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Fatty acid, micronutrient, proximate composition and phytochemical analysis of red seaweed *Tricleocarpa fragilis* (L.) Huisman & R.A. towns from Andaman Sea, India

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Abstract
In the present study red seaweed *Tricleocarpa fragilis* from Andaman Sea was investigated for its proximate composition viz., Carbohydrate (28.76%), Ash (42.29%), Moisture (24.05%), crude fiber (12.05%), lipid content (0.84%) and Protein (4.07%). The quantitative phytochemical analysis revealed that the total phenol and flavonoid content were (0.26%) and (0.83%) respectively. DPPH free radical scavenging activity showed 76% of antioxidant activity. Elemental composition (in ppm) of N (6500±0.72), B (61.342±0.22), Ca (26200±0.54), Cu (7.114±1.39), Fe (3000±0.63), Mg (5600±0.15), Mn (5600±0.15), K (20900 ±0.33), Na (16900±0.20) and Zn (11.37±0.46) were estimated. There were six types of fatty acids in the thallus of *T. fragilis* i.e. one type of monounsaturated fatty acids (MUFA) upto 58%, four types of saturated fatty acid (SAFA) upto 38%, and another polyunsaturated fatty acid (PUFA) upto 3.86% composition. These results suggest that the species has greater potential to be used as fuel, fodder and fertilizer and can be utilized as seaweed based industry from the Andaman Sea, India.

Keywords: macroalgae, *Tricleocarpa fragilis*, micronutrient, fatty acid, south andaman

1. Introduction
Seaweeds are macrothalic marine algae, which are a renewable natural resource and found luxuriantly along the coasts of India. Their distribution along the Indian coast is mainly reported from Gulf of Mannar (Sathianeson and Samuel, 2012; Doss and Rukshana, 2016; Mary *et al*. 2013; Rani *et al*. 2015) [43, 10, 29, 41], Gulf of Kutch (Malsatar and Mehta, 2017; Suparna *et al*. 2015) [25, 50] and Andaman & Nicobar Islands (Gopinathan and Panigrahy, 1983; Mantri, 2005; Subba Rao and Mantri, 2006; Palanisamy, 2012; Karthick *et al*. 2013) [14, 27, 49, 38, 21]. As it is well recorded that marine seaweed resources have been used as food, feed, fertilizer and also as an unique source of traditional medicines in many countries (Zahid, 1999; Lakshmana *et al*. 2013; Evans and Critchley, 2014; Massoumeh *et al*. 2014; Maria and Combet, 2015) [55, 24, 12, 30, 28]. To mention a few, seaweeds also serve as a major sources of vitamins, proteins, carbohydrates, minerals and other bioactive compounds (Manivannan *et al*. 2008; Seenivasan *et al*. 2012; Zeliha, 2012; Isaiah *et al*. 2015) [26, 44, 57, 15]. Simultaneously other studies have reported that seaweeds contain more than 60 trace elements in much higher concentration than in terrestrial plants including the essential primary and secondary micro and macro nutrients, which is required for plant cell division, growth and development and making it as excellent fertilizer (Chennubhotla *et al*. 1991; Zopade, 2001; Mohanty *et al*. 2013; Jayasree *et al*. 2012) [7, 58, 32, 17]. In this regard several other studies on the biochemical and nutritional composition of various seaweeds collected from different parts of the world have been conducted with a view to utilize their nutritional value (Dhargalkar *et al*. 1980; Manivannan *et al*. 2008; Jayshree *et al*. 2012; Kiuomars *et al*. 2012; Cosman *et al*. 2013; Norziah and Ching, 2000; Parthiban *et al*. 2013; Rajababu *et al*. 2017) [9, 26, 17, 23, 8, 37, 39, 40]. In recent years, great interest has been taken to develop commercial seaweed farming to harness seaweeds as food, feed and fuel (Chennubholta *et al*. 1991; Vinoj and Kaladharan, 2007; Bindu and Levine, 2010; Bjorn *et al*. 2012) [7, 52, 3, 4]. Study by Gopinathan and Panigrahy (1983) [14] reported that seaweeds are found abundantly in Andaman Sea and is potential to be used commercially. One such red seaweed *Tricleocarpa fragilis*, which grows luxuriantly in Andaman Sea was found to be underutilized and remains unexplored. Also this species lacks any specific study pertaining to its biochemical composition. Thus this species was studied for its biochemical composition and to ascertain their potential for use in different forms as food, feed and fuel with probable commercial use for Island economy development.

In the present study the proximate, fatty acid and mineral composition of the red seaweed *T. fragilis* collected from the Coast of South Andaman, Andaman Sea, India was investigated.

