16:0     | 1.76        | 1.91       | 2.68       |
| C18:0     | 0.32        | 0.36       | 0.47       |
| C20:0     | 0.27        | 0.31       | 0.26       |
| C20:1n9   | 0.08        | 0.06       | 0.05       |
| C20:5n3   | 0.64        | 0.71       | 0.33       |
| C20:5n6   | 0.73        | 0.86       | 0.72       |
| C22:5n6   | 0.14        | 0.17       | 0.19       |
| C22:5n3   | 0.58        | 0.58       | 0.46       |
| C22:6n3   | 0.05        | 0.05       | 0.06       |

### Discussion

Our study examined the chemical composition of 15 seaweed species in terms of their possible applications in food and health products. Many useful seaweed species have insufficient scientific data especially in terms of nutritional and medicinal benefits, despite their widespread use since the prehistoric times. Furthermore, several...
Caulerpa (G1) and antennina Chaetomorpha (G1) contain the lowest amount of ash. High amount of ash was also observed in green seaweeds: protein content was recorded in red algae: contained higher protein content than the Rhodophyta and Chlorophyta seaweed species. The lowest amount of Stoechospermum edible red seaweeds ranged from 19.07 ± 0.61% to 34.00 ± 0.11%33. The intake of seaweed ash has been reported and was detected in the brown seaweeds, family Gracilariaceae, Lomentariaceae, Corallinaceae, and Rhodomelaceae. The highest amount of crude protein and Halimedaceae. Brown seaweeds belong to the family Dictyotaceae, Sargassaceae. Red seaweeds belong to the samples tested in the present study belong to the green seaweed family Ulvaceae, Cladophoraceae, Caulerpaceae, concentration, and reduces the risk of weight gain and obesity41,42. We found that green seaweeds species are rich sources of total dietary fiber: total dietary fiber (TDF) (5%), brown algae is (66.5%) and red algae is (33%)31. The nutritional compositions of 15 seaweed species tested in the present study belong to the green seaweed family Ulvaceae, Cladophoraceae, Caulerpaceae, and Hallimedaceae. Brown seaweeds belong to the family Dictyotaceae, Sargassaceae. Red seaweeds belong to the family Gracilaria, Lomentaria, Corallina, and Rhodomelaceae. The highest amount of crude protein was detected in the brown seaweeds Sargassum ilicifolium (B9) when compared to green seaweeds Ulva lactuca (G1) and Caulerpa racemosa (G2). Minimum protein content was recorded in brown seaweed species namely: Stoechospermum polyiodoidei (B5). According to the previous study, Ochrophyta/Phaeophyceae seaweed species contained higher protein content than the Rhodophyta and Chlorophyta seaweed species. The lowest amount of protein content was recorded in red algae: Gracilaria corticata32. It is important to note that the protein content of macroalgae is affected by both geographical location and seaweed species.

Present study revealed that the green alga: Halimeda opuntia (G3) yields highest ash content and Ulva lactuca (G1) contain the lowest amount of ash. High amount of ash was also observed in green seaweeds: Chaetomorpha antennina (G7), and brown seaweed: Padina antillarum (B8). A previous study showed that the ash content of edible red seaweeds ranged from 19.07 ± 0.61% to 34.00 ± 0.11%33. The intake of seaweed ash has been reported to prevent many diseases such as arthritis, fever, gout, fluid retention, bladder problems and constipation, and to improve intelligence34. Moreover, ash is used in treatment for childhood constipation35 and helps in maintaining bowel health36.

We found that green seaweeds species are rich sources of total dietary fiber: Ulva lactuca (G1) 81.59 ± 0.40%, and Caulerpa racemosa (G2) 81.70 ± 0.91%. The highest proportion of dietary fiber content has been reported in green seaweed, Caulerpa chemnitzia, which is significantly prominent when compared to dietary fiber levels of Acanthophora spicifera (Rhodophyta), Ulva intestinalis, Ulva rigida (Chlorophyta), and Sargassum wightii (Ochrophyta/Phaeophyceae)37. In a study conducted by Labayen, seaweed contained between 33 and 50% of dietary fiber38. Accordingly, the fiber content of green seaweed species is higher than those found in red and brown seaweeds. The consumption of this dietary fiber has been linked to the growth and protection of the beneficial intestinal flora39,40. Furthermore, the intake of dietary fiber helps in regulating calories and free cholesterol concentration, and reduces the risk of weight gain and obesity41,42.

