The Pharma Innovation Journal 2023; 12(3): 2637-2641

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 2637-2641

www.thepharmajournal.com
Received: 13-01-2023
Accepted: 21-02-2023

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Screening of micronutrient, trace element and phytochemical of Seaweed: *Sargassum wightii* Greville and *Kappaphycus alvarezii* (Doty)

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Abstract
Microalgae are an important source of biologically active metabolites with pharmacological significance. A preliminary study was conducted to identify the therapeutic compounds using phytochemical screening and micronutrient analysis for food fortification. In this research, the methanolic extract of *Sargassum wightii* (SW) and *Kappaphycus alvarezii* (KA) was screened for micronutrient, trace element and phytochemical content. The screening was done by Inductively coupled optical emission spectrometry (ICPOES) for minerals, inductively coupled plasma mass spectrometry (ICPMS) for Trace elements and Gas chromatography Mass Spectrometry (GCMS) for phytochemical screening. The results suggest that KA and SW possess high sodium (8079.00 & 7910.53 ppm), potassium (7543.96 & 7871.84 ppm) and manganese content (5792.50 & 6277.70 ppm) respectively. The major phytochemicals present were Hexadecenoic acid, methyl ester, heptadecane, naphthalene and 10-octadecanoic acid (Z, Z)-, methyl esters. The heavy metals found were above the permissible limit and are to be reduced to apply in food application. Therefore, these findings suggest that SW and KA extracts have potential applications in agriculture and pharmacology

Keywords: Kappaphycus alvarezii, Sargassum wightii, minerals, heavy metal and phytochemicals

1. Introduction

Human interventions and flooded population have become a challenge for the short comes of available natural resources. Demand and supply chain management of food and other bio resources attains huge attention to meet the requirement in 2050 (Tiwari & Troy, 2015) [22]. In the mean course of time, agricultural land, production and consumption would not be sufficient for near future to feed the population. Now a days food deception increased owing to reduction in overall food production and care should be taken will going for food alternatives (Kaavya *et al*., 2020) [9]. Thus an alternative source is required and so coastal environment, includes natural seaweed communities having balanced ecosystem, have attained popularity in the past decade and in recent years (Chung *et al*., 2017) [3]. Seaweed is a marine macroalgae contains a plant like structure usually attached to rock or other hard substrate in coastal areas. Seaweed in numerous countries, used as source of food, food packaging aid and food ingredient (Pati *et al*., 2016) [15]. Apart from food, it has widespread application in the field of pharmacological, clinical, biochemical, industrial, cosmetic and fertilizer (Couteau & Coiffard, 2016) [4]. Among the seaweed varieties, *Kappaphycus alvarezii* (KA) stands 5th in the world's largest cultivated macroalgae. It is a major industrial source of k-carrageenan with huge market potential. KA also called cotonii, belongs to the family Rhodophycear family. The polysaccharide contents were about 37% weight dry basis of algae. The products made from k-carrageenan are utilized as thickeners, stabilizers, emulsifying agents, a gelling agents for food application and help to assist in drug delivery systems, tooth paste, edible packaging, edible coating and antimicrobial packaging material (Rudke *et al*., 2020) [16].

Another major variety, *Sargassum* spp brown seaweed are known for their abundant vital amino acids including tryptophan, arginine and phenylalanine. They also contain fair content of vitamins, minerals and Beta-carotene, The *Sargassum wightii* (SW) grows abundantly during all the year in the Indian coastal line. Commercial use of *Sargassum spp is explored, in* its use in food, fuel and pharmaceutical products (Milledge *et al*., 2016) [14]. Thus, this study focused on both the variety of seaweed for their phytochemical content, minerals and trace elements.

KA was studied for its heavy metal bioaccumulation from seawater of Indonesia and found that Copper, Cadmium and lead accumulation was much high since it absorbs metals from seawater of different origin in Malaysia. The results also suggest that these accumulated metals again leach into water (i.e., the accumulation was inconsistent in nature) at the time of post harvesting process and during carrageenan production. It was also concluded that the accumulation was not permanent and so it is safe for human health (Tresnati *et al*., 2021) [23]. Another study on KA and *Kappaphycus* striatus in Malaysian wild offshore water was investigated for its heavy metals, minerals, soluble and fat-soluble antioxidant capacities and found that heavy metals (cadmium, lead, mercury and arsenic) were accumulation was not significantly different in all striatus and KA, whereas the mineral content namely Mn, Fe, Cu, Ni, Zn, Se and the antioxidants were predominant in KA species in comparison with *Kappaphycus* striatus and which could be suggested for use as feed additives owing to its chemical and nutritional endeavor (Ariano *et al*., 2021) [1]. (Suresh Kumar *et al*., 2015) [19] studied the seasonal variation of KA micronutrient content in Gujarat northwest coast of India were the mineral content recorded highest in April 2005, and least in January 2006.

