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S Adithiyalakshmi
PG Research Scholar, Department of Food Service Management and Dietetics Avinashilingam Institute of Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India

Salnamchi J Sangma
PG Research Scholar, Department of Food Service Management and Dietetics Avinashilingam Institute of Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India

PL Sridevi Sivakami
Associate Professor, Department of Food Service Management and Dietetics Avinashilingam Institute of Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India

Corresponding Author:
S Adithiyalakshmi
PG Research Scholar, Department of Food Service Management and Dietetics Avinashilingam Institute of Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India

From ocean depths to nutrient rich plates: A comparative study on the toxicity, nutritional composition and antioxidant potential of selected seaweeds

S Adithiyalakshmi, Salnamchi J Sangma and PL Sridevi Sivakami

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Abstract
Seaweeds, thriving in intertidal, shallow, and deep waters, are rich reservoirs of proteins, essential amino acids, fatty acids, vitamins, minerals, and phytochemicals. Their unique ability to flourish in environments laden with toxic minerals underscores their potential as absorbers of such elements, necessitating a thorough toxicity analysis for safety assessment. This study focuses on *Sargassum wightii* and *Ulva lactuca*, aiming to investigate their toxicity, nutritional composition, and antioxidant potential as contributors to overall health. Collected from the Mandapam coastal area of Rameswaram, Tamil Nadu, India, both seaweeds underwent rigorous processing, including washing, drying, and powdering, followed by comprehensive analyses. Primary toxicity assessment revealed concentrations of heavy metals (Mercury, Lead, Cadmium, Arsenic) below quantifiable limits for both species, affirming their non-toxic nature. Secondary toxicity analysis, employing a brine shrimp lethality assay, indicated low toxicity levels for both *Sargassum wightii* and *Ulva lactuca*. Nutritional composition analysis unveiled diverse profiles: *Sargassum wightii* exhibited richness in Carbohydrates (54.97 g), Protein (5.13 g), Fat (0.21 g), Fiber (24.1 g), Calcium (32.64 mg), and Iron (0.77 mg), while *Ulva lactuca* excelled in Fiber (27.6 g), Calcium (878.2 mg), and Iron (2.11 mg). Phytochemical screening highlighted various bioactive compounds, and antioxidant potential, assessed through the DPPH method, showcased significant activity in *Sargassum wightii* compared to *Ulva lactuca*. In conclusion, both seaweeds demonstrated edibility, absence of toxic heavy metals, and low toxicity levels, suggesting their potential as health-promoting dietary components. Their diverse nutritional profiles and notable antioxidant activity position *Sargassum wighti*i and *Ulva lactuca* as promising candidates for inclusion in balanced and nutritious diets, emphasizing their safety and potential health benefits.

Keywords: Sargassum, *Ulva*, toxicity, nutritional composition, health benefits

Introduction

Seaweeds are floating, submerged macroalgae find their life in intertidal, shallow and deep waters of seas and estuaries [1] They are dense source of proteins, essential amino acids, essential fatty acids, vitamins, minerals also phytochemicals [2], found to have anti-oxidant activity in seaweeds. Seaweeds exhibit remarkable biodiversity, encompassing a vast array of species that range from microscopic unicellular forms to large multicellular macroalgae.

The seaweeds can be classified based on the presence of pigments, *Phaeophyceae* (red), *Chlorophyceae* (green), *Rhodophyceae* (brown). The colour of the seaweeds are due to the prominent pigment whereas phycoerythrin gives characteristic red colour to the *Phaeophyceae* family, presence of chlorophyll in *Chlorophyceae* provides green colour and *Rhodophyceae* has its xanthophyll pigment. The seaweed from the families *Chlorophyceae* and *Rhodophyceae* are selected for this study [3]. *Chlorophyceae* green seaweeds are widely used as food in many parts of the world, *Ulva* has the characteristic polysaccharide ulvan which has various potential biological activities such immune-stimulative, anti-depressant, anti-tumor, anti-anxiolytic activities also helps in improving anaemic condition [4]. Phloro tannins, laminarin, fucoidan are major bioactive substance of marine macro algae especially brown algae (*Sargassum*), which is proven to have anti adipogenicity, immunostimulatory, anti-hypertensive effects respectively [5-7].

