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Potential of *Gracilaria tenuistipitata* var. *liui* grown in Nuniachara, Cox's Bazar, Bangladesh

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Abstract

During selection of *Gracilaria tenuistipitata* var. *liui* (red seaweed) growing naturally at Nuniachara sand-flat south-east of Moheshkhali channel, Cox's Bazar local fishermen informed that Rakhaine and Chakma tribes of Cox's Bazar and Bandarbon consume the seaweed. To ascertain the quality of the organism as "Seafood" analysis of its nutritional status was carried out using three replicates in many cases. Percentage composition of total crude protein, crude fiber, crude lipid, carbohydrates, ash and moisture were 25.55 ± 0.18, 5.65 ± 0.13, 0.16 ± 0.03, 45.93 ± 1.53, 10.61 ± 0.69 and 12.10 ± 0.25%, respectively. Mineral contents such as phosphorus, calcium, magnesium, iron and copper were 596.90 ± 10.4, 132.75 ± 3.4, 3.90 ± 1.2, 80.13 ± 2.45 and3.99 ± 1.2mg/100gdry wt., respectively. Heavy metals such as Pb, As, Cr and Cd were 0.031, 0.01, 0.06 and 0.02 mg/kg dry wt. respectively, which are below tolerance level. The seaweed contained 9.03% nine essential amino acids and 7.52% five non-essential amino acids. The quantity of total energy, β-carotene and vitamin C were 294.56 kcal/100g, 11.54 ± 1.20 and 2.5 mg/100g, respectively. Presence of high crude protein, amino acid profiles, β-carotene, phosphorus and low crude lipid and heavy metals made *Gracilaria tenuistipitata* var. *liui* to be considered as a good human food supplement.

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Introduction

Seaweeds of family Gracilariaceae (Rhodophytceae) are economically very important for valuable sources such as agar-agar (Santos 1990; Kumar and Fotedar 2009; Sousa *et al.,* 2008), protein, fiber, fatty acids, vitamins, macro and trace elements as well as important bioactive compounds (Darcy-Vrillon 1993; Mabeau and Fleurence 1993; Fleurence 1999; Ortiz *et al.,* 2006). Consumption of edible seaweeds has a positive effect on human health reducing blood lipid levels, obesity, and risk of coronary heart diseases (Critchley *et al.,* 2006; Benjama and Masniyom, 2011). In the Philippines *Gracilaria tenuistipitata* var. *liui* is consumed as a sea vegetable, and intensively cultivated in China and Taiwan for food (Haglund and Pedersen, 1993; Tseng and Xia, 1999). In Malaysia, several species e.g. *G. changii* and *G. tenuistipitata* are used as salads and for agar extraction (Phang, 2006). Agar is extensively used in the production of Jam and jelly, cosmetics, etc. and pharmaceutical industries, as well as in microbiological researches.

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Due to the combined impacts of climate change, current cropping and consumption patterns, the seemingly plentiful and cheap resources are becoming limited and has been estimated that an equivalent of more than two Earths will be needed by 2050 to support the rapidly growing global population (Tiwari and Troy, 2015). Bangladesh is affected by population boom and frequent tidal bore along its 710 km coast against Bay of Bengal. Once crop fields are flooded with saline water about 7 years needed to produce land crops. Under the circumstances Bangladesh Agricultural Research Institute coordinated by BARC took a project on "Capacity building for conducting Adaptive Trials on Seaweed Cultivation in Coastal Areas" to provide nutritious seaweeds as food supplement (Anonymous, 2019; Aziz, 2015; Ito and Hori 1989). In fact the *G. tenuistipitata* var. *liui* growing on the sand-flat of Nuniachara beside Moshekhali Channel, Cox's Bazaris is being consumed by Rakhaine and Chakma tribes. Therefore, nutritional analysis of the seaweed grown in large scale at Nuniachara has been carried out.

Materials and methods

Samples collection

Sundried sample of *G. tenuistipitata* var. *liui* (which on thorough study has been found to be a new record for Bangladesh (Aziz, 2020)) grown at Nuniachara sand-flat southeast of Moheshkhali Channel, Cox's Bazar under the Project "Adaptive Trials on Seaweed Cultivation in Coastal Areas", implemented by Bangladesh Agricultural Research Institute, Gazipur, Coordinated by Bangladesh Agricultural Research Council, Farm Gate, Dhaka was supplied in zip-lock polybags for nutritional analysis.

