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Nutritional Assessment on Brown Macro algae Lobophora variegata from GOMBR, Tamil Nadu, India

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Abstract
Nutrition is very important to all living organisms including human beings which obtained from various kinds of resources. Seaweed as renewable marine natural resources to provide nutrition for maintains good health. Based on that reason, the present study was conducted to analysis nutritional profile (Proximate composition, mineral content, amino acid composition and fatty acid profile) and antioxidant potential of brown seaweed Lobophora variegata (J.V. Lamouroux) Womersley ex. Oliveira collected from the Gulf of Mannar Biosphere, Tamil Nadu, India. The proximate composition (moisture, ash, protein, lipid, carbohydrate, and dietary fiber, mineral, amino acid, fatty acid and antioxidant activity (2-dipheny- 1 – picrylhydrazyl (DPPH) and Reducing power assays) of L.variegata. The results exhibited significant profile of proximate composition, mineral content, fatty acids and amino acids recorded in the brown algae L. variegata. The brown seaweed L.variegata showed excellent antioxidant potential through DPPH scavenging and reducing power assay. According to the current findings, brown seaweed, L. variegata, appears to be a viable marine natural resource for generating innovative nutraceutical and antioxidant products.

Keywords
Macro algae, antioxidant, L. variegata, minerals and amino acids.

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1. Introduction

Seaweed is evolutionary primitive eukaryotic and multicellular photosynthetic thallus[1]. Seaweeds are grown luxuriantly in the coastal region of Tamil Nadu, Kerala, Gujarat, Lakshadweep, Andaman and Nicobar Islands in India [2]. Seaweed products are available in the form of food and medicine due to their myriad beneficial biomolecules like anti-diabetic, anti inflammation and antioxidant compounds. [3]. According to recent studies, seaweeds as nutraceuticals or functional foods which may help prevent or even cure diseases in modern society [4],[5],[6]. Now-a-days the different types of seaweeds have been cultivated in devolving countries for the purpose of various commercial applications including nutraceutical, pharmaceutical and fertilizers [7]. Due to the numerous research claims regarding richness of seaweed bioactive compounds, they are all using in health beneficial product making industries in a prolonged time [8], [9]. Marine seaweed is a rich source of multi variant micro and macro nutrients including carbohydrates, protein, vitamin, minerals and polyphenols [10]. Seaweeds generally have high nutritional value. They have low lipid content, along with richness of essential amino acids, fiber carotenes, vitamin C, vitamin B12 and polyunsaturated fatty acids [11], [12] Seaweed are nutritionally balanced and good source of essential trace elements such as Mg, Cu, Fe, Zn, macronutrients with the role of coenzyme and minerals [13]. In recent times, based on their nutritional and medicinal properties, western countries have an increasing interest in edible seaweeds as ”sea vegetables.”[14]. In 2016, 31 million tones of seaweeds were harvested for direct human consumption which amount is more than twice in 2005 [15]. Besides biological compounds brown seaweeds play a vital role in various applications of nutraceuticals and functional foods [16]. The brown algae have the highest non starch polysaccharides, while compare to red and green algae [17]. Nowadays modern society consumed seaweed product and 66.5% brown seaweed are the most consumed species followed by 33% red and 5% green seaweed [18]. Brown seaweeds are a rich source of natural bioactive compounds with potent biological and antioxidant compounds like Phlorotannins [19],b carotene, fucoxanthin, catechin, flavonoids, fucoidan [20], [21]. The genus Lobophora J. Agardh is distributed tropical and subtropical seas. Currently the genus of Lobophora contains 28 accepted species in worldwide [22]. L. variegata is the most important species of the genus. The species L. variegata contains a wide range of bioactive compounds [23]. Phaeophyta (brown algae) members are most significant group of seaweed having rich source of implicit modernized medicines. L. variegata is comes under the phaeophyta and its availability

are large in Tamil Nadu oceans. Therefore the present study has been made an attempt to assess the preliminary bioactive and nutritional potential of them. However the present study focused on nutritional composition and in vitro antioxidant of L. variegata.