2. Materials and Methods

2.1 Sample Collection and preparation

Tricleocarpa fragilis (Fig. 1) was collected by hand picking from the coast of Marina Park (Sesostris Bay) (Lat. 11˚66.927' N; Long. 92˚74.9347' E) along South Andaman coast during low tide. The collected seaweed was first washed with seawater and then brought to the laboratory and washed again under tap water to remove any sand particles and epiphytes followed by washing with distilled water. The moisture content of the fresh sample was immediately analyzed. The remaining cleaned sample was shade dried for a week at ambient temperature and then the dried sample was powdered using electronic blender. The powdered sample was then stored in 4 ˚C for further analysis.

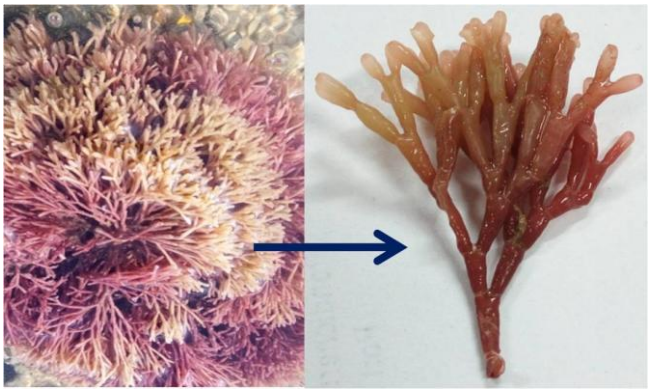

Fig 1: Image of *Tricleocarpa fragilis* (L.) Huisman & R.A. Towns from South Andaman Sea, India

2.2 Proximate composition analysis

2.2.1 Estimation of Moisture Content

Moisture content of *T. fragilis* was determined according to the method described by AOAC (2000) [1] with modifications. Samples (2 g) were put in a petridish and dried in a hot air oven at 105 °C until constant weights were obtained.

2.2.2 Estimation of Carbohydrate Content

The total carbohydrate was estimated by following the phenol-sulphuric acid method by Dubois *et al*. (1956) [11]. The carbohydrate content was calculated by referring to a standard D-glucose and the results are expressed in percentage.

2.2.3 Estimation of Ash Content

Ash content of *T. fragilis* was determined according to the method described by AOAC (2000) [1] with slight modifications. Dried samples obtained from the moisture content analysis were burnt and ashed in a muffle furnace (Kasba PID-964) at 525 °C overnight.

2.2.4 Estimation of Crude Fiber

Crude fiber was determined by sequential extraction of seaweed samples with 1.25% H_2SO_4 and 1.25% NaOH using the fibre-bag as a container. For drying and ashing, the crucible with sample was dried in an oven for 5 hours at 105 °C and ashed in the muffle furnace (Kasba PID-964) at 525°Covernight. The weight of crucible with sample after drying and ashing was recorded and the fiber content was calculated (AOAC, 2000) [1].

2.2.5 Estimation of Lipid

The lipid was estimated by using chloroform-methanol mixture as described by Folch *et al*. (1956) [13]**.** In this 10 mg of dried powder sample was taken in a test tube and 5 ml of chloroform- methanol mixture (2:1) was added. The mixture was then incubated at room temperature for 24 hrs followed by filtration using a filter paper. The filtrate was collected in a 10 ml pre weighed beaker, which was kept on a hot plate. The chloroform-methanol mixture was evaporated leaving a residue at the bottom of the beaker. The beaker with the residue and the weight of the empty beaker was calculated to know the weight of the lipid present in the sample.

2.2.6 Estimation of Protein

The protein content was estimated by Biurette method followed by Raymont *et al*. (1964) [42]. In this 5 mg of dried powdered sample was taken in a tube and 1ml of distilled water was added to this followed by addition of 4ml of biurette reagent and the sample was incubated for 30 minutes in room temperature. Then the mixture was centrifuged for 10 minutes at 4000 rpm. The supernatant solution was collected and the optical density was measured in a Spectrophotometer (Eppendorf AG- 6135) at 540 nm.

2.3 Phytochemical analysis

2.3.1 Total Phenolic content

The amount of total phenolic content was determined with Folin and Ciocalteu's reagent according to the method of Singleton and Rossi (1965) [46] with Gallic acid as the standard. Briefly 0.1 ml of sample extract was mixed with 1 ml of Folin and Ciocalteu's reagent (1:2 with water) was added and kept at room temperature for 5 min and then 1 ml of 7% sodium carbonate solution was added to the reaction mixture and incubated at room temperature for 90 minutes. The colour developed was read at 750 nm.