Chemical analysis

Determination of crude protein

Crude protein content was determined by Micro-Kjeldahl method (Guebel *et al.,* 1991). Powdered 0.5g sample was mixed in 10 ml of concentrated H_2SO_4 and 1:1 g digestion mixture (sodium sulphate and mercuric oxide) and heated for 6 h. It was diluted up to 100 ml with distilled water 10 ml of which was transferred in a micro-Kjeldahl distillation apparatus and distilled for 10 min. The distillate was collected in excess of 2% boric acid solutions with indicator and was titrated by 0.02 N HCl. It was compared with a similar blank digestion.

Determination of crude lipid

Crude lipid content of seaweed was determined by following Mehlenbacher (1960). Powdered 10 g sample was mixed with 150 ml n-Hexane and petroleum benzine solvents at 90:60 ratio. Crude lipid was extracted using Soxlet apparatus. Powdered 10 g extract was kept in the extraction thimble for eight hours and weighed again. Deference in the weight represented crude lipid content.

Determination of crude fiber

Crude fiber content was measured following AOAC (2000). In the moisture and fat free 5.0 g sample 200 ml of 0.255 N sulphuric acid was added and boiled for 30 min and after that 200 ml of 0.313 N (1.25%) NaOH solution was added to the solution and boiled for another 30 min. The filtrate was dried at room temperature and weighed. The sample was then kept in muffle furnace at 650 ºC for 2-3 h, cooled and weighed again. The deference in weight represents the amount of crude fiber.

Determination of moisture

One gram-powdered sample at 25 °C was taken onto the tray of automatic moisture determination machine, Model Chyo, IB-30 for 10-15 min and moisture content was recorded.

Determination of ash

Ash content was determined by following AOAC (2000). The residue of crude lipid determination described earlier was considered as ash content.

Determination of carbohydrate

Total carbohydrate percentage was determined following Edeogu *et al.* (2007) using following formula- [Percentage of total carbohydrate content = 100 – (moisture + protein + lipid+ minerals)]

Determination of available energy

Available energy was calculated using the formula [available energy = (9.3 × fat) + (4.1 × carbohydrates) + (4.1 × protein)] described by Eneche (1991), Chinma and Igyor (2007) and Nwabueze (2007). The proportion of protein, fat, and carbohydrate was multiplied by their physiological fuel values of 4.1, 9.3, and 4.1 kcal, respectively, and the sum of the values was considered as available energy.

Determination of amino acids

Amino acid analysis was conducted by following the Amino Acid Analysis System Instruction Manual (Anonymous

1993). The amino acid analyzer uses the system of a column (Amino-Na) packed with a strongly acidic cation exchange resin for separation. Amino acids injected and separated using a binary gradient eluting method and detected by post-column derivation detection and fluorescence detector of each amino acid at high sensitivity and 120 kg/cm^2 pressure.

Determination of vitamins

β-carotene content was determined by column chromatography and quantification by visible spectroscopy as described in AOAC (1990). Vitamin C was measured by HPLC method (Lakshanasomya 1998; Knelfel and Sommer, 1985).

Determination of minerals

The mineral contents (Ca, Mg, Fe, Cu, and P) were determined following the methods described in the Manual of Laboratory Techniques (Anonymous, 2006; Boltz, 1958; Rupérez, 2002).

Determination of heavy metals

Heavy metals were analyzed using Atomic Absorption Spectrometer (AAS). The quantities of chemical elements present were determined by measuring the absorbed radiation of the chemical element of interest. This was done by reading the spectra produced when the sample is excited by radiation (García and Báez, 2012).

Results and discussion

Nutrient composition

Utilization of seaweeds as plant protein, vitamins, etc. sources is becoming more popular in the food industry in developing countries (Fleurence, 1999; Wong and Cheung, 2000) and also in developed countries like USA where every super shop displays seaweed food item (Prof. Charles Yarish, Connecticut University, pers. Communication).

