International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 8, Issue 6, pp: 106-112, 2023
https://doi.org/10.55126/ijzab.2023.v08.i06.016 **http://www.ijzab.com**

Research Article

EVALUATION OF THE PROXIMATE COMPOSITION, MINERALS, AND HEAVY METALS OF BROWN SEAWEEDS:*SARGASSUM WIGHTII* AND *SARGASSUM THUNBERGII* COLLECTED FROM THE MANDAPAM COASTAL REGIONS, TAMIL NADU

[1,3]Preethy Treesa Paul, [1,3]Jean Mary Joy, [4]Fazil T.S, [1,3]Akshay P,
[2*]Niladri Sekhar Chatterjee, [1]Suseela Mathew

[1]Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India-682029
[2]Quality Assurance and Management Division, ICAR-Central Institute of Fisheries Technology,
Cochin, Kerala, India-682029
[3]Faculty of Marine Sciences, Cochin University of Science and Technology, Cochin, Kerala, India-682016
[4]Research Institute for Electronic Science, Hokkaido University, N20, W10, Sapporo, Hokkaido 001-0020, Japan

Article History: Received 17th November 2023; Accepted 20th December 2023; Published 31st December 2023

ABSTRACT

A vital renewable resource for the marine environment, seaweed naturally supplies nutrients and has several health benefits. The nutritional properties of seaweed compounds have yet to be well investigated; much research has been done on their chemical and biological makeup. Seaweeds provide an alternate supply of these essential nutrients, including protein, fibre, and necessary and non-essential trace elements. In the present study, two brown seaweeds-*Sargassum wightii* and *Sargassum thunbergii,*collected from the Mandapam coastal region of Tamil Nadu, were assessed for their proximate composition, minerals, and heavy metals.The findings on dry weight basis showed that carbohydrates (65.1–67.9%) predominated in the proximal analysis, followed by ash content (12.5–16.3%). On a dry weight basis, the critical trace elements found in seaweeds, such as copper (Cu), zinc (Zn), and iron (Fe), were between 8.14–12.41 ppm, 3.95–4.86 ppm, and 648.92-898.45 ppm, respectively. Both seaweeds have a very high calcium (Ca) concentration, ranging from (14,805.08-16,235.62) ppm. The seaweed species did not contain any measurable levels of dangerous heavy metals such as cadmium (Cd), beryllium (Be), lead (Pb), or mercury (Hg). The results demonstrated how seaweed or marine macroalgae may be helpful for essential nutrient supply.

Keywords: Marine, Algae, Seaweeds, Phaeophyceae, Sargassum, Nutrition.

INTRODUCTION

Novel technologies and heightened consciousness regarding the correlation between diet and health have resulted in noteworthy progressions in nutrition and product creation and unparalleled levels of mass manufacturing. Research indicates that the kind and origin of food might affect one's general health (Lordan *et al.*, 2011). A new category of foods dubbed "functional foods" has arisen in recognition of food's role in promoting health. Its goals are to lower disease risk and improve general well-being (Honkanen & Frewer, 2009). These foods frequently include nutrients or bioactive substances with possible health benefits (Siró *et al.*, 2008), or they contain components that have been scientifically created with particular health benefits (Niva, 2007).

Algae are multicellular, photoautotrophic organisms divided into two size categories: macroalgae (seaweeds) and microalgae. Shallow ocean waters are home to non-flowering macroalgae that lack natural root stems and leaves. Algae are, in the broadest sense, photosynthetic, oxygen-producing organisms that are neither lichens nor embryophyte terrestrial plants (Cavalier-Smith, 2007).

---

*Corresponding Author: Dr. Niladri Sekhar Chatterjee, Senior Scientist, Quality Assurance and Management Division, ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India-682029. Email: niladri.sekhar.chatterjee@gmail.com.

Abiotic stressors that affect microalgae include freezing, desiccation, salinity fluctuations, temperature extremes, UV and intertidal species, and intense photon irradiance. It is thought that bioactive substances first appeared as chemical defence mechanisms against other living things and as reactions to physical stress. Therefore, it is possible to consider the bioactive substances as a modification for the sessile benthic lifestyle. The same bioactive substances can show multiple biological activities and functions.