2.3.2 Total flavonoid content

Total flavonoid content was estimated using aluminium chloride method (Chang *et al*. 2002) [5] with quercetin as standard. 0.5 ml of sample extract was mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of 1M potassium acetate and the final volume was adjusted by adding 2.8 ml of distilled water. The reaction mixture was mixed properly and allowed to stand for 30 minutes at room temperature after which the absorbance was measured at 415 nm.

2.4 Total antioxidant activity DPPH radical scavenging activity

Total antioxidant activity was determined using 1, 1-Diphenyl-2-Picrylhydrazine (DPPH) method (Yen and Chen, 1955) [54]. Briefly, 2 ml of test sample was added to 2 ml of 0.16 mM DPPH methanolic solution. The mixture was mixed for one minute and then left to stand at room temperature for 30 minutes in dark condition, and then its absorbance was measured at 517 nm. The percentage of scavenging activity of the DPPH radical was calculated using the following equation; DPPH scavenging activity (%) = [(Ac-As) /Ac] ×100 where Ac is the absorbance of the control (2 ml of distilled water with 2 ml of 0.16 mM DPPH methanolic solution) and As is the absorbance of the sample.

2.5 Micronutrient Estimation

The samples for micronutrient estimation were prepared using muffle furnace and elements were analysed through Atomic

Absorption Spectrophotometer (AAS; Shimandzu AA 6200, Scientific Instruments Inc. Columbia, USA).

2.6 Fatty acid Estimation

Fatty acids were analysed for the sample using direct transesterification method, adapted from Bjorn *et al*. (2012) [4] briefly 2ml freshly prepared methylation mixture [methanol, acetyl chloride, 20:1 (v/v)] and 300µl internal standard solution of nonadecanoic acid at a concentration of 0.2 mg/ml in methanol was added to approximately 50 mg of dried seaweed powder. The samples were heated at 100 ˚C for 1 hour, and then allowed to cool down before 1ml of hexane was added. To ensure complete partitioning of FAMEs into the hexane phase, samples were again heated to 100 ˚C for 1 minute, during which a single methanol/hexane phase formed. Two ml of deionized water was then added to facilitate phase separation. The hexane upper phase containing the FAMEs was collected and filtered through a 0.2 µm syringe filter prior to injection on the GC column.

3. Result and Discussion

3.1 Proximate composition analysis

The proximate composition analysis of *Tricleocarpa fragilis* is presented in Table -1. Six components such as total moisture, total carbohydrate, total Ash, Crude fiber, total lipid and total protein content were estimated. Total moisture content in fresh *T. fragilis* sample was estimated to be 24.05%. The total carbohydrate content was found to be 28.76%, which happens to be the most important component for metabolism as it supplies the energy needed for respiration and other metabolic processes (Murugaiyan *et al*. 2012) [34]. Dhargalkar *et al*. (1980) [9] reported higher values of carbohydrate content found in red algae than in brown and green algae and suggested that this might be due to the higher phycocolloid content in their cell walls.

The percentage of ash content in *T. fragilis* was 42.29%, which is higher than other red seaweeds, when comparable to those reported in other species i.e. *Hypnea japonica* (22.10%), *Hypnea charoides* (22.80%), *Hypnea musciformis* (21.57%) and *Gracilaria changgi* (22.70%) (Norziah and Ching, 2000; Wong and Cheung, 2000; Siddique *et al*. 2013) [37, 53, 45]. In general, high level of ash is associated with the amount of mineral elements.

The crude fiber content in *T. fragilis* was estimated at 12.05%. Although fiber cannot be digested in the intestinal digestion, it helps bind digestive enzymes, cholesterol, glucose, and subsequent toxin excreted through faeces. Consumption of fiber containing components protects organism against a number of chronic disease like colon cancer (Kaliaperumal *et al*. 1987) [19].

The total lipid content was estimated as 0.84% in *T. fragilis.* Protein content of *T. fragilis* was estimated as 4.07%. This high protein content of the species is one of the important constituents for projecting the species as supplemental food and can also be beneficial as animal feed, including aquaculture, farm animals and pets. Protein has crucial functions in all the biological process and an estimated 30% of global algal production is used for animal feed due to its excellent nutritional profile (Becker, 2004) [2]. It has been reported that macroalgae can be incorporated as protein sources into the diets of poultry, pigs, cattle, sheep, and rabbits (Stephen and Maria, 2017) [48].