Seaweeds are rich in PUFA from n3-PUFAs and n6-PUFAs series, thus, they could be an alternative valuable source of these compounds for human and animal health43,44. In this study we examined fatty acids profile by using the standard mix (Supplementary file 1). In general, a higher amount of PUFAs were reported from seaweeds compared to other groups of fatty acids including monounsaturated fatty acids (MUFA). This study showed that omega-3 polyunsaturated fatty acids was not present in some red algae species. Long-chain PUFA, fatty acid C20:5n3 and C22:6n3 were found in seaweeds. Some PUFA are interesting since they serve as the precursors for

| Fatty acids | Chlorophyta | Phaeophyta | Rhodophyta |
|------------|-------------|------------|------------|
| Total MUFA | 4.95 ± 1.38 | 3.99 ± 1.70 | 0.78 ± 0.22 |
| Total SFA  | 4.63 ± 0.55 | 12.48 ± 1.22 | 13.73 ± 1.46 |
| Total PUFA | 11.78 ± 1.96 | 20.53 ± 0.43 | 14.71 ± 0.31 |
| Total PUFAs| 11.08 ± 1.09 | 18.46 ± 0.47 | 14.37 ± 0.32 |
| Total MUFA | 4.95 ± 1.38 | 3.99 ± 1.70 | 0.78 ± 0.22 |
| Total SFA  | 4.63 ± 0.55 | 12.48 ± 1.22 | 13.73 ± 1.46 |
| Total PUFA | 11.78 ± 1.96 | 20.53 ± 0.43 | 14.71 ± 0.31 |
| Total PUFAs| 11.08 ± 1.09 | 18.46 ± 0.47 | 14.37 ± 0.32 |

Table 5. Fatty composition values and the respective Green, brown and Red seaweed group (mean ± SE) %.

– ; not available. Total saturated fatty acids (SFAs) = the sum of C8 to C20. Total mono unsaturated fatty acids (MUFA) = the amount of C18:1. Total poly unsaturated fatty acids (PUFAs) = the sum of C18:2 and C18:3. Data are expressed as values: mean ± SE and analysed by one-way analysis of variance.
Table 6. The percentage as a mean value (mg/100 g) of mineral elements composition for each seaweeds species. (G1) Ulva lactuca, (G2) Caulerpa racemosa, (G3) Halimeda opuntia, (G4) Caulerpa racemosa, (G5) Caulerpa serrulatiorides, (G6) Ulva lactuca, (G7) Chaetomorpha antennina, (G8) Chaetomorpha crassa, (B1) Padina antillarum, (B2) Sargassum silicifolium, (B3) Sargassum polycystum, (B4) Turbinaria ornata, (B5) Stoechospermum polyphodzioides, (B6) Sargassum silicifolium, (B7) Sargassum illicifolium, (B8) Padina antillarum, (B9) Sargassum illicifolium, (R1) Gracilaria corticata, (R2) Gracilaria corticata, (R3) Acanthophora spicifera, (R4) Gelidiopsis variabilis, (R5) Gracilaria corticata, (R6) Jania adhaerens.

| Minerals | Chlorophyta | Phaeophyta | Rhodophyta |
|----------|-------------|-------------|------------|
| Cu (µg/L) | 8.95 ± 6.46 | 3.56 ± 0.82 | 3.16 ± 0.57 |
| Pd (µg/L) | 2.29 ± 0.33 | 2.34 ± 0.34 | 2.47 ± 0.36 |
| Cd (µg/L) | 2.35 ± 1.20 | 1.18 ± 0.59 | 2.95 ± 2.78 |
| Cr (µg/L) | 5.02 ± 1.10 | 4.32 ± 1.15 | 3.29 ± 1.21 |
| K (µg/L) | 1254 ± 16.76 | 1256 ± 11.48 | 1267 ± 16.60 |
| Zn (µg/L) | 200.10 ± 111.10 | 50.69 ± 3.61 | 163.10 ± 109.50 |
| Mn (µg/L) | 20.87 ± 5.74 | 20.63 ± 5.91 | 15.32 ± 4.61 |
| Ni (µg/L) | 18.69 ± 2.97 | 12.86 ± 3.03 | 11.88 ± 3.75 |
| Co (µg/L) | 1.33 ± 0.94 | 0.34 ± 0.08 | 0.66 ± 0.44 |
| Fe (µg/L) | 1049 ± 182.7 | 887.40 ± 137.90 | 475.60 ± 64.47 |
| Mo (µg/L) | 52.02 ± 39.43 | 7.70 ± 0.87 | 8.93 ± 1.84 |
| Li (µg/L) | 14.43 ± 11.15 | 16.95 ± 3.28 | 17.43 ± 4.68 |
| Se (µg/L) | 5.87 ± 0.45 | 6.29 ± 0.27 | 5.39 ± 0.43 |
| Na (µg/L) | 1070 ± 428.30 | 662.20 ± 103.70 | 1272 ± 549.80 |
| Ca (µg/L) | 1893 ± 48.63 | 1887 ± 29.76 | 1793 ± 10.77 |
| Mg (µg/L) | 731.10 ± 54.31 | 780.30 ± 37.50 | 843.70 ± 36.12 |
| Sr (µg/L) | 728.70 ± 528.20 | 1186 ± 290.40 | 45.52 ± 6.54 |