Chemically mediated biological activities have been reported for over 150 taxa of benthic sea algae (Rindi *et al*., 2008). Antioxidant activity is a significant bioactivity among the several bioactivities observed. With the ability to use these chemicals as natural antioxidants in various food and pharmaceutical products, seaweeds are waiting for a breakthrough in culinary and medical applications. As Gupta and Abu-Ghannam (2011) outlined, brown seaweeds are notably abundant in phytochemicals such as terpenes, carotenoids, and phenolic compounds. Furthermore, they serve as a substantial source of polysaccharides with diverse biological applications (Synytsya *et al*., 2015). These seaweeds are employed in both culinary and medicinal contexts, addressing various health issues like gallstones, stomach problems, eczema, cancer, renal disorders, skin conditions, respiratory problems, and ulcers, among others, owing to their elevated nutritional and pharmaceutical value (Besada *et al*., 2009). The known species of brown algae have surpassed 1,500, with the genus *Sargassum* comprising the most significant number. Despite the numerous benefits of seaweeds, there exists limited awareness among Indians about their potential uses. *Sargassum wightii*, a marine brown alga, exhibits anti-tumour, anti-inflammatory, antioxidant, and antibacterial properties (Yuvaraj *et al*., 2014). Notably, *S. wightii*, a critical species in the *Sargassum* genus, demonstrates various bioactive qualities (Mizukoshi *et al*., 1993). The antioxidant action of *S. wightii* is attributed to numerous flavonoids (Meenakshi *et al*., 2009), suggesting its potential to benefit food systems as a safe substitute for synthetic antioxidants. While seaweeds are increasingly becoming part of diets globally, their consumption varies. Brown seaweeds, for example, are incorporated into soups and stews as a vegetable, allowing for a consumption of up to 10 g dry weight or more per serving. Some are used as seasonings, with consumption seldom exceeding 1 g dry weight. The current study explores the proximal levels, minerals, and heavy metals of *S. wightii* and *S. thunbergii* harvested from the Mandapam coastal region of Tamil Nadu, India.

MATERIALS AND METHODS

Seaweed collection and preparation

The research was conducted at the Indian Council of Agricultural Research-Central Institute of Fisheries Technology in Cochin, Kerala, India, from April to October 2021. Two varieties of brown seaweed, namely *S. wightii* and *S. thunbergii*, were collected from the Mandapam coastal area in Tamil Nadu, India (Figure 1). The seaweed specimens underwent a thorough cleaning process involving tap water to eliminate sand, salt, shells, epiphytes, debris and other foreign substances adhering to the thalli. Following sorting, the samples underwent meticulous cleaning with distilled water. Subsequently, the cleaned seaweeds were subjected to a 48-hour drying period in a hot oven set at 45°C. Once dried, the samples were finely ground into powder using a dry grinder. The resulting powder was then stored in an airtight, dry, and clean container for subsequent chemical analysis.

Chemicals

Sulfuric acid, copper sulphate, potassium sulfate, phenolphthalein indicator, sodium hydroxide, boric acid, Tashiro's indicator, petroleum ether, glucose, anthrone, hydrogen peroxide, and nitric acid were purchased from Sigma-Aldrich. All the other chemicals used in this study were of analytical grade.

Proximate composition analysis

The approximate compositions of *Sargassum* sp. were evaluated by AOAC 2000 techniques. The crude protein concentration was determined using the Kjeldahl Nitrogen technique, which includes titration, distillation, and digestion.

Moisture

As per AOAC (2000) criteria, the moisture content was determined by drying a 20 g sample at 105°C in a hot air oven with accurate temperature control. The samples were put on Petri dishes that had been previously weighed and were cooled in desiccators until a steady weight was reached, allowing us to track the weight reduction. After that, the moisture content was computed and given as a percentage.

$$\text{Moisture content (\%)} = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

W1= Weight of the dry petri dish
W2= Weight of the petri dish with sample
W3= Weight of the petri dish with sample after drying