The use of seaweeds were recorded first in Japan, 15,000 years ago. In 17th century, they were used for the treatment of goitre where as in 19th century isolation of its bioactive components such as alginic acid and carrageenan were done, also it was used as the medicine for constipation and obesity. Twentieth century had seen lot of improvement in seaweeds utilization including development of seaweeds based industries there by rises the demand for seaweeds [8] It also has anti thrombotic, anti carcinogenic and anti-microbial characteristics, all of which help to reduce obesity and fat deposition. These are pharmaceutical properties of several bioactive compounds found in seaweeds (agar, carrageenan, algin, fucoidans, mannitol, laminarin, carotenoids, fucoxanthin, phloro tannins), hence can be used in various dietary matrices [9]. Seaweeds are used in a variety of industries, including foods, pharmaceuticals, dairy, paper, and textiles. Agar and alginate are utilised as food, animal feed, fertilisers, bio filters, antibacterial agents, bio fuels, pigments and pharmaceuticals in India. *Gracillaria edulis* has been used as a raw material in the cottage industry for numerous decades [10].

Being finding their lives un environment with high toxic minerals, their surface has the characteristic of absorbance of toxic minerals. Hence it is essential to analyse its toxicity. This study aims to investigates the toxicity, nutritional composition, and antioxidant potential of selected seaweeds (*Sargassum wightii* and *Ulva lactuca*), offering insights into their potential as nutrient-rich and health-promoting components for consumption. The current research addresses the link between oceanic biodiversity and the development of beneficial, sustainable dietary options.

Methodology

Collection and processing of seaweeds

The seaweeds of family *Rhodophyceae* (*Sargassum wightii*) and family *Chlorophyceae* (*Ulva lactuca*) are selected for this study. The Seaweeds are collected from Mandapam coastal area of Rameswaram, Ramanathapuram district, Tamil Nadu, India in the month of December – January. The preliminary processing of washing, drying and powdering are done.

Fig 1: Dry seaweeds *Sargassum wightii* and *Ulva lactuca*

They were washed in laboratory for two to three times with portable water and made into dried powder by shadow drying the fresh seaweeds for one to two days till all its moisture got evaporated and become crisp and sound. It was then finely powdered in mixer grinder and collected in zip lock covered, stored in room temperature. The processing of seaweeds *Sargassum wightii* and *Ulva lactuca* were shown in figure 1