The crude protein content in *G. tenuistipitata* var. *liui* was 25.55 ± 0.18%, higher than *G. cervicornis, G. changgi* and *G. cornea* but significantly lower than *Spirulina platensis* (58.42 ± 1.07%), a cyanobacterium known as super food (Table 1). Crude fiber 5.65 ± 0.13% was similar to *G. cervicornis* and *G. cornea* and slightly lower than *S. platensis* (7.83 ± 0.26%) (Table 1). Crude lipid in the present seaweed was much lowe 0.16 ± 0.03% than *G. cervicornis* and *G changgi* also significantly lower than *S. platensis* (12.25 ± 0.39%) (Table 1). Ash content in *G. tenuistipitata* var. *liui* was 10.61 ± 0.69% similar to *G. cervicornis*, higher than *S.platensis* (8.51 ± 0.42%), but lower than *G. changgi* and *G. cornea* (Table 1). Moisture content in *G. tenuistipitata* var. *liui* was 12.10 ± 0.25% significantly lower than *G. cervicornis* and higher than *S.platensis* (8.83 ± 0.26%) (Table 1). However, moisture content usually depends on the drying process. Carbohydrate content in *G. tenuistipitata* var. *liui* was 45.93 ± 1.53%, which is significantlylower than *G. cervicornis*, but higher than *G. cornea* (Table1). The energy

Table I. Nutrient contents (% dry wt.) of G. *tenuistipitata* var. *liui,* comparison with species reported by others and *Spirulina platensis* (used as natural vitamins)

Seaweed	Crude protein	Crude lipid	Crude fiber	Moisture	Ash	Carbohydrate	Available Energy (kcal/100g)	Reference
G. tenuistipitata var. *liui*	25.55 ± 0.18	0.16 ± 0.03	5.65 ± 0.13	12.10 ± 0.25	10.61 ± 0.69	45.93 ± 1.53	294.56	Present research
G. cervicornis	19.70 ± 2.7	0.43 ± 0.06	5.69 ± 0.74	14.33 ± 1.78	10.51 ± 1.56	63.13 ± 3.5	356.84	Marinho - Soriano *et al.* (2006)
G. changgi	6.90 ± 0.10	3.30 ± 0.2	24.7 ± 0.7	-	22.70 ± 0.6	-	-	Norziah and Ching (2000)
G. cornea	5.47 ± 0.44	-	5.21 ± 0.12	-	29.06 ± 0.50	36.29 ± 0.44	201.25	Robledo and Freile -Pelegrin (1997)
Spirulina platensis	58.42 ± 1.07	12.25 ± 0.39	7.83 ± 0.26	8.83 ± 0.26	8.51 ± 0.42	Nd	Nd	Toyub *et al.* 2011

(n = 3, Mean ± SE)

content of *G. tenuistipitata* var. *liui* was good enough (294.52 kcal/100g) and higher than *G. cornea* but lower than other *G. cervicornis* (356.84 kcal/100g) (Table I).

Amino acid profile

G. tenuistipitata var. *liui* contained higher amount of crude protein and thus amino acid profile was determined. As many as 15 amino acids were found of which eleven were essential amino acids (EAA), lysine percentage was highest and histidine was lowest, and four were non-EAA of which glutamate was highest. Total Amino acids were 16.55% of which total EAA was 9.03% (Table II). Total non-EAA was 7.52% of which glutamic acid was highest followed by aspartic acid (Table II). *G. tenuistipitata* var. *liui* had significantly higher total amino acids than *G. corticata* but much lower than *G. changgi* (Table II).

Vitamins

Vitamin C was approximately 2.5 mg/100g in *G. tenuistipitata* var. *liui* lower than *G. lemaneiformis* (3.4 mg/100g) (Fig. 1). The lower value may be due to the fact that vitamin C contentvaries depending on heating time and drying processes (Igwemmar *et al.,* 2013; Santos and Silva, 2008). β-carotene content was found to be significantly higher(11.54 ± 1.20 mg/100g) than *G. changgi* (5.02 ± 0.40 mg/100g) and other species (*G. birdiae, G. caudate, G. domingiensis* and *G. ferox*) (Fig. 2).