Crude Protein

The Kjeldahl Nitrogen Method was used to determine the crude protein content. A 100 mL Kjeldahl flask containing glass beads, a tiny pinch of digestion mixture ($CuSO_4$, K_2SO_4), and 10 mL sulfuric acid were filled with around 100 mg of the sample. Over a hob, this mixture was heated until the solution lost all colour. Distilled water was added to the digested and cooled solution to make it up to 100 ml. Then, 10 ml of this mixture was transferred to the micro-Kjeldahl distillation device. Two drops of phenolphthalein indicator and 40% NaOH were added after washing with distilled water until the indicator became pink. After five minutes of distillation, ammonia was collected in 10 mL of 2% boric acid and a drop of Tashiro's indicator.

$$\text{Protein Content (\%)} = \frac{x \times 0.14 \times V \times 6.25}{V1 \times W \times 1000} \times 100$$

x= Titer value of the sample
V= Total digest volume
V_1= Volume of digest taken for distillation
W= Sample weight

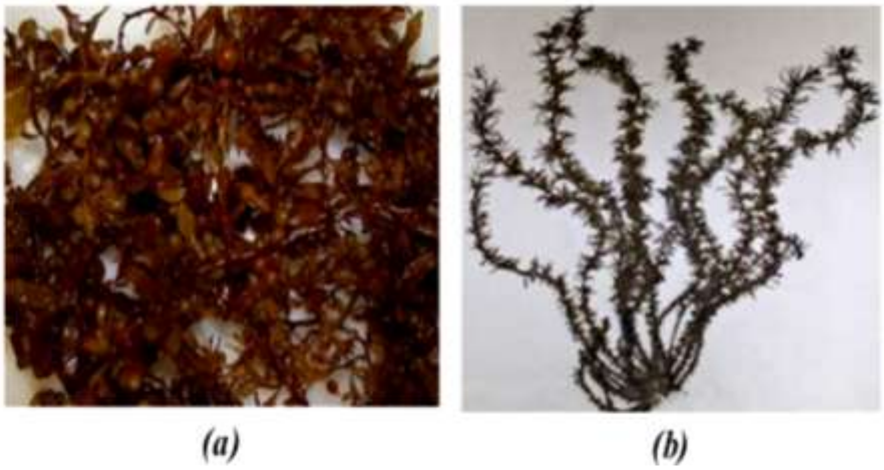

Figure 1. Images of the two brown seaweeds (a) *Sargassum wightii* (b) *Sargassum thunbergii.*

Crude Fat

The Soxhlet method was used to calculate the crude fat content (AOAC, 2000). 1 g of the sample was weighed and put into a thimble and then into a Soxhlet device. Petroleum ether was used to extract the material, taking two and a half hours at 90°C. After the extraction procedure, the solvent evaporated between 80 and 100℃. The fat content was measured after chilling it in a desiccator (W3). The weight of the fat content in the sample was determined by comparing the weights of the pre-weighed flat-bottom flask (W2) and the flask containing the fat after the petroleum ether had evaporated entirely.

$$\text{Fat content (g/100g)} = \frac{W3 - W2}{W1} \times 100$$

W_1= Sample weight
W_2= Soxhlet beaker weight
W_3=Weight of fat and beaker

Ash

Using the AOAC technique (2000), the ash content of the *Sargassum* species was determined. After carefully cleaning and heating the silica crucibles to 600°C for an hour in a muffle furnace, they were cooled in a desiccator, and the weight of the empty crucible (W1) was noted. To find the amount of ash, a 2 g sample was burned in a crucible until it was blackened. The charred material was then transferred to a muffle furnace prepared to 650°C. It was kept there for 6-8 hours or until the material transformed into ash that was either white or greyish-white in colour. The crucibles were cooled in a desiccator (W3), and the final weight was documented.

$$\text{Ash content \%} = \frac{W_3 - W_1}{W_2} \times 100$$

W_1= Crucible weight
W_2= Sample weight
W_3=Weight of ash + crucible

Carbohydrates

The anthrone technique (JE, H., 1962) was employed to assess the total carbohydrate content of seaweed, utilizing glucose as a reference standard. Various volumes of glucose solution were dispensed into a series of test tubes from the stock solution (200µg/ml), and the volume was adjusted to 1 mL with distilled water. Subsequently, 5 mL of the anthrone reagent was added to each tube and thoroughly mixed by vortexing. The lines, covered with marbles, were cooled and incubated at 90℃ for 17 minutes in a water bath. After cooling to room temperature, the optical density at 620 nm was measured against a blank. The quantity of glucose in the unknown sample was determined by constructing a standard curve.