2. Materials and methods

2.1 Collection of L. variegata

The brown seaweed, Lobophora variegata (J.V.Lamouroux) Womersley ex E.C.Oliveira (Fig 1) was collected from Mandapam region (09º 28 º 177. N, 79º 18 536 E) South east coast of Tamil Nadu, India. The collected algal material washed thoroughly on the spot and removes adherent debris on their surfaces. Seaweed was thoroughly washed in distilled water after dried under shade for one week. After drying, it was powdered. The powdered sample was used for nutraceutical and pharmaceutical analysis. Based on the morphological characters L. variegata was identified by using the taxonomic keys provided by [24] and [25]. They were made herbarium and a voucher specimen was maintained in Department of by Botany, Algal Laboratory (PU-BOT-SH-BS0108-2018) Periyar University Tamil Nadu.

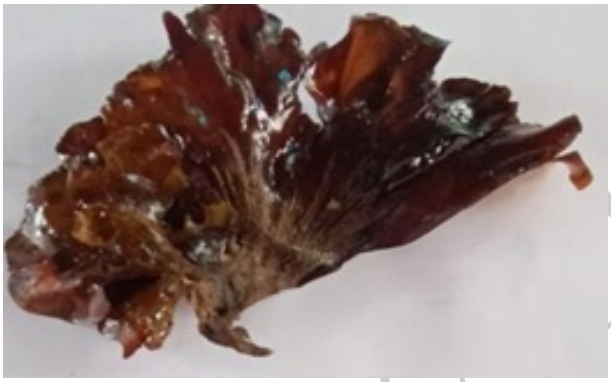

Figure 1. Morphology of L. variegata

2.2 Proximate analysis

The proximate composition of L. variegata was determined by [26] and [27]. The moisture and ash content was determined by using gravimetric techniques according to [28]. Assessment of crud lipid was done by method of [29]. The concentration of nitrogen of protein content (N × 6.25) was established by the Kjeldahl method, [30]. The carbohydrate content (%) was estimated by [31]. Dietary fiber (soluble and insoluble fiber) determined by [32]

2.3 Determination of Minerals

Minerals content of L. variegata was analyzed by using Inductively Coupled Argon Plasma (ICAP200). The mineral value was expressed in mg/100g dry weight basis of sample.

2.4 Fatty acids composition of seaweed

Gas Chromatography Mass Spectrometry (GC-MS) was used to evaluate, the fatty acid profiles of L. variegata. The fatty acid methyl ester (FAMEs) was prepared using boron trifluoride/methanol [33]. The methyl esters extracted from the algal powder were detected using a gas chromatograph equipped with a mass spectrometer (Make-Thermo Fischer, Model- TQ MS System). By experimenting with the following conditions: 30 m DB-WAX capillary column (internal diameter 0.025 cm, film thickness 0.2 m; Agilent Technologies, USA). Mass spectra of substances were identified and compared to NIST mass spectral libraries using the mass spectra derived from their associated chromatographic peaks.

2.5 Amino acid profile of seaweed

The marine brown algae L. variegata dried powder (0.5 mg) were taken for 10 mL /3 min LC-MS ultra pure water before being sonified in an ultrasonic bath for 15 minutes. To extract were centrifuged at 11000 rpm for 15 minutes at 4°C. Further hydrolyzed with 2N HCL and neutralized with 2N NaOH.

2.6 Liquid Chromatography (LC)

The amino acid composition was quantified by liquid chromatography using Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Mass Spectrometer system. The chromatographic separation was performed by using an AAA-MS column, 3m, 250 x 2.0 mm, and a flow rate of 0.25 mL min-1. Separation was carried out in reverse phase. The column was maintained at a temperature of 35°C. The mobile phase was composed of water and methanol (A/B) gradient containing both 10 mM of Ammonium formate. A sample was examined using the chromatographic conditions described before, with a sample volume of 10µl injected into the system.

