



RESEARCH ARTICLE

CHN CONTENT AND BIOACTIVE POTENTIALS OF *GRACILARIA CORTICATA* (J.AGARDH) J. AGARDH FROM SURATHKAL BEACH, KARNATAKA

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ABSTRACT

Gracilaria corticata, a red seaweed collected from Surathkal beach, Karnataka India was studied for the estimation of Carbon, Hydrogen and Nitrogen using CHN analyzer whose percentage was found to be $36 \pm 0.64\%$, $6.47 \pm 0.09\%$ and 4.99 ± 0.055 respectively. The mineral content Na and K of *G.corticata* was found to be 12.84mg and 5.34mg respectively. The values of elements such as Zn, Fe, Cu, Cr, Pb and Ni were determined spectrometer and their concentration was found to be 12.06 ± 0.004 , 4.88 ± 0.15 , 4.77 ± 0.01 , 6.69 ± 0.002 , 0.193 ± 0.001 and 0.088 ± 0.004 in ppm respectively. The pigments such as chlorophyll a, chlorophyll b, and Phycobilins were also estimated. The protein content of extracts was determined out of which methanol extract showed higher value of $23.35 \pm 0.21\%$ dry weight (dw) and lowest was with water extract of *G. corticata* ($14.23 \pm 0.01\%$ dw). Also the extract of *G. corticata* was subjected to determine the carbohydrate content in which water extract was to be highest (6.47 ± 0.3) % dw followed by ethanol (4.91 ± 0.03) % dw, while the lowest carbohydrate value was found in chloroform extract (2.1 ± 0.03) % dw.

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INTRODUCTION

Seaweeds are the marine macro algae which are renewable living resources used for food, feed and fertilizer in many parts of the world. Marine algae are known to contain more than 60 trace elements in a concentration which is very much higher than in the terrestrial plants. Seaweeds are also potentially known as good sources of proteins, polysaccharides and fibers in addition to that of vitamins and minerals (Lahaye, 1991; Darcy-Vrillon, 1993). The red alga *Gracilaria corticata* (J. Agardh) J. Agardh is one of the algae which are commonly found in the Indian coast and found mostly in the lower littoral zone. It also inhabits rarely in the intertidal rock pools as submerged population. Seaweeds are well known for their high nutritive value containing essential fatty acids, vitamins, minerals, proteins and fiber contents (Ortiz et al., 2006). The seaweeds are rich in valuable resource such as carbohydrates, protein, minerals, lipids, vitamins, iodine and dietary fiber, thus they are also used up in animal nutrition. Trono 1999 has examined the seaweeds to contain bioactive products that possess antibacterial, antiviral and antifungal properties (Jr.Trono., 1999). However, the seaweeds available worldwide is underexploited and hardly used commercially, due to the

lack of knowledge on their potential activity, negligence while harvesting or discordancy as a nutritional supplement (Marsham et al., 2007). Algae belonging to the genus *Gracilaria* and *Gracilariopsis* are of certain interest since they are found to contain eicosanoids, distinctive of higher plants and human and thus considered as beneficiary to health (Norziah et al., 2002). Seaweeds have potential to selectively absorb minerals from the surrounding seawater and also to accumulate them in their thalli (Azmat et al., 2006). For this reason, their mineral composition and concentration are species and location specific. So far, there is no currently published data on the chemical composition of seaweeds from Surathkal Beach, Karnataka. Thus this study aims at proximate biochemical analysis of *G. corticata*, their CHN content and their photosynthetic pigments value.

MATERIALS AND METHODS

Study area

The algal samples were handpicked between intertidal rocks from Surathkal beach, Karnataka, India ($13^{\circ}00'34.1''$ N lat. & $74^{\circ}47'16.1''$ E long). The collected seaweed was authenticated as *Gracilaria corticata* (J.Agardh) J.Agardh by Dr. C.R.K Reddy, CSIR- Central Salt and Marine Chemicals Research Institute, Bhavnagar, India.