Heavy metal analysis of seaweeds

Approximately 0.25 g of finely powdered seaweed samples were measured and placed into microwave digestion vessels. 4 ml of nitric acid (HNO_3) and 1 ml of 30% H_2O_2 were added to these vessels. The samples underwent digestion at a temperature of 190°C for 10 minutes, utilizing temperature feedback control in microwave digestion. The method performance data provided in this procedure was generated using a Bergh of Speed Wave 4 microwave digestion system. Upon completion of the acid breakdown, the vessel was cautiously removed from the hot plate, allowing its contents to cool for thirty minutes to facilitate acid drainage. Following the evaporation of the acid, the disintegrated samples were subsequently transferred to a 50 ml volumetric flask containing deionized water that had been diluted with acid (ranging between 1 and 5%). The assessment of minerals such as Iron (Fe), Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K), and Phosphorus (P), alongside other elements, including Selenium (Se) and heavy metal ions, was conducted using inductively coupled plasma mass spectrometry (ICP-MS). Calibration curves of standard elements were employed to determine the concentrations of each component, with the analysis performed in triplicate.

Statistical analysis

The experimental values were presented as mean ± standard deviation (SD). Statistical significance was evaluated utilizing 2-wayANOVA tests conducted, SAS). A probability value of $P \leq 0.05$ was considered indicative of relevance.

RESULT AND DISCUSSION

Seaweeds were found to be rich in micronutrients such as vitamins and minerals. The proximal composition of two *Sargassum* species was evaluated regarding moisture, crude protein, crude fat, ash, and carbohydrates using the standard AOAC methods (Table 1). The highest protein content was expressed by *S. thunbergii* (12.3 ± 1.0%), followed by *S. wightii* (10.5±1.0%). The crude fat content in *S. wightii* is 3±0.3%, and *S. thunbergii* showed only 0.8±0.1%. The ash content represents the mineral content of the samples; the higher ash content was in *S. thunbergii* (16.3 ± 0.9), followed by *S. wightii* (12.5±1.2%).The primary organic elements of both seaweeds were 67.9 ± 1.0% for *S. thunbergii* and 65.1±1.0% for *S. wightii*, with ash content coming in second. Seaweeds are extensively acknowledged for their abundant minerals and polysaccharides, evidenced by their notably high ash and carbohydrate content (Matloub *et al*., 2012).

Table 1. Proximate composition of the brown seaweeds.

Components (%)	*S. wightii*	*S. thunbergii*
Moisture	7.0 ± 0.4	3.0 ± 0.3
Crude protein	10.5 ± 1.0	12.3 ± 1.0
Crude fat	3.0 ± 0.7	0.8 ± 0.1
Ash	12.5 ± 1.2	16.3 ± 0.9
Carbohydrates	65.1 ± 0.1	67.9 ± 1.0

Macro elements refer to the essential natural ingredients the body necessitates in more significant quantities, prioritizing their significance over other minerals. The macro elements are phosphorus, sulphur, sodium, calcium, potassium, chloride, and magnesium. Conversely, trace elements are indispensable in minimal quantities for sustaining a healthy bodily state. Primarily serving as constituents of enzymes and hormones or participating in initiating enzymatic reactions, these trace elements play a pivotal role in bodily functions. It is crucial to maintain adequate levels of trace elements such as manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), and selenium (Se) for human health. Deficiencies in these trace elements can lead to various health issues and complications. Both seaweeds included the trace elements (Se,Fe, Cu,Mn, Zn) and critical macro minerals (Mg,Ca, K,P, Na, Cl, S) vital to human health.