(Thodhal Yoganandham *et al*., 2019) [21] studied the mineral and trace metals concentration of mandapam, coastal region, South India and the results found that there was a significant difference in mean concentration of all mineral and trace elements except Co, Cu and Zn. Similar study by (Y. Kumar *et al*., 2021) on *Sargassum wightii* and *Ulva rigida* of Kanyakumari, Mandapam. Tamil Nadu India was procured during September 2018 and its properties were evaluated. SW contains high amount of potassium (1.36± mg g^{-1}), magnesium (8.39±0.80 mg g^{-1}) and calcium content (14.03±3.46 mg g^{-1}) respectively. Ulva rigida contains high crude protein. Iron, carbon and Sulphur content. The results suggest that both varieties could possibly be used for manufacturing of functional food products.

Thus, seaweed has a shift in its nutrient content, minerals, heavy metals and phytochemical content based on factor such as geographical location, season, temperature, solar radiation, pH, salinity, nutrient availability, water quality, type of aquaculture, environmental pressure at the targeted location and socioeconomic impact. Region specific research has to be emphasized to understand the flora of particular region and this study is such an attempt to explore the micronutrient content and safety aspect for its application in pharmacology, agriculture and food.

2. Materials and methods

Seaweed samples were collected from Private firm that procures raw material from the Gulf of Mannar region (latitude 9˚ 17' N, longitude79˚ 22' E), Tamil Nadu, India. Freshly procured seaweeds were cleaned with distilled water and sun-dried for 3-4 days to reduce the moisture content and milled using mechanical grinder. All the dried samples were stored airtight in the freezer at -20°C until further processing.

2.1 Mineral analysis (ICPOES)

Reagents required - Ultra pure Milli-Q water, nitric acid from Sigma Aldrich, were used in all the digestion experiments. Ultra-pure argon (99.99%) and nitrogen (99.99%) was used as carrier gas.

The method mineral analysis was adopted from (Selmi *et al*., 2021) [17] with slight modification. In brief, the first step of mineral analysis was digestion performed using Microwave-assisted acid digestion system. The glassware's were washed with 10% nitric acid solution followed by Milli Q water wash for 24 hours prior to digestion. The seaweed sample of 0.5 g was pre-digested with 5mL of nitric acid in Teflon vessel capped for 20 minutes. The digestor was pre-heated at about 200°C for 20 minutes, followed by placing of Teflon vessels to digest in microwave digestor for 20 minutes. The digested samples were then transferred into 50mL polyethylene centrifuge vials. ICP-OES system used was made of Perkin Elmer, Avio 560 Max, USA. Argon gas was used as plasma gas and nitrogen as collision gas in the system. The standard reference material was used and compared with seaweed samples using inbuilt software.

2.2 Heavy metal content (ICPMS)

The heavy metal content of the dried KA and SW was estimated by AOAC, 21st Edition, 2019, method 2011.14. The digestion procedure in brief, the sample of 0.5g was mixed with concentrated HNO_3 for 15 minutes. The mixture was subjected to microwave digestion (Multiwave GO, Anton-Paar, Graz, Australia) heated to 120°C for 8 minutes, then temperature ramped up to 180°C and held for 15 minutes. Then the sample was cooled to room temperature and injected into ICP-MS (Nexion 800X, Perkin Elmer,Waltham, USA) system. The Mass spectra was analysed using Helium KED mode and quantification done by multi-element standard for ICP (LOBA Chemie, Mumbai, India).

2.3 Phytochemical content (GC-MS)

The standard protocol described by (Chakraborty & Paulraj, 2010) [2] & (Dakshayani *et al*., 2021) [5], was followed to perform phytochemical screening on both seaweed samples. The samples were extracted using methanol as a solvent and analyzed using TSQ 9000 Triple Quadrupole with TSQ Quadrupole Mass Spectrometer. The resulting chromatograms were used to determine the relative percentage of each component by comparing the peak area with the total area, using Turbo Mass software, which helps in handling chromatogram and spectra. The GCMS/MS conditions used were as follows.