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Preparation of extracts

The collected samples were transferred to the laboratory in plastic bags. Those were washed with tap water several times to remove dirt and debris and was shade dried for 5-9 days which was further powdered. The powdered seaweed sample was subjected to Soxhlet apparatus and was sequentially extracted using solvents based on their polarity namely: chloroform, acetone, methanol, ethanol, and water. The resulting pasty extracts were used for further analysis. The standard method to study moisture content of seaweeds was carried out by drying 2g of samples in a thermostatically regulated incubator at 105°C until constant weight was obtained (Arlington, 1997); while the Ash content was determined by heating the samples in a muffle furnace at 500°C for 4 hours (ASTA, 1999).

CHN content of *G. corticata*

The total carbon hydrogen and nitrogen content was determined by combusting the dried samples using a CHN Analyzer (Leco- TruSpec® CHN, USA), calibrated using EDTA as a reference standard.

Flame photometric detection of sodium and potassium (Jackson, 1971)

The seaweed sample was dried and one gram of dried sample was used for detection of sodium and potassium, 10ml of triple acid mixture of H₂SO₄: HNO₃:HCl(9:3:1 v/v/v) was used for digesting the sample. Further Liquid ammonia was added into the digested sample to alter the pH7 and 100ml of Distilled water was used and thus the volume was made up. Later through Whatmann No.40 filter paper the contents were filtered and obtained filtrates were stored in sterile glass bottles until analysis was made. Flame photometer (Elico, India) was used for the analysis of Na⁺ and K⁺ using a suitable filter against deionized water as a blank.

Atomic absorption spectrophotometer for detection of elements

One gram of seaweed powder sample was taken in 100 ml beaker to which 5 ml of conc. HNO₃ was added and was left overnight. Further the solution was digested on a hotplate at 80°C for 10 min and later cooled at room temperature. To this, 20 ml of sub-boiled distilled water was added and filtered through Whatman filter paper No.42 into a standard flask. Make up the final volume to 100 ml with sub-boiled distilled water. All essential precautions were taken to avoid possible contamination of the samples. Instrument used was AAS GBC Avanta ver. 1.33 for detection of Zn, Fe, Cu, Cr, Pb and Ni. The instrument was calibrated using standard solutions using the concentration and thus analysis was conducted.

Estimation of Chlorophyll

The chlorophyll content present in seaweed was estimated following the standard method of Arnon (1949). 500 mg of fresh tissue was homogenized with 10 ml of 80% acetone in a pestle and mortar. This homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was collected. The pellet was re extracted by repetitive washing with 5 ml of 80% acetone until it became colorless. All the extracts were pooled and thus used for chlorophyll determination. The absorbance

was measured at 645 nm and 663 nm in a spectrophotometer. The chlorophyll content was determined by using the following formula.

$$\text{Chlorophyll 'a'} = \frac{[12.7(A_{663}) - 2.69(A_{645})] \times \text{vol. of extraction}}{\text{Weight of the sample}}$$

$$\text{Chlorophyll 'b'} = \frac{[22.9(A_{645}) - 4.68(A_{663})] \times \text{vol. of extraction mg/g}}{\text{Weight of the sample}}$$

$$\text{Total Chlorophyll} = \frac{[20.2(A_{645}) + 8.02(A_{663})] \times \text{vol. of extraction mg/g}}{\text{Weight of the sample}}$$

Where A₆₆₃ = absorbance at 663 nm

A₆₄₅ = absorbance at 645 nm

Extraction and estimation of Phycobiliproteins by the method of Padgett and Krogman (1987)

The Absorbance was measured at 615 nm and 652 nm against 0.05 M phosphate buffer blank using spectrophotometer. The concentration of phycocyanin, phycoerythrin and allophycocyanin in the extracts were calculated (in milligrams / milliliter) using the following equations (Official methods of analysis. Arlington, VA, 1997).

$$\text{Phycocyanin (PC)} = \frac{(\text{OD}_{615}) - 0.474(\text{OD}_{652})}{5.34}$$

$$\text{Allophycocyanin (APC)} = \frac{(\text{OD}_{652}) - 0.208(\text{OD}_{615})}{5.09}$$

$$\text{Phycoerythrin (PE)} = \frac{(\text{OD}_{652}) - 2.41(\text{OD}_{615}) (\text{PC}) - 0.849 (\text{APC})}{9.62}$$

Biochemical studies of extracts of *G. corticata*

Estimation of protein

The total protein was estimated by following procedure of Lowry *et al.*, 1951.