The heavy metal content of *S. wightii* and *S. thunbergii* is displayed in Table 2. Iron (Fe) was higher in *S. thunbergii* 898.45 ± 9.40 ppm than in *S. wightii* (648.92±13.80 ppm); Mn, Cu, Zn, and Se were all expressed by the seaweeds. *S. wightii* (16235.62±80.91 ppm) expressed higher Ca content than *S. thunbergii* (14805.08±32.92 ppm). The elements that are not necessary for human survival are lead, mercury, cadmium, and beryllium. Beyond a particular threshold, both non-essential and essential components can become poisonous. While the concentrations of Be and Hg were not found, the harmful trace elements, such as Cd and Pb, varied from 0.45-2.12 ppm and 0.24-0.42 ppm, respectively, and the levels of Cd and Pb in this investigation were below the suggested limit threshold of 3 ppm, the amounts of mercury and beryllium were not traceable. Micronutrients include vitamins, sterols, and minerals that are abundant in seaweeds (Patarra *et al*., 2011; Peña-Rodríguez *et al*., 2011; Ferraces-Casais *et al*., 2012). Significant concentrations of trace elements, as well as essential minerals like magnesium, sodium, calcium, and potassium, have been found in seaweeds. Seaweeds can be used as dietary supplements to feed certain minerals and trace elements because of their substantial mineral content (Teas *et al*., 2004; Villares *et al*., 2002).

The European Commission (EC) Regulations No. 629/2008 (EC, 2008) and No. 488/2014 (EC, 2014) explicitly state the maximum permissible concentration of Cadmium (Cd) in algae, set at 3 ppm. However, these regulations do not specify particular limits for Aluminium (Al) and Lead (Pb) in algae. In France, the Center d'Etude et de Valorization des Algues (CEVA) has established its standards for toxic metals, defining levels at 0.5 ppm dry weight for Cd and 5 ppm dry weight for Pb in edible French algae. Given seaweed's substantial tendency to serve as a primary accumulator of Arsenic (As) in the oceanic environment and its crucial position in the trophic network (Ghosh *et al*., 2022), monitoring Arsenic levels in seaweed becomes imperative. Despite the absence of a specific regulatory limit (Banach *et al*., 2019), the Australia New Zealand Food Standard Code (FSANZ) stipulates a maximum threshold of 1 ppm for inorganic Arsenic (i-As). Contrastingly, in France and the USA, authorization allows for a maximum of 3 ppm dry weight of i-As in products derived from seaweed. Significantly, prior studies (Sim *et al*., 2023; Lee *et al*., 2022) have validated that brown algae manifest the most elevated levels of Arsenic content. This emphasizes the heightened overall levels of Arsenic in brown seaweed and the predominance of Arsenic within the taxonomic class Phaeophyta (Van Netten *et al*., 2000; Dawczynski *et al*., 2007).

Table 2. List of macro and micro minerals present in the brown seaweeds.

Elements (ppm)	***S. wightii***	***S. thunbergii***
Al	21.33 ± 2.72	88.40 ± 2.47
As	2.82 ± 0.25	3.75 ± 0.32
B	68.10 ± 0.40	61.76 ± 0.35
Ba	22.57 ± 0.11	27.42 ± 0.01
Be	-	-
Ca	16,235.62 ± 80.91	14,805.08 ± 32.92
Cd	0.45 ± 0.01	2.12 ± 0.01
Co	-	-
Cu	8.14 ± 0.13	12.41 ± 0.19
Fe	648.92 ± 3.80	898.45 ± 9.40
Hg	-	-
K	11,437.83 ± 29.67	8,952.57 ± 91.79
Li	0.47 ± 0.02	0.57 ± 0.02
Mg	6,392.99 ± 36.08	6,834.99 ± 95.77
Mn	15.41 ± 0.31	54.80 ± 0.53
Na	5,598.03 ± 84.19	6,463.78 ± 73.43
Ni	1.77 ± 0.09	2.09 ± 0.01
P	390.71 ± 2.65	307.39 ± 0.43
Pb	0.24 ± 0.06	0.42 ± 0.03
Se	0.31 ± 0.02	0.40 ± 0.05
Zn	3.95 ± 0.13	4.86 ± 0.02