Minerals

Among the minerals P content (596.90 ± 10.4mg/100g) was similar to *S. platensis* (600.0 mg/100g), and was much higher than other species reported by others (Table III). Other minerals determined in *G. tenuistipitata* var. *liui* was compared with different species of *Gracilaria*: Ca was 132.75 ± 3.4 mg/100g significantly lower than *S. platensis* (700.0 mg/100g), much lower than other compared species such as *G. changgi* (651 ± 5.2 mg/100g), *G. gracilis* (429.11 mg/100g) and *G. lemaniformis* (139.13mg/100g); Fe was (80.13 ± 2.45 mg/100g) significantly lower than *G. changgi* (95.6 ± 3.7 mg/100g) and *S. platensis* (150.0 mg/100g), but far more higher than *G. lemaniformis* (26.09 mg/100g) and *G. gracilis* (15.20 mg/100g); Cu was (3.99 ± 1.2 mg/100g) significantly higher than *G. changgi* (0.8 ± 0.1 mg/100g), *G. lemaniformis* (0.28 mg/100g), and *S. platensis* (1.20 mg/100g), (Table III).

Table II. Amino acid (% dry wt.) profile of *G. tenuistipitata* var *.liui* and comparison with species reported by others

Sl no.	Amino acids	*G. tenuistipitata* var.*liui* (Present Research)	*G. corticata* (Kumar and Kaladharan, 2007)	*G. changgi* (Norziah and Ching, 2000)
1.	Arginine	1.62	0.89	3.55
2.	Histidine	0.50	0.33	1.91
3.	Iso-Leucine	0.84	0.63	2.94
4.	Leucine	1.54	1.06	3.66
5.	Lysine	1.66	0.86	1.66
6.	Methionine	0.66	0.15	2.0
7.	Threonine	0.69	1.23	3.98
8.	Tyrosine	0.63	0.44	0.94
9.	Valine	0.89	0.90	3.17
	Total EAA	9.03	6.49	23.81
10.	Alanine	1.42	0.81	6.33
11.	Aspartate	1.64	1.99	4.01
12.	Glutamate	2.63	1.85	6.36
13.	Glycine	0.89	1.03	0.71
14.	Serine	0.94	1.12	Not determined
	Total Non-EAA	7.52	6. 8	17.41

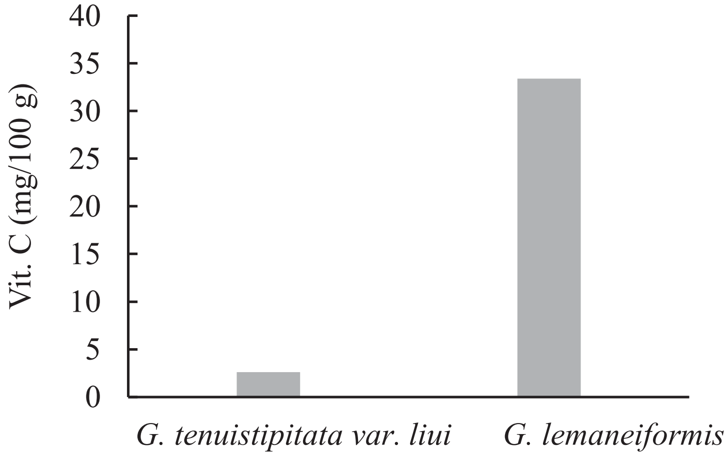

Fig. 1. Vitamin C in *G. tenuistipitata* var. *liui* and comparison with G. *lemaneiformis*