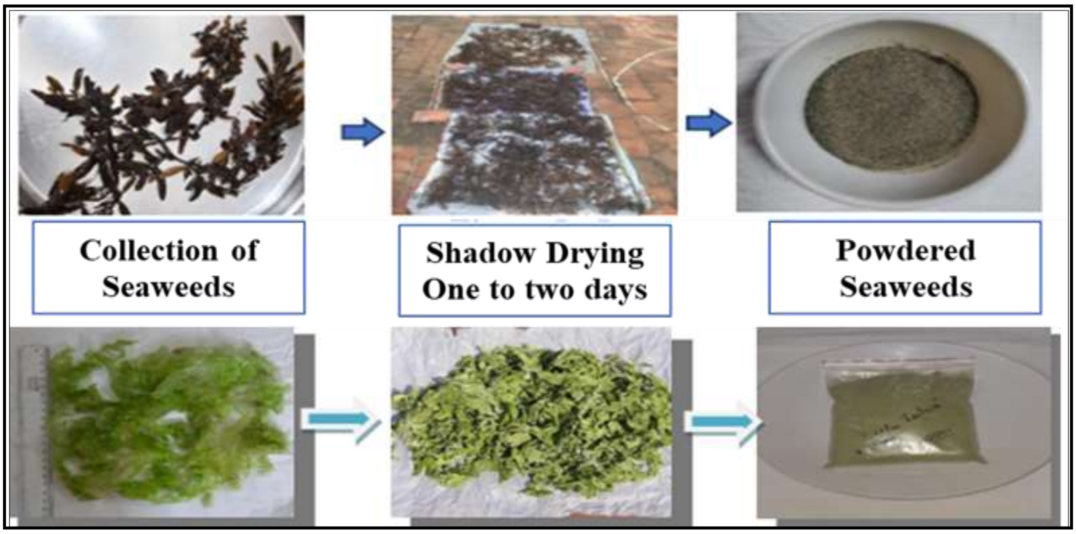

Fig 2: Processing of seaweeds

Analysis for toxicity level

Primary toxicity analysis

Seaweeds can be key sources of increased exposure to persistent and potentially hazardous elements due to their high biosorption and accumulation capability (iAs) (Monteiro *et al*., 2019).The heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg) and arsenic were analysed *lactuca* according to AOAC 21st edition for both selected seaweeds *Sargassum wightii* and *Ulva Lactuca.*

Secondary toxicity analysis

For analyzing the cytotoxicity of the selected seaweeds, brine shrimp lethality assay was done. The brine shrimp lethality assay is simple, very rapid and non time consuming, inexpensive and requires small amounts of test materials. It is considered as the useful tool for preliminary assessment of toxicity and it is the general bio assay to investigate the pharmacological activities natural marine products. The samples were given at different volumes (100, 250, 500, 1000 and 1500 µl) to 30 shrimps and it was monitored at the intervals of 2, 4, 6, 8, 24 hours, the mortality of shrimps was calculated after 24 hour and it was compared to control potassium dichromate ($K_2Cr_2O_7$) at volume of 1 mg/ ml (Sarah *et al*., 2017)

Analysis of nutritional composition

Analysing the nutritional composition of seaweeds is crucial for understanding their potential as a sustainable and nutrient-rich food source, contributing valuable insights to enhance dietary diversity and address global food security (FAO 2018). The dried seaweeds powder are chemically analysed for nutrients such as moisture, ash, energy, carbohydrate, protein and fat, fibre, calcium, iron and iodine (AOAC method, 2016).

Qualitative screening for phytochemicals

Some plants include phytochemicals, which are physiologically active substances that are responsible for and demonstrate potency in biological activity. Their confirmations, identifications, and characterizations are critical and several standard approaches were used to detect these phytochemicals (Amir *et al*. 2020). The dry seaweeds samples *Sargassum wightii* and *Ulva lactuca* are qualitatively analysed to know the presence or absence of phytochemicals. The test for phytochemicals such as alkaloids, flavonoids, sterols, terpenoids, phenolic compounds, cardiac glycosides and carbohydrates.

Analysis of antioxidant potential

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a popular, quick, easy and affordable approach for the measurement of antioxidant properties that includes the use of the free radicals used for assessing the potential of substances to serve as hydrogen providers or free-radical scavengers (FRS). The technique of DPPH testing is associated with the elimination of DPPH, which would be a stabilized free radical (Baliyan *et al*., 2022). The antioxidant assay for the seaweed samples (*Sargassum wightii* and *Ulva lactuca*) was conducted using DPPH method where ascorbic acid was used as the standard at different concentrations.

Results and Discussion

Processing of seaweeds

The selected brown seaweeds *Sargassum wightii* of *Rhodophyceae* family and green seaweeds *Ulva lactuca* of Chlorophyceae family were handpicked and cleaned with seawater to remove foreign particles, grit particles and epiphytes, shadow dried for one to two days. The dried seaweed was then pulverised and stored at room temperature in zip lock bags and analysed for primary and secondary toxicity, nutritional composition, phytochemicals and antioxidant potential.

Analysis for Toxicity

Primary toxicity analysis

The primary toxicity analysis of seaweeds involves the analysis of presence of heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg) and arsenic in the processed seaweed powders. The findings shows that, all the heavy metals are below the limit of quantification (table I). This proves that both selected seaweeds does not accumulate harmful heavy metals on its surface, ensuring their safety for consumption and minimal environmental impact in compliance with safety standards. The findings suggest these seaweeds pose no immediate health risks based on the analysed heavy metal content.