2.7 Mass spectrometry

The analysis was performed using a mass spectrometer equipped with ultra-fast polarity switching and MRM transitions. Nitrogen was used as both the drying and nebulizing gas. It was produced from compressed air using a N2 LC-MS pump operating at rates of 15 and 3 µL min-1, respectively. At a pressure of 230 kPa, the collision induced dissociation gas (CID) was 99.99 % argon. Data processing and analysis were performed using X calibre software.

2.8 Preparation of seaweed extracts

The dried seaweed samples were ground and extracted for 5 hours using the Soxhlet equipment with 15 g of L.variegata were pulverized and extracted with 150 ml of methanol solvent. The extract collected and then dried through rotary vapor. The seaweed sample was lyophilized and kept at temperature of -20ºc for future tests using a freeze drier.

2.9 In Vitro Antioxidant Activity

2.10 DPPH (2, 2-dipheny- 1 – picrylhydrazyl) radical scavenging assay

DPPH-free radical-scavenging activity was assessed by [34]. About 3 mL of a 0.1 mM of methanolic solution of DPPH was added to the aliquots of samples and vortexes well. And

tubes were kept by dark 30 min. The absorbance was measured at 517 nm using UV –Vis Spectrophotometer (UV-1800, Shimudzu, Torrance,CA). As a positive control were ascorbic acid was utilized (Merck Life Sciences, India). DPPH radical activity was calculated by Scavenging effect (percentage) = [1(Acontrol - A sample)/Acontrol] X100.

2.11 Reducing Scavenging Assay

The reducing scavenging assay of crude methanol extract (25–200 g ml-1) was carried out by [35]. Vitamin C used as a standard. 1.25 ml of 0.2 M phosphate buffer adjusted to pH 6.6 and 1.25 ml of 1 percent K3[Fe(CN)6] were added to the sample. In additional, the mixture was kept in a water bath for 20 minutes at 50°C. After the completion of reaction, 10% C2HCl3O2 and 1% FeCl3.6H2O were added. After that, the mixture was cooled for 10 min at room temperature. At 700 nm, the absorbance was measured.

2.12 Statistical analysis

All analysis experiments were triplicates. The results were described as mean ± SD assays which were subjected to P¡0.05 one way variance considered significant differences.

3. Results and Discussion

3.1 Proximate composition of L. variegata

In the present investigation the results of nutritional composition of L. variegata are presented in Table 1. The proximate composition includes carbohydrates, proteins, lipids, total dietary fiber (Tdf), soluble and insoluble fiber, moisture and ash content were determined and the values are expressed in g/100g/DW. The derivative organic compound of carbohydrate of powder sample was found 28.81 ± 0.08 (g/100g/DW). In compared to other forages, carbohydrates of brown seaweeds such as Padina gymnospora (26.9% DW) and Sargassum ilicifolium (32.9% DW) [36]. The similar results were observed in Sargassum ilicifolium (27.33% DW) and Sargassum polycystum (25.0) [37]. The protein content is 34.2 ± 1.06 g/100g in L. variegata. The result was similar to Undaria pinnatifida (21 g/100 g) [38] and Turbinaria ornata 4.85% studied by [39]. In previous study of in the species reported that maximum protein content of 23.13 % of Lobophora variegata collected from Mandapam coast reported by [40]. In current study the lipid content of L. veriegata was found to be 2.65 ± 0.45 g/100g/DW. Similarly the lipid content of S. linifolium presented (2.16%) of algal dry weight [41]. Total lipid content of seaweeds ranged from 0.60% to 4.14 % reported by [42]. Similarly [43] reported that total lipids were comparatively low in U. fasciata was the maximum (2.96%) and C. officinalis was the minimum (1.37%). The macro algae have lipid content below 5%. Despite the fact that seaweed has low lipid content, and the lipid fraction have a variety of useful components such omega-3 polyunsaturated fatty acids, fucoxanthin and polyphenol [44]. Ash content ranges from 16.61 ± 0 .29 g/g was considered to be high and associated high mineral elements. Total dietary fiber content of L. variegata noticed as 2.78±0.37 g/100g/DW, In our study confirmed that fiber content higher than the other species, such as Hypnea pannosa (8.5%) and D. dichotoma (2.5%) reported by[45]. The brown seaweeds contain higher ash content when compared with red and green seaweeds. Ash and moisture content of L. variegata (16.61 g/100g & 9.34 g/100g) in previous studies of [46], [47]. In Total dietary fiber was comprised 1.28 ± 0.07 g/100g/DW and 1.50 ± 0.07 g/100g/DW of soluble and insoluble fiber, respectively.