3.2 Phytochemical analysis

The quantitative estimation of phytochemicals including phenolic and flavonoid compounds is presented in Table -1. The total phenolic and flavonoid were estimated to be 0.26% and 0.86% respectively. Both phenolic and flavonoid are reported to have antioxidant properties responsible for quenching the reactive oxygen species from body system and boost the homeostasis mechanism in cells for neutralizing the free radicals. There is evidence that under oxidative stressful conditions, oxygen radicals such as superoxide anion (O_2), hydroxyl radical (OH) and peroxyl radicals (H_2O_2) are produced in biological systems. These reactive oxygen species (ROS) lead to oxidative damage to cellular components such as proteins, lipids and DNA, which enhance the degenerative processes such as ageing, cardiovascular diseases, cancer, Alzheimer's disease and other neurodegenerative diseases (Smith *et al*. 1996) [47].

3.3 Antioxidant activity

The antioxidant activity of *T. fragilis* was estimated to be 76%. DPPH is a stable free radical in methanol and accepts an electron or hydrogen radical to turn into stable diamagnetic molecule. It is simple and most commonly used as a substrate to evaluate the antioxidant activity (Parthiban *et al*. 2013) [39].

Table 1: Proximate composition analysis of *Tricleocarpa fragilis*

Sl. No	Proximate composition	Composition (%)
1.	Moisture content	24.05
2.	Carbohydrate	28.76
3.	Total Ash content	42.29
4.	Dietary Fiber	12.05
5.	Total lipid content	0.84
6.	Total protein content	4.07
	Phytochemicals	
1.	Total Phenolic compounds	0.26
2.	Total Flavonoid compounds	0.83
	Antioxidant activity	
1.	DPPH free radical scavenging activity	76

3.4 Micronutrient estimation

A total of ten elements were estimated in *T. fragilis* (Table - 2). The result shows that *T. fragilis* is rich in micro and macro nutrients and can be used as biofertilizer, which will support the plant growth and development and also it will be highly applicable to treat the leached acid soil due to the presence of high calcium content. Kiuomars *et al*. (2012) [23] evaluated mineral composition of green, brown and red seaweeds from the Persian Gulf of Iran and it was reported that seaweeds contained higher amount of K, Mg, Fe, Mn, Cu, Zn and Co compared to terrestrial vegetables. As per their report, seaweeds could potentially be used as feed additive, which is in concurrence with the present investigation involving red seaweed *Tricleocarpa fragilis*. Another study by Manivannan *et al*. (2008) [26] reported that mineral composition in Chlorophyceae, Phaeophyceae and Rhodophycaee and reported the concentration of Fe (10.88± 1.68 ppm), Mg (39.89± 4.56 ppm), Mn (11.22±0.505 ppm) and Na (293.3± 15.2 ppm) in Rhodophycaea *Hypnea valentiae*. Similarly, Narasimman and Murugaiyan (2013) [35] reported that in red seaweed *Amphiroa fragilissima* from Gulf of Mannar region, Southeast coast of India, the concentration of elements (in ppm) found to be as Al (97.68±7.93), B (9.17±0.61), Cd (0.38±0.63), Co (1.29±0.71), Cr (7.36±0.96), Cu (3.42±0.73), Fe (100.6±7.89), Mg (529.1±45.96), Mn (29.67±2.39), Ni (1.89±0.53), Pb (4.69±0.86), Zn (3.75±0.89). Another study

by Rajubabu *et al*. (2017) reported concentration of K, Ca, Mo, Mn, Fe, Co, Ni, Cu, Zn, I, Na, Mg, Se and P in seaweeds of Vishakhapatnam coast (India). There are many reports suggesting the seaweeds can be used as biofertilizer due its rich mineral content in the era of green agriculture. (Zahid 1999 and Mohanty *et al*. 2013) [55, 32].

Table 2: Concentration of micronutrients present in *Tricleocarpa fragilis* (in ppm)

S. No	Name of the Element	Values (in ppm)
1.	Nitrogen	6500 ± 0.72
2.	Boron	61.342 ± 0.22
3.	Calcium	26200 ± 0.54
4.	Copper	7.114 ± 1.39
5.	Iron	3000 ± 0.63
6.	Magnesium	5600 ± 0.15
7.	Manganese	101.33 ±0.81
8.	Potassium	20900 ±0.33
9.	Sodium	16900 ± 0.20
10.	Zinc	11.37 ± 0.46

3.5 Fatty acid profiling

Based on GC-MS analysis, various fatty acids identified into six different types, which consist of four types of saturated fatty acids (SAFA), one type of monounsaturated fatty acids (MUFA) and one type of polyunsaturated fatty acids (PUFA). The types of fatty acids and its concentration were shown in (Table-3). The dominant fatty acid identified is Oleic acid which is a monounsaturated fatty acid and constitute 58% of the total fatty acid followed by group of saturated fatty acids (Tridecanoic acid, Capric acid, Palmitic acid and Stearic acid), which constitute 38% of the total fatty acid and the minimum constitute 3.86% of total fatty acid by polyunsaturated fatty acid 11, 14 Eicosadienoic acid (Fig. 2).