Table I: Primary toxicity analysis of selected seaweeds

Heavy metals	*Sargassum wightii*	*Ulva lactuca*
Mercury	Below Limit of Quantification (LOQ* – 0.2)	Below Limit of Quantification (LOQ* – 0.2)
Lead	Below Limit of Quantification (LOQ* – 0.2)	Below Limit of Quantification (LOQ* – 0.2)
Cadmium	Below Limit of Quantification (LOQ* – 0.2)	Below Limit of Quantification (LOQ* – 0.2)
Arsenic	Below Limit of Quantification (LOQ* – 0.2)	Below Limit of Quantification (LOQ* – 0.2)

Secondary toxicity analysis

The brine shrimp assay was used to determine the toxicity of selected seaweed samples (*Sargassum wightii* and *Ulva lactuca*) to determine their toxicity (*Sargassum wightii* and *Ulva lactuca*). By sonication for 60 minutes, the seaweed samples (*Sargassum wightii* and *Ulva lactuca*) were weighed and dissolved in water to make a 1 mg/ml stock solution. The samples (*Sargassum wightii* and *Ulva lactuca*) are added to each beaker containing saline solution in the following amounts: 100, 250, 500, 1000, 1500 µl. the seaweeds samples *Sargassum wightii* and *Ulva lactuca* are comparatively less toxic than potassium dichromate (K2Cr2O7) which shows maximum lethality of shrimps at higher concentration (1500 µg/ml). The shrimps in both the samples were found to be very less toxic in lower as well as higher concentration. Even after 24h, only 2-3 shrimps were found to be mortal at lowest (100 µg/ml) and highest concentration (1500 µg/ml). On comparison of brown and green seaweeds, *Sargassum wightii* and *Ulva lactuca, Sargassum wightii* was less toxic than *Ulva lactuca.* The table II provides the toxicity analysis of selected seaweeds.

Table 2: Toxicity analysis of selected seaweeds

Seaweeds	Concentration (μg/ml)	Mortality of Brine shrimp (No. of shrimps dead) (hour)						
		1	2	4	6	8	24	Mortality percentage (24hour)
Sargassum wightii	100	0	0	0	0	0	0	0
	250	0	0	0	0	0	1	3
	500	0	0	0	0	0	1	3
	1000	0	0	0	0	0	0	0
	1500	0	0	0	0	0	3	10
Ulva lactuca	100	0	0	0	0	1	1	3
	250	0	0	0	0	1	1	3
	500	0	0	0	0	1	2	6
	1000	0	0	0	0	2	2	6
	1500	0	0	0	0	1	3	10
Control ($K_2Cr_2O_7$)	1 (mg/ml)	30	-	-	-	-	-	100
Blank (Saline water)	Saline water	0	0	0	0	0	0	0

Analysis of nutritional composition

The analysis of nutritional composition of both seaweeds resulted as given in the table III, the micronutrient content shows that the species *Ulva lactuca* was rich in calcium (878 mg) and iron content (2 mg) where iodine content was rich in brown seaweeds *Sargassum wightii*. *Sargassum wightii* generally exhibits higher ash content, carbohydrate, and fibre, while *Ulva lactuca* has higher moisture, protein, fat, calcium, iron, iodine, and energy levels. On comparing the macronutrients, the brown seaweeds *Sargassum wightii* has highest amount of carbohydrates (55 g), protein (5 g), fat (0.21 g). Energy content (246 kcal) was also high in brown seaweeds of *Phaeophyceae* family *Sargassum wightii*. The green seaweeds *Ulva lactuca* has high composition of fibre (27.6 g) on comparing to *Sargassum wightii*.

Table 3: Nutritional composition of selected seaweeds

Nutrients	Composition of nutrients	
	Sargassum wightii	*Ulva lactuca*
Moisture (%)	10.98	9.59
Ash (%)	28.7	36.7
Carbohydrate (g)	54.97	48.53
Protein (g)	5.13	4.95
Fat (g)	0.21	0.27
Fiber (g)	24.1	27.6
Calcium (mg)	32.64	878.2
Iron (mg)	0.77	2.11
Iodine (mcg)	6.14	2.98
Energy (Kcal)	246	219.9

These differences highlight the diverse nutritional profiles of the two seaweeds, The incorporation of these seaweeds into diverse diets not only broadens nutrient intake but also offers a balanced array of essential elements, addressing various dietary requirements and promoting overall health and well-being.