Table 7. The percentage as a mean value and the respective green, brown and red seaweed group (mean ± SE) %. Data are expressed as values: mean ± SE of three replicates and analysed by one-way analysis of variance.
the biosynthesis of regulating/signalling molecules like prostaglandins, thromboxanes and other bioregulators of many cellular processes. However, there is significant difference (p < 0.05) between brown and red seaweeds in terms of SAFAs, MUFAs and PUFAs. Amounts of saturated fatty acids (SAFAs) and PUFAs were higher in red seaweeds. MUFAs were higher in green seaweeds (4.95 ± 1.38%). In contrast, green seaweeds, like Ulva lactuca (formerly Ulva pertusa) (Chlorophyta), are characterised by the presence of hexadecatetraenoic (16:4 (n-3)), oleic (C18:1) and palmitic acids (C16:0). SAFAs and MUFAs were generally low in green and red seaweeds. SAFAs were higher in Sargassum polyoidoioides, a brown seaweed (B5). MUFAs was higher in Sargassum ilicifolium (B7), and PUFAs was detected highest in red algae Acanthophora spicifera, and green algae Ulva lactuca.

The 20:4 n-6 and 20:5 n-3 fatty acids were the predominant PUFAs found in red algae and hexadecatetraenoic acid is prominent in Ulva sp. Acanthophora spicifera (R3) contained 17.98% of PUFAs followed by Linoleic, γ-linolenic, homo-γ-linolenic and docosapentaenoic acid (DPA).

Fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are known to prevent cardiovascular diseases and inflammation caused by many chronic diseases. Brown seaweeds contain higher levels of beneficial PUFAs, especially DHA and EPA. Furthermore, seaweeds contain a favorable ratio of omega-6 to omega-3 fatty acids. The red seaweeds contain 0.34 ± 0.14% n3-PUFAs and 14.37 ± 0.32% n6-PUFAs and the ratio of ω-3/ω-6 was 0.023%. Higher accumulation of n3-PUFAs was observed in brown seaweeds (02.07 ± 0.62%) and that of n6-PUFAs was recorded in red seaweeds. The ω-3/ω-6 ratio is a good index for comparing the relative nutritional values of seaweed oils of different species, and a higher ratio of n-3/n-6 PUFAs has often been quoted as an index of higher nutritional value. Although many omega-3 fatty acids occur in nature, DHA and EPA are not synthesized by humans at a rate that can meet our metabolic needs, making a dietary source necessary. Differences in fatty acids of marine seaweeds should only be considered with respect to species habitat. The difference in fatty acids composition varies depending upon the environmental conditions, especially water temperature. Most of the seaweed varieties showed differences in their strain, appearance, geographical distribution and nutrient content.

Seaweeds are known as an excellent source of essential mineral, especially Na and Ca. Minerals have a major role in synthesising hormones and enzymes. Trace elements such as Iron (Fe), Copper (Cu), Cobalt (Co), Nickel (Ni), Zinc (Zn), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Chromium (Cr), Lithium (Li), Selenium (Se), Fluorine (F) and Iodine (I) play an important role in healing and prevention of many diseases. Sodium (Na), Calcium (Ca), Potassium (K) and Magnesium (Mg) are among the minerals which are present in significant amounts in marine algae. Mineral concentrations such as Cu, Zn and Co were higher in the green