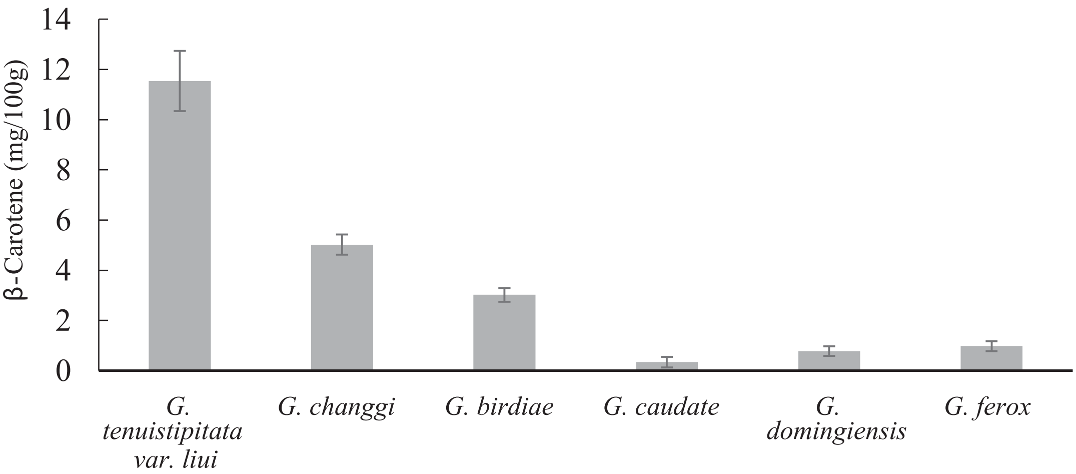

Fig. 2. β-carotene content in *G. tenuistipitata* var. *liui* (mg/kg) and comparison with species reported by others

Table III. Minerals (mg/100g dry wt.) in *G. tenuistipitata* var. *liui* and comparison with species reported by others and *Spirulina platensis* (used as natural vitamins)

Seaweeds	Ca	Mg	Fe	Cu	P	Reference
G. tenuistipitata var. *liui* (present sample)	132.75 ± 3.4	3.90 ± 1.2	80.13 ± 2.45	3.99 ± 1.2	596.90 ± 10.4	Present research
G. changgi	651 ± 5.2	Nd	95.6 ± 3.7	0.8 ± 0.1	Nd	Norziah and Ching, 2000
G. gracilis	429.11	Nd	15.20	Nd	57.01	Rasyid *et al.,* 2019
G. lemaniformis	139.13	Nd	26.09	0.28	189.97	Wen *et al.,* 2006
Spirulina platensis	700.0	400.0	150.0	1.2	600.0	Jung *et al.,* 2019

Heavy Metals

Heavy metals determined in *G. tenuistipitata* var. *liui* was compared with the Provisional Tolerable Daily Intake (PTDI) recommended by Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food Safety Authority of Ireland (Anonymous, 2009), and *G. foliifere*: the determined amount of heavy metals in the present organism were, Pb 0.031 mg/kg (tolerable limit is 0.1-0.3 mg/kg) lower than *G. foliifere*; total As was 0.01 mg/kg (tolerable limit is 0.12 mg/day for a 60kg adult), lower than total As of *G. foliifere* (0.033 mg/kg); Cr content in present sample was 0.06 mg/kg (tolerable limitis 0.1 mg/kg) and higher than *G. foliifere* (0.007 mg/kg); Cd was 0.02 mg/kg (tolerable limitis 0.2 mg/kg) significantly lower than *G. foliifere* (0.055 mg/kg) (Table IV). All the four heavy metals determined for *G. tenuistipitata* var. *liui* were lower than the permissible limit and *G. foliifere* in most cases.

Table IV. Heavy metals (mg/kg dry wt.) found in *G. tenuistipitata var. liui* and comparison with species reported by others

Seaweeds	Pb	As	Cr	Cd	Reference
G. tenuistipitata var *.liui* (present sample)	0.031	0.01	0.06	0.02	Present research
G. foliifere	0.091	0.033	0.007	0.055	Nabil *et al.* 2008

From the above analytical results and discussions, it can be concluded that the red seaweed can be used as enriched health seafood like *Spirulina platensis* in Bangladesh (Table I and III) (Toyub *et al.,* 2011; Jung *et al.,* 2019).

Conclusion

G. tenuistipitata var. *liui* (red seaweed) growing/grown at Nuniachara sand-flat, Moheshkhali Channel, Cox's Bazar is a rich sources of protein, β-carotene and mineral content, a moderate quantity of crude fiber, ash, moisture, carbohydrate, and total energy; low fats and heavy metals, and balanced amino acid profile made it a nutritionally rich and healthy sea vegetable. In the wake of global warming, Bangladesh's coastal areas being more prone to natural disasters that affect crop-field soil by increased salinity, selection of crops and cropping patterns should be changed and seaweed crop appears to be a suitable candidate, adapting to the changing coastal environment, ensuring food security.

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