The extensive historical use of seaweed has led to the realization that certain elements are more beneficial and superior to those found in terrestrial counterparts. Apart from their applications in biotechnology, bioremediation, and aquaculture, seaweeds are gathered for use as food, feed, phycocolloids, fertilizer, energy, pharmaceuticals, cosmetics, and nutraceuticals (Hold & Kraan, 2011; Rao P. S *et al*., 2018; Mohamed *et al*., 2012). Despite the significant potential of seaweeds as nutritional sources, therapeutic health enhancers, and beauty products in India, they are primarily utilized as raw materials for agar, alginates, and seaweed liquid fertilizer. Therefore, promoting and developing seaweed as a nutritious dietary option is crucial. Several investigations focusing on the nutritional and biochemical makeup of seaweeds from diverse geographic locations have been carried out by researchers like McDermid & Stuercke (2003), Marsham *et al*. (2007), Ortiz *et al*. (2006), and Chakraborty & Santra (2008); Rupérez (2002). These studies aimed to explore and utilize the nutritional potential of seaweeds comprehensively. Unfortunately, limited research has been carried out on compounds specific to seaweed of this nature. The existing knowledge primarily focuses on their chemical and biological composition rather than comprehensive empirical investigation. The biochemical composition of marine seaweeds is significantly influenced by geographical location and local climatic conditions (Rohani-Ghadikolaei *et al*., 2012). The analysis of proximate composition and heavy metal content in *Sargassum wightii* and *Sargassum thunbergii*, conducted in this current study, will serve as a valuable resource for assessing their nutritional significance in human and animal diets.

CONCLUSION

Marine macroalgae, commonly known as seaweed, serve as a valuable reservoir of essential trace elements. The present study reveals that both types of seaweed excel in providing carbohydrates and minerals. An analysis of the proximate composition of different species shows that *S. thunbergii* outperforms others, exhibiting lower crude fat and higher levels of crude protein, carbohydrates, and ash than *S. wightii.* This investigation underscores the rich nutritional content of both *S. wightii* and *S. thunbergii*. Consequently, it can be inferred from the research mentioned above that both species hold promise for diverse culinary and medicinal applications. These seaweeds can serve as natural antioxidants in various food and medical contexts. Therefore, these species might be valuable sources of components for the advancement of functional food products and animal feed. Further research is essential to deepen our understanding and promote the utilization of these marine algae as food resources. This research should explore additional nutritional components, including the concentrations of phenolic compounds and other bioactive chemicals.

ACKNOWLEDGEMENT

The authors are grateful to The Director, ICAR-CIFT, Cochin, for providing the necessary facilities and support for the research. The authors express their gratitude to Mrs. PA Jaya (Technical Officer), Mrs. N. Lekha (Technical Officer), Mr. P. Suresh (Senior Technician) and at ICAR-Central Institute of Fisheries Technology (CIFT), Cochin, Kerala, for their valuable technical assistance in conducting the analyses.

REFERENCES

AOAC (2000) Association of Official Analytical Chemists. Official Methods of Analysis. Vol. II, 17th Edition, AOAC, Washington DC

setting maximum levels for certain contaminants in foodstuffs. Off. *Journal of European Union Letters*, 173, 6-9.

EC (European Regulation), 2014. Commission Regulation (EU) No 488/2014 of 12 May 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs. Off. J. Eur. Union L138, 75-79.

Ferraces-Casais, P., Lage-Yusty, M. A., Rodríguez-Bernaldo de Quirós, A., & López-Hernández, J. (2012). Evaluation of bioactive compounds in fresh edible seaweeds. *Food Analytical Methods*, *5*, 828-834.

Ghosh, D., Ghosh, A., & Bhadury, P. (2022). Arsenic through aquatic trophic levels: effects, transformations and biomagnification—a concise review. *Geoscience Letters*, 9(1), 1-17.

Gupta, S., & Abu-Ghannam, N. (2011). Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science & Technology*, 22(6), 315-326.

Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, *23*, 543-597.

Honkanen, P., & Frewer, L. (2009). Russian consumers' motives for food choice. *Appetite*, *52*(2), 363-371.

JE, H. (1962). Determination of reducing sugars and carbohydrates. *Methods of Carbohydrate Chemistry*, *1*, 380-394.

Lee, K. J., Kang, E. H., Yoon, M., Jo, M. R., Yu, H., Son, K. T & Kim, J. H. (2022). Comparison of heavy metals and arsenic species in seaweeds collected from different regions in Korea. *Applied Sciences*, 12(14), 7000.

Lordan, S., Ross, R. P., & Stanton, C. (2011). Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Marine Drugs*, *9*(6), 1056-1100.