Figure 2. FT-IR spectra of raw seaweeds samples: (A) Chlorophyta, (B) Phaeophyta, (C) Rhodophyta. (a) C=O stretching (1650–1600 cm⁻¹), (b) C–H bending (1465–1450 cm⁻¹), (c) N–O stretching (1550–1500 cm⁻¹), (d) O–H bending; carboxylic acid (1440–1395 cm⁻¹), (e) C–O stretching (1275–1200 cm⁻¹), (f) S=O stretching (1070–1030 cm⁻¹), (g) anomeric region (950–700 cm⁻¹), (h) C–O–C bridge in 3,6-anhydro-l-galactose (930 cm⁻¹). (G1) Ulva lactuca, (G2) Caulerpa racemosa, (G3) Halimeda opuntia, (G4) Caulerpa racemosa, (G5) Caulerpa sertularioides, (G6) Ulva lactuca, (G7) Chaetomorpha antennina, (G8) Chaetomorpha crassa, (B1) Padina antillarum, (B2) Sargassum ilicifolium, (B3) Sargassum polycystum, (B4) Turbinaria ornata, (B5) Stoechospermum polyoidoioides, (B6) Sargassum ilicifolium, (B7) Sargassum ilicifolium, (B8) Padina antillarum, (B9) Sargassum ilicifolium, (R1) Gracilaria corticata, (R2) Gracilaria corticata, (R3) Acanthophora spicifera, (R4) Gelidiopsis variabilis, (R5) Gracilaria corticata, (R6) Jania adhaetereus.
alga, Mg and Sr were high in the brown alga, and Na was found highest in the red alga. Calcium is the leading mineral to help build strong bones and healthy teeth. A higher level of Cd was found in red seaweeds when compared with other groups/classes of seaweeds. The presence of Cd could be due to exposure to human activities such as fishing and drainage system of the settlements in the coastal areas. This study explored the presence of heavy metals accumulation in red seaweeds. Heavy metals such as Lead (Pb), Arsenic (As), Cadmium (Cd) and Mercury (Hg) are toxic for health when exposed for a long time even at the lowest levels of their concentrations. According to the World Health Organization (WHO, 1989) report, the maximum allowed levels of heavy metals such as Pb, As and Cd in food and drug products are 10, 1.0 and 0.3 mg/kg, respectively. Higher level of Pd (4.45 µg/L) has been shown in the brown alga, Sargassum polycystum (B3), and Cr was high in Padina antillarum (B1), species collected from south coastal algae bed of Sri Lanka. The mineral fraction of some seaweeds has been shown up to 40% of dry matter, however mineral content of marine algae is recorded even higher than that of terrestrial plants.

The FTIR spectroscopy is a fast, cost-effective, and extremely useful technique in identifying compounds present in the crude extracts. Besides, FTIR spectra can be applied to distinguish agar and for estimating total sulphate content of carrageenans and agars. A broad band at 3257–3333 cm⁻¹ and medium signal at 2922–2928 cm⁻¹ can be assigned to O–H, N–H functional groups and C–H stretching vibrations. Absorption peak ranging between the 4000–2000 cm⁻¹ were common in all studied seaweeds and phycocolloid (carrageenans, agars and alginates). The absorption peaks at 3550–3200 cm⁻¹ (Fig. 2) indicated the presence of O–H and the absorption peaks at 3000–2840 cm⁻¹ can be attributed to C–H stretching vibrations (possibly −CH2 functional group), while 1650–1600 cm⁻¹ were attributed to the C–H bending overtone band of aromatic compounds. Bands in the region 1440–1395 cm⁻¹ corresponded to the asymmetric stretching vibration of the sulfate group O–H bending, whereas peak at 1275–1200 cm⁻¹ was assigned to the C–O stretching band related to the C–O–SO3 group. The absorption peaks at 950–700 cm⁻¹ revealed the existence of aromatic C–H bending band. One of the significant peaks was found at 930 cm⁻¹ in the spectra of red seaweeds, which is associated with the stretching vibration mode of C–O–C bridge in 3,6-anhydro-l-galactose. IR absorption bands at 2960, 2920, 2845, 1640, 1370, 1250, 930, 900, 845, 805 and 705 cm⁻¹ are used to obtain information on the structure of agars and carrageenans. The seaweed chemical composition identified by FT-IR and its vibrational spectra has allowed more accurate and an easier monitoring of chemical composition of seaweeds. This study outcomes can be applied in nutritional quality control and authentication of seaweeds.

The study shows that brown and green seaweeds are the most abundant seaweed species in the Sri Lankan coastal areas. Brown seaweed was a most common species in areas that were exposed to constant sea waves and where dissolved oxygen levels are high, whereas, the green seaweed species is the most common in deeper oceanic areas. Moreover, we observed that the most diverse seaweed communities are found close to the coast, where the depth gradient is changing their distribution patterns. The south location had a higher density of Sargassum ilicifolium species than other coastal locations. As well, a positive correlation (P < 0.05) was apparent between Sargassum spp. and other seaweeds. Both north and south coastal areas exhibit marked differences in seaweed communities. For assertive correlations to be established between these locations’ seaweed communities and their distributions, needs further studies.