Estimation of carbohydrate

The total carbohydrate was estimated by following the method of Dubois *et al.*, 1956.

RESULTS

The red seaweed *G. corticata* was subjected to determine the Biochemical composition and CHN content of the seaweed. The moisture content and ash content of *G. corticata* was found to be 5.08 mg/g and 3.96 mg/g respectively. The Carbon, Hydrogen and Nitrogen were investigated using CHN analyzer whose percentage was found to be 36 ± 0.64%, 6.47 ± 0.09% and 4.99 ± 0.055 respectively (Figures 1 and 2). The Na and K values of *G. corticata* were determined using a flame photometer and the values were found to be 12.84 mg and 5.34 mg respectively (Table: 1). The values of elements such as Zn, Fe, Cu, Cr, Pb and Ni were determined using Atomic

Absorption Spectrometer and their concentration was found to be 12.06 ± 0.004 , 4.88 ± 0.15 , 4.77 ± 0.01 , 6.69 ± 0.002 , 0.193 ± 0.001 and 0.088 ± 0.004 in ppm respectively (Table 2).

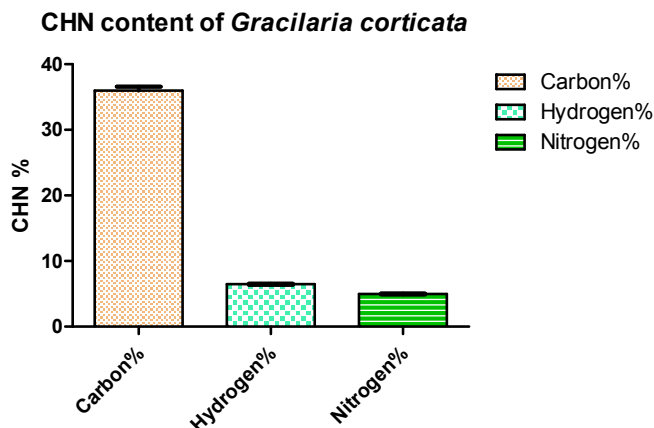


Fig.1. CHN content of Gracilaria corticata

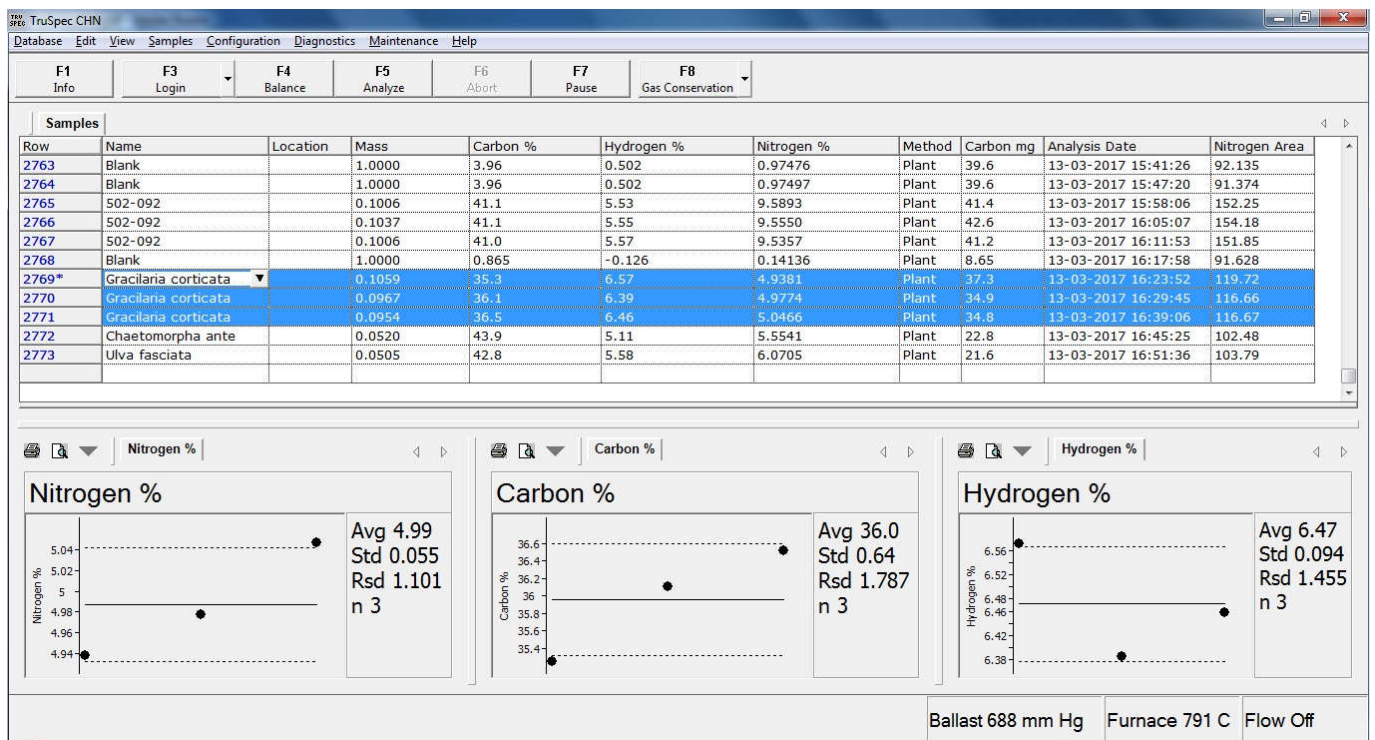


Fig.2. CHN analyzed chart of Carbon, Hydrogen and Nitrogen

Table 1. Sodium and Potassium content in *G. corticata*

Minerals	Mineral content(mg/g)
Na	12.84
K	5.34

Table 2. Elements present in *G. corticata* using Atomic Absorption Spectrometer

Elements	Concentration in ppm
Zn	12.06 ± 0.004
Fe	4.88 ± 0.15
Cu	4.77 ± 0.01
Cr	6.69 ± 0.002