Qualitative Screening for Phytochemicals

Phytochemicals serve as natural defense mechanisms in plants, helping them cope with environmental challenges like salinity and temperature. Table IV reveals that the brown seaweed Sargassum wightii possesses a more diverse array of phytochemicals, including flavonoids, alkaloids, sterols, cardiac glycosides, and carbohydrates.

In contrast, *Ulva lactuca* lacks sterols and cardiac glycosides. The presence of carbohydrates in both seaweeds implies the presence of various polysaccharides, suggesting potential therapeutic properties. These distinct phytochemical compositions highlight the unique bioactive compounds associated with each seaweed species, emphasizing their potential roles in contributing to human health and well-being.

Table 4: Phytochemicals screening of selected seaweeds

Phytochemicals	Test and observation	*Sargassum wightii*	*Ulva lactuca*
Flavonoids	+H_2SO_4 – Presence of reddish orange color	+	+
	+lead acetate – presence of white precipitate		
Alkaloids	+Dragendorff's reagent – presence of reddish brown precipitate	+	+
Sterols	Libermann test +$CHCl_3$ + Acetic anhydride + Conc H_2SO_4 – Presence of reddish brown ring	+	-
Cardiac glycosides	Keller-killani test – presence of brown ring	+	-
Carbohydrates	Molisch's test – presence of violet ring	+	+

+ indicates presence, - indicates absence

Analysis of antioxidant potential

From table V, it can be understood that when the concentration was 10 ml, the inhibition of Sargassum wightii was 53.91 percent and when the concentration was increased from 10 m to 50 ml, 150 ml, 250 ml, 350 ml and 750 ml the inhibition also increased as 54.74, 67.83, 68.70, 77.39 and 78.26 respectively. As the concentration increased, the inhibition also increased, hence it can be inferred that *Sargassum wightii* has remarkable antioxidant activity. The inhibition of *Ulva lactuca* at 10 ml was 60.00 percent and 54.78 percent at 50 percent. It can be seen from the table that as the concentration increases from 50 ml to 150 ml,250 ml, 350 ml and 750 ml there was an inverse in the inhibition of *Ulva lactuca*. Therefore, it can be deduced that *Ulva lactuca* had antioxidant activity and when compared with *Sargassum wightii*, the antioxidant activity of *Sargassum wightii* considerably greater than *Ulva lactuca*.

Table 5: Antioxidant activity of selected seaweeds

Concentration (ml)	DPPH % inhibition of *Sargassum wightii* at concentration of 8.54 mg/ml H_2O	DPPH % inhibition of *Ulva lactuca* at concentration of 8.1 mg/ml H_2O
10	53.91	60.00
50	54.74	54.78
150	67.83	51.30
250	68.70	49.57
350	77.39	49.57
750	78.26	47.83

These findings imply that both seaweeds possess antioxidant properties, with *Sargassum wightii* demonstrating a notably higher inhibition rate compared to *Ulva lactuca*. Antioxidants play a crucial role in neutralizing oxidative stress, which is linked to various chronic diseases. Therefore, the observed antioxidant potential suggests that these seaweeds could contribute to promoting health and preventing oxidative damage when incorporated into dietary patterns.

Conclusion

The analysis confirms *Sargassum wightii* and *Ulva lactuca* seaweeds are non-toxic, free from heavy metals, and exhibit low toxicity levels in the brine shrimp assay. Both seaweeds showcase diverse nutritional profiles, with *Sargassum wightii* being rich in carbohydrates, protein, and fat, while *Ulva lactuca* excels in fibre, calcium, and iron. Phytochemical screening reveals unique bioactive compounds in each species. *Sargassum wightii* exhibits significant antioxidant activity, surpassing *Ulva lactuca*. Overall, these findings emphasize the safety, nutritional richness, and potential health benefits of both seaweeds, positioning them as promising candidates for sustainable and nutritious dietary inclusion.

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