There are large populations of Sargassum ilicifolium in tropical and temperate oceans, a marine macroalga of the Phaeophyceae family. It is a member of the marine genus Sargassaceae and order Fucales. Recently, there has been an increasing discovery of seaweed metabolites presenting biological activities. For examples, it has been reported that their bioactive compounds possess antibacterial, cytotoxic, cell stimulation, antitumoral, and immune-modulating properties. Sargassum ilicifolium has shown different amounts of protein when compared to other coastal species (B2, B6, B7, B7). Species of Sargassum spp (B6) collected from the north coastal area of Sri Lanka (Kankasanthurai, GPS point: N 09°48.592’ E 080°02.546’) contained the highest amount of protein. When compared with Sargassum ilicifolium species collected from different coastal areas, Sargassum ilicifolium (B7, collected from Negombo, GPS point: N 07°12.170’ E 079°48.570’) displayed the lowest amount of protein and lipid content.

As for red seaweeds, Gracilaria corticata (R1, R2) from the north and south areas have different ratio of ash, lipid, protein, and dietary fiber concentrations. Additionally, Ulva spp. showed different nutritional characteristics depending on the collection site. The results of this study indicated that nutritional properties vary depending on where they are collected. Due to their sensitivity to changes in water quality parameters, seaweed distribution patterns and abundance might change over time. Additionally, other environmental factors could influence the nutritional composition of seaweeds. A further study is highly recommended to explain how water quality parameters affect nutrition levels. According to our previous study, diluted S. ilicifolium extracts induced promising cell proliferation and migration activity against the L929 cell line. There were significant differences (p < 0.05) in cell proliferation activity between S. ilicifolium samples collected from the south algae bed and samples collected from other algae beds of Sri Lanka. The results of the previous study indicated that the cytotoxic effects were also dose-dependent. Furthermore, it was discovered that the nutritional value of seaweeds varied depending on geographical distribution. Present investigation is the first report on the biochemical profile of seaweeds in Sri Lanka, and more research studies are needed in determining the nutritional profile and toxicological issues in using seaweed as a food and feed resource for humans and animals.

Conclusions
This study is the first to highlight the nutritional significance of available seaweed species in Sri Lanka and all 15 species were found to be sources of proteins, total dietary fiber, lipids and minerals. Seaweed species belonging to Ochrophyta/Phaeophyceae was the dominant marine alga that contained highest protein. Dietary fiber and ash contents were more prominent in seaweed species of Chlorophyta. The present investigation brings
out comprehensive data on the biochemical (including fatty acids) and mineral compositions of three types of seaweeds. These baseline data will be beneficial in studying and establishing nutritional quality of seaweeds and their industrial applications. However, in vitro and in vivo therapeutic activities of most of these seaweeds and their compounds are yet to be determined. Thus, further studies would be required to determine specific phytocomponents and their health benefits.

Data availability
The datasets used and/or analysed in this study can be obtained from the corresponding author upon reasonable request.

Received: 14 June 2022; Accepted: 2 November 2022
Published online: 15 November 2022

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the authors wish to thank Miss. Krinsli Pius who is Research Laboratory Manager, and Miss. Clarisa Naa Shormeh Darke and Miss. Sanjida Humayan, in School of Natural Sciences and Health, Tàllinn University, Estonia. The authors also wish to thank Mr. K.A Wijesekera, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, and Emeritus Professor, Andrew J Sinclair, Deakin University, Australia is appreciated. Technical support was given by Mr. N.A.N.D Perera, Mr. K.B.A.T Bandara and D.P.G.S.P Jayasinghe, Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science is also appreciated.

Acknowledgements

A.D. conceived the study, concept, and design and conducted the laboratory experiments, data analyzed and interpreted experimental results and manuscript preparations. R.P.V.J., P.H.P., A.P., and R.T. contributed to the proposal for study design and supervision of the study and drafting of the proposal. T.H. and M.C.N. supported carrying out laboratory experiments. P.W., M.H. and R.A. contributed with interpretation of data and critical revision of the manuscript. All authors read and approved the final manuscript. We certify this manuscript has not been published elsewhere and is not submitted to another Journal.
Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-23609-8.

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