Table 1: GCMS/MS operating condition

GC parameter	Operating condition
GC column	30m x 0.25 mm ID x 1.4μm df
Sample injection volume	1μl
Oven Temperature program	110°C for 3.50 minutes, to 200°C then to 280°C at the rate of 5°C/min-12 min hold
Injector temperature	280°C
MS Programme	
NIST Version	2011
Inlet line Temperature	290°C
Electron energy	70eV
Mass scan (m/z)	50-500amu

2.4 Statistical Analysis
The analysis of the results was carried out using one-way analysis of variance (ANOVA) through SPSS version 10.0. A significant level of 95% (p=0.05) was maintained throughout the analysis and differences between the various treatments were determined through post hoc measurements. The measurements were conducted thrice (n=3) to ensure accuracy ant the values obtained were presented as the mean of the three replicated determinations.

3. Results and Discussion
3.1 Mineral analysis (ICPOES)
Minerals are the elements present on the earth and in foods required for our body organs to develop and needs for proper functioning of the body. The mineral content of SW and KA were given in the table 2. The KA of this study possessed higher Fe, Mg, Mn content and lower Ca, P, Zn content when compared to study by (Suresh Kumar *et al*., 2015) [19]. Another study on mineral content by (K. S. Kumar *et al*., 2022) [10] was performed and compared with this results found that Na, Ca, Mg and Mn were found high in this study, whereas K, P, Zn, Cu and Fe were lower. In all the studies the Mg and Mn content were found to be high in this study, the Mg could help in regulating muscles, nerve function, blood sugar level, blood pressure and making protein, bone and DNA, whereas the Mn helps in fat and carbohydrate metabolism, calcium absorption, brain and nerve function. The above studies were done in same location at different time period with respect to our study. The SW possessed higher Cu, Mn, K, Zn, Na in comparison with study by (Y. Kumar *et al*., 2021) [12] whereas the other minerals found to be likely lower in concentration namely Ca, Fe, Mg and P. Another study on mineral content of SW by (S. Kumar *et al*., 2015) [11] was compared with our sample and found that Cu content was found high in this study, on the other hand the Zn and Fe content was reported lower in this study by comparison. Yet another study by (Syad *et al*., 2013) [20] on SW in which samples were collected from same region of our study were evaluated and found that Na, K, Ca, Fe, Mg, Zn was higher in this study in comparison with the other studies. The major minerals content in this study of SW was reported as Cu, K, Zn where Cu helps our body to regulate red blood cells, keeps nerve cells and immune system healthy, K helps in maintaining normal level of fluid inside our cells, Zn much needed for the body defense mechanism. Thus, from the above study it could be concluded that though the same sample from same location at different time period could definitely affect the bioaccumulation in seaweed. These variation in KA and SW sample with respect to mineral content of other comparative study may be due to the elemental bioaccumulation of seaweed. These bioaccumulations were greatly affected by season, thallus age, pH, habitat and exposure to residential and industrial effluents of the water similarly reported by (K. S. Kumar *et al*., 2022) [10]. This study was also compared with the recommended dietary allowance per day and found that all the minerals analyzed were within the upper bound of the daily intake.

3.2 Heavy metal content (ICP-MS)
Heavy metals are metal chemical element that possesses a high density and toxic or poisonous at short concentration. These metals are naturally occurring components of the earth's crust and cannot be degraded or destroyed. The heavy metal content namely arsenic, lead, cadmium and mercury were majorly found in seaweed were measured for both KA and SW samples presented in table 3, it was found that Arsenic content was very high in KA and above the acceptable limit for food application, whereas the SW had arsenic content below the limit of FSSAI (1.1 ppm) standards. The KA variety also had lead content above the permissible limit of FSSAI, whereas the cadmium and mercury content were found to be below the limit described by FSSAI standards. The SW was found to have all the heavy metal content namely lead, cadmium, mercury below the permissible limit (FSSAI, 2011) [8].