Table 3: Fatty acid profiling of *Tricleocarpa fragilis*

S. No	Fatty acid Profile	Carbon chain -numbers	Chemical formula	Type of Fatty acids	Result (%)
1.	Tridecanoic acid	C13:0	$CH_3(CH_2)_{11}COOH$	SAFA	1.83
2.	Capric acid	C10:0	$CH_3(CH_2)_8COOH$	SAFA	1.51
3.	Palmitic acid	C16:0	$CH_3(CH_2)_{14}COOH$	SAFA	23.23
4.	Stearic acid	C18:0	$CH_3(CH_2)_{16}COOH$	SAFA	11.44
5.	Elaidic acid	C18:1n9t	$C_{18}H_{34}O_2$	MUFA	58.13
6.	11, 14 Eicosadienoic acid	C20:2	$C_{20}H_{36}O_2$	PUFA	3.86

SAFA- Saturated fatty acids; MUFA- Monounsaturated fatty acids; PUFA- Poly unsaturated fatty acids.

Similarly Jayasree *et al*. (2012) [17] reported 12 fatty acid components in red seaweed *Amphiroa anceps*. The report revealed its saturated fatty acids include palmitic acid (57.57%), myristic acid (4.25%), stearic acid (2.70%) and lignoceric acid (0.34%). Monounsaturated fatty acids include palmitoleic acid (2.48%), oleic acid (5.77%), erucic acid (0.11%), and polyunsaturated fatty acids are lignoceric acid (4.51%), arachidonic acid (18.67%), eicosapentaenoic acid (2.46%), docosahexaenoic acid (0.96%). A study in the thallus of brown algae, *Sargassum duplicatum* reported nine types of saturated fatty acid (SAFA) constituting 64.32%, three types of monounsaturated fatty acids (MUFA) upto 29.16% and five types of polyunsaturated fatty acid (PUFA) upto 5.80% (Zailanie and Kartikaningsih, 2016) [56]. Similarly, Kamariah *et al*. (2017) [20] invetigated the fatty acids compositions of *Sargassum granuliferum* and *Dictyota dichotoma* and their anti-fouling activities, which revealed that palmitic acid, elaidic acid, stearic acid, *cis*-11, 14, 17-eicosatrienoic acid and erucic acid were dominant in both seaweeds with co poly- and mono-unsaturated fatty acids were higher than the saturated fatty acids. Khotimchenko *et al*. (2002) [22] reported four dominant fatty acids viz., palmitic (16:0), oleic (18:1n-9), arachidonic (20:4n-6) and eicosapentaenoic (20:5n-3) in 7 red algal species belonging to the orders Cryptonemiales, Gigartinales and Ceramiales. A study of fatty acids of red seaweed *Gracilaria manilaensis* reported 35 fatty acids, where 25.47% accounted for saturated fatty acids, 35.70% for monounsaturated fatty acids and 42.18% for polyunsaturated fatty acids (PUFAs) (Nor *et al*. 2013) [36].

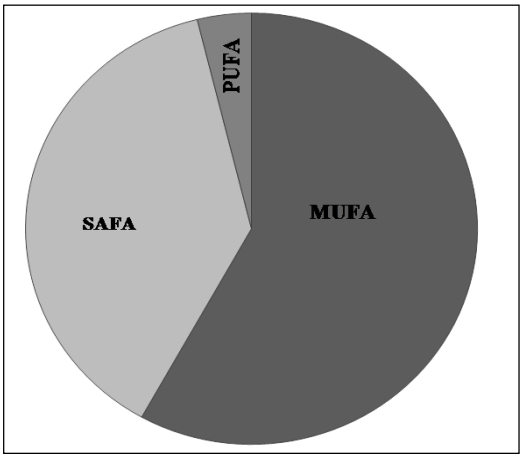

Fig 2: Total fatty acid content of *T. Fragilis*

4. Conclusion

This study revealed that extracts of *T. fragilis* has a good proximate composition of carbohydrate, protein, crude fiber and lipid which can be used as fodder for the livestock. *T. fragilis* being a rich source of minerals and growth promoters, they can be of immense help to the coastal farmers for their use as a source of organic fertilizer. The high content in important fatty acids especially the SAFA and MUFA make both species good candidates for biodiesel, and PUFA for nutritive food source.

5. Acknowledgement

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