Table 1. Proximate Compositions of L. variegata

Proximate composition	L. variegata g/100g
Carbohydrate	28.81±0.08
Protein	34.2±1.06
Lipid	2.65±0.45
Total Dietary fibers	2.78±0.37
Soluble fibers	1.28±0.07
Insoluble fibers	1.50±0.07
Ash	16.61±0.29
Moisture	9.34±0.25

All values represent the mean of triplicates ± Standard deviation

Table 2. Mineral composition of L.variegata g/100g

Minerals	L .variegata
Calcium(Ca)	1020.6±0.99
Magnesium(Mg)	1712.6±2.75
Iron(Fe)	913.6±1.49
Sodium(Na)	1516.6±3.47
Potassium(K)	1078.3±1.23
Phosphorus(P)	1477.3±5.24
Zinc(Zn)	813.6±0.04
Copper(cu)	427.3±0.08

All values represent the mean of triplicates ± Standard deviation

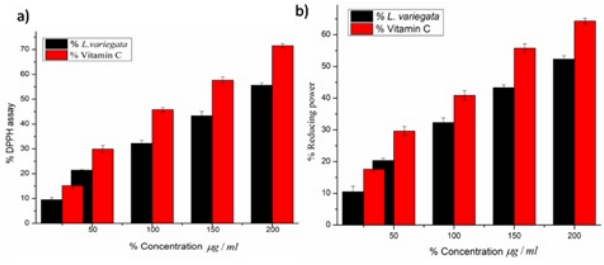

Figure 2. Antioxidant activity of L. variegata a) DPPH scavenging activity b) Reducing power

3.1.1 Mineral composition

The present study different mineral content of Lobophora variegata are shown in Table 2. The mineral content may change significantly by the seasons. The element composition of Lobophora variegtata magnesium (1712.6 ± 2.75 g/100g DW) was the most abundant element followed by phosphorus (1477.3 ± 5.24 g/g DW)), sodium (1516.6 ± 3.47 g/100g DW)),

Table 3. Table 3. Amino acids profile of L.variegata using LCMS

Amino acids	L. variegate (mg/100g)/ Dw
Essential amino acids	
Threonine	0.89±0.01
Valine	1.26±0.04
Methionine	0.34±0.013
Isoleucine	1.83±0.05
Ieucine	0.28±0.004
Phenylanaline	1.83±0.008
Histidine	1.82±0.02
Lysine	1.89±0.0.7
Tryptophan	1.02±0.06
Non-essential amino acids	
Serine	1.98±0.07
Glutamic acid	1.48±0.14
Proline	0.91±0.11
Alanine	1.84±0.04
Tyrosine	0.94±0.07
Glycine	2.61±0.11
Cysteine	0.46±0.02
Aspartic acid	0.96±0.03
Asparagine	2.73±0.06
Essential amino acids	12.43±0.04
Non-essential amino acids	16.99±0.05
Total amino acids	29.42±1.10