3.3 GCMS
The phytochemical screening of seaweeds KA and SW was done using GC-MS and are provided in table 4. The phytochemicals are phytonutrients, potentially helpful compounds in plant foods, which helps preventing chronic diseases. The results of both the seaweed were almost similar and found about eight components in KA and 14 components in SW. The major phytochemical revealed in KA was Naphthalene 33.87% peak area, heptadecane of 26.26%, hexadecenoic acid, methyl ester was 24.31%, whereas SW reported was hexadecenoic acid, methyl ester with peak area of 39.19% and Naphthalene of 20.82% respectively. The biological activity of naphthalene was antimicrobial activity, antioxidant activity, anti-inflammatory, anti-protozoal and anti-platelet aggregation activity reported by (Ibrahim & Mohamed, 2016) [7], hexadecenoic acid, methyl ester was anti-fungal and anti-bacterial in nature reported by (Stabili *et al*., 2012) [18]. The heptadecane was majorly found unsaturated hydrocarbon in brown and red seaweeds reported by (López-Pérez *et al*., 2017) [13] with antimicrobial activity (Goudarzian *et al*., 2017) [6] respectively.

Table 1: Nutritional profile of KA and SW

Mineral Content (ppm)	Ca	Cu	Fe	Mg	Mn	K	Zn	Na	P
KA	1120.62±0.99b	91.70±0.52a	491.70±0.44a	1192.42±0.4b	5994.28±1.4b	7562.60±1.4b	72.52±2.05a	8077.17±1.8a	278.533±0.80a
SW	1218.36±0.9a	85.633±0.61b	343.97±0.81b	2825.40±0.9a	6283.23±2.3a	7858.20±1.52a	49.67±1.89b	7912.08±1.38b	397.567±0.41b

Table 2: Heavy metal content of Seaweed (KA and SW)

Sample Name	Arsenic (mg/kg)	Lead (mg/kg)	Cadmium (mg/kg)	Mercury (mg/kg)
KA	56.53	253	1.33	ND
SW	1.08	ND	1.48	ND
FSSAI standard	1.1	5	1.5	1.0

Table 3: Phytochemical screening of seaweed (KA and SW)

No	RT (min)		Name of the compound	Molecular	Molecular weight	Peak area %	
	KA	SW				KA	SW
1.	5.27	5.26	Naphthalene	C10H8	128	33.87	20.82
2.	10.49	-	Ethisterone	C21H28O2	312	02.67	-
3.	12.54	-	Heptadecane	C17H36	240	26.26	-
4.	12.83	-	Oxiraneundecanoic acid,3-pentyl-methyl ester, cis-	C19H36O3	312	01.29	-
5.	14.23	-	11,14-Eicosadienoic acid, methyl ester	C20H38O2	322	04.55	-
6.	15.07	-	Cyclo propane octanoic acid,2-octyl-, methyl ester	C20H38O2	310	01.48	-
7.	15.20	15.20	Hexadecanoic acid, metyl ester	C17H34O2	270	24.31	39.19
8.	16.09	-	Cyclopentaneundecanoic acid, methyl ester	C17H32O2	268	05.57	-
9.	-	9.66	Cis-5,8,11,14,17- Eicosapentaenoic acid	C20H30O2	302	-	2.05
10.	-	12.29	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis	C28H44O4	444	-	3.40
11.	-	12.29	Myristic acid, methyl ester	C15H30O2	242	-	3.75
12.	-	13.15	3-buten-2-one,4-(6,6-dimethyl-1-cyclohexen-1yl)	C12H18O	178	-	5.41
13.	-	14.16	Z, E-3,13-Octasecadien-1-ol	C18H34O	266	-	1.76
14.	-	14.24	Dotriacontane	C32H66	450	-	1.78
15.	-	14.96	9-Hexadecanoic acid, methyl ester	C17H32O2	268	-	2.05
16.	-	17.26	Cis-13-Eicosenoic acid	C20H38O2	310	-	3.07
17.	-	17.44	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294	-	2.70
18.	-	17.51	10-Octadecenoic acid, methyl ester	C19H36O2	296	-	10.86
19.	-	17.85	Docosanoic acid, methyl ester	C23H46O2	354	-	1.57

4. Conclusion

The study has identified the high risk of heavy metal contaminant in KA and are not suitable for food application, whereas SW could possibly apply in area of food application. If in case, it has to be applied in food, measures for reduction of heavy metal contamination have to be addressed. The KA and SW samples contains rich source of minerals and have greater antioxidant potential. There is a huge scope on extraction of particular mineral for the seaweed and could potentially be applied in food as bio-fortification agent. The majorly found component of GCMS possess antioxidant, antimicrobial, anti-inflammatory, anti-protozoal, anti-fungal and antibacterial effect. Therefore, extraction of bioactive compounds from KA and SW species could also be a potential area of future research and could possibly be a natural source of antioxidant in near future as an alternative to Butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) respectively. Therefore, in comparison with both variety, SW could be used in both food and non-food application.

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