Marsham, S., Scott, G. W., & Tobin, M. L. (2007). Comparison of nutritive chemistry of a range of temperate seaweeds. *Food chemistry*, *100*(4), 1331-1336.

Matloub, A. A., Awad, N. E., & Khamiss, O. A. (2012). Chemical composition of some Sargassum spp. and their insecticidal evaluation on nucleopolyhedrovirus replication in vitro and in vivo. *Egyptian Pharmaceutical Journal*, *11*(1), 53.

McDermid, K. J., & Stuercke, B. (2003). Nutritional composition of edible Hawaiian seaweeds. *Journal of Applied Phycology*, *15*, 513-524.

Meenakshi, S., Gnanambigai, D. M., Mozhi, S. T., Arumugam, M., & Balasubramanian, T. (2009). Total flavanoid and in vitro antioxidant activity of two seaweeds of Rameshwaram coast. *Global Journal of Pharmacology*, *3*(2), 59-62.

Mizukoshi, S., Matsuoka, H., Nakamura, K., & Kato, F. (1993). Effect of seaweed preparations on murine immunocytes. *Journal of Applied Phycology*, *5*, 629-637.

Mohamed, S., Hashim, S. N., & Rahman, H. A. (2012). Seaweeds: A sustainable functional food for complementary and alternative therapy. *Trends in Food Science & Technology*, 23(2), 83-96.

Niva, M. (2007). 'All foods affect health': understandings of functional foods and healthy eating among health-oriented Finns. *Appetite*, *48*(3), 384-393.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., & Rios, A. (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulva lactuca and Durvillaea antarctica. *Food chemistry*, *99*(1), 98-104.

Patarra, R. F., Paiva, L., Neto, A. I., Lima, E., & Baptista, J. (2011). Nutritional value of selected macroalgae. *Journal of Applied Phycology*, *23*, 205-208.

Peña-Rodríguez, A., Mawhinney, T. P., Ricque-Marie, D., & Cruz-Suárez, L. E. (2011). Chemical composition of cultivated seaweed Ulva clathrata (Roth) C. Agardh. *Food Chemistry*, *129*(2), 491-498.

Rao, P. S., Periyasamy, C., Kumar, K. S., Rao, A. S., & Anantharaman, P. (2018). Seaweeds: distribution, production and uses. *Bioprospecting of algae. Society for Plant Research*, 59-78.

Rindi, F., Guiry, M. D., & López-Bautista, J. M. (2008). Distribution, morphology, and phylogeny of klebsormidium (Klebsormidiales, Charophyceae) in urban environments in Europe 1. *Journal of Phycology*, *44*(6), 1529-1540.

Rohani-Ghadikolaei, K., Abdulalian, E., & Ng, W. K. (2012). Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. *Journal of Food Science and Technology*, 49, 774-780.

Rupérez, P. (2002). Mineral content of edible marine seaweeds. *Food Chemistry*, *79*(1), 23-26.

Sim, R., Feldmann, J., Stengel, D. B., & Pétursdóttir, Á. H. (2023). Temporal and intra-thallus variation in arsenic species in the brown macroalga *Laminaria digitata*. Environmental Chemistry.

Siro, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance-A review. *Appetite*, *51*(3), 456-467.

Synytsya, A., Čopíková, J., Kim, W. J., & Park, Y. I. (2015). Cell wall polysaccharides of marine algae. *Springer handbook of Marine Biotechnology*, 543-590.

Teas, J., Pino, S., Critchley, A., & Braverman, L. E. (2004). Variability of iodine content in common commercially available edible seaweeds. *Thyroid*, *14*(10), 836-841.

Van Netten, C., Cann, S. H., Morley, D. R., & Van Netten, J. P. (2000). Elemental and radioactive analysis of commercially available seaweed. *Science of the Total Environment*, *255*(1-3), 169-175.

Villares, R., Puente, X., & Carballeira, A. (2002). Seasonal variation and background levels of heavy metals in two green seaweeds. *Environmental Pollution*, *119*(1), 79-90.

Yuvaraj, N., & Arul, V. (2014). In vitro anti-tumor, anti-inflammatory, antioxidant, and antibacterial activities of marine brown alga *Sargassum wightii* collected from Gulf of Mannar. *Global Journal of Pharmacology*, *8*(4), 566-577.