All values represent the mean of triplicates ± Standard deviation

potassium (1078.3 ± 1.23 g/100g DW), calcium (1020.6 ± 0.99 g/100g DW) and the moderate level of minerals like Iron (913.6 ± 1.49 g/100g DW), Zinc (813.6 ± 0.04 g/100g DW), Copper (427.3 ± 0.08 g/100g DW). Previous study report that the high potassium, magnesium and iron levels, but low copper and cobalt levels by [48]. Similar results were reported in brown seaweed are [49]& [50]. Generally 8-40% of minerals, essential amino acids and trace elements are required for human nutrition [51. Brown seaweeds contain rich, major Na and K contributors, as salt replacers in food formulations. [52]. Brown seaweeds (Ascophyllum nodosum& Laminaria digitata) are rich in potassium (2–3% & 3–4%) and sodium (1.3–3.8%& 0.9–2.2%) [53]. besides, the amounts of the trace elements were greater than in earlier studies [54] and [55].

3.1.2 Amino acid

Amino acids composition of L. variegata was determined and the resultsare presented in Table 3. The total amino acid of L.variegatais (29.42 ± 1.10 mg/g), that values are comparable to their equivalent crude protein content of 34.2 ±0.06 mg/100g. Nine essential amino acids (EAAs) and 10 non-essential amino acids (NEAAs) were found in L. variegata. Total EAAs significantly present (12.43 ± 0.04 mg/g), and total NEAAs presented in the maximum of (16.99 ± 0.05 mg/g). the previous study reported that, high level of histidine, glutamine, aspargine and alanine in the net content of total amino acid(32.9%) was observed in Undaria pinnatifida [56]

3.1.3 Fatty acid

The fatty acid composition (as methyl esters) of L. variegata was determined and presented in Table 4. The Docosahexanoic acid l ester (omega-3) and Palmitic acid was most abundant fatty acids followed by the Lauric acid, Stearic acid and Myristic acid Tetracosenoic acid, Eicosapentaenoic acid low in Linoleic acid, Oleic acid. A similar result was reported by [57]. On the contrary, [58] reported that C22; 6n3 and Palmitic acid C16; 0 most abundantly fatty acids in seaweed G.changgii and B.bifurcata, respectively. The highest saturated fatty acids content was found in Sargassum wightii. [59] One of the most abundant fatty acids in seaweeds is oleic acids. FAMEs are environmentally benign, non-hazardous, and eco-friendly chemicals used for biodiesel and antibacterial activity reported by [60].

3.2 Antioxidant activity

The DPPH radical scavenging assay and reducing power were used to determine the antioxidant potential of L.variegata. The results showed the percentage of inhibition are increasing while concentration of extract increased in both L.variegata extract and standard (Vitamin C) by the dose dependent manner. The IC50 value of L.variegata and Standard are in DPPH and reducing power (175.41 µg/ml; 118.43µg/ml) and (186.29 µg/ml; 130.76 µg/ml) respectively. The similar results were reported previously in the brown seaweeds Cystoseira sedodes, Cladostephus spongeosis and Padina pavonica [61] Cystyscira compressa [62]. Generally, brown seaweeds are significant source of unique bioactive compounds, which have antioxidant potential [63]. These bioactive compounds of seaweeds play a vital role in scavenging and neutralizing the ROS. carotene, fucoxanthin, catechin, flavonoids, fucoidan and phloratannin are the chief source of antioxidant compounds found surplus in seaweed [62].

4. Conclusion

Brown seaweed L.variegata is a good source of high-quality protein with a high concentration of important amino acids, as well as minerals, and fatty acids, all of which are good antioxidants. In addition, L.variegata contains all essentials amino acid and micronutrients which found to be excellent source for pregnant women for cognitive development of fetus. Our results showed that the crude methanol extract having the significant antioxidant activity. It has the potential to be commercialized as a natural antioxidant. In Tamil Nadu, exploitation of seaweed utilization is still emerging; therefore more studies are required to evaluate the application of seaweeds for nutraceutical and pharmaceutical industries. Further, it is believed to be used L. variegata more potent in nutraceutical and pharmaceutical analysis.

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