Pb	0.193 ± 0.001
Ni	0.088 ± 0.004

Photosynthetic pigments

The photosynthetic pigments like chlorophyll 'a' ($2.582 \pm 0.05 \text{ mg/g}$), chlorophyll 'b' ($0.11 \pm 0.01 \text{ mg/g}$) and total chlorophyll content ($2.8 \pm 0.03 \text{ mg/g}$) were determined and presented in Table- 3a.

Similarly, the phycobilins content was observed in red algae and are tabulated in Table - 3b, wherein the values are Phycocyanin ($3.03 \pm 0.16 \mu\text{g/g}$), Allophycocyanin ($0.51 \pm 0.04 \mu\text{g/g}$) and phycoerythrin ($6.15 \pm 0.007 \mu\text{g/g}$).

Table 3a. Photosynthetic pigments of *G. corticata* (mg/g fresh sample)

<i>G. corticata</i>	Photosynthetic pigments value (mg/g)
Chlorophyll-a	2.582 ± 0.05
Chlorophyll-b	0.11 ± 0.01
Total chlorophyll content	2.8 ± 0.03

Table 3b. Phycobilin Content of *G. corticata* ($\mu\text{g/g}$ fresh sample)

<i>Gracilaria corticata</i>	Phycobilins value($\mu\text{g/g}$)
Phycocyanin	3.03 ± 0.16
Allophycocyanin	0.51 ± 0.04
Phycoerythrin	6.15 ± 0.007

The protein content of extracts was determined, out of which methanol extract showed higher value of $23.35 \pm 0.21\%$ dry weight (Fig.3) and lowest was with water extract of *G. corticata* with value of $14.23 \pm 0.01\%$ dry weight. The carbohydrate content was the highest in water extract (6.47 ± 0.3) % dry weight followed by ethanol (4.91 ± 0.03) % dw, while the lowest carbohydrate value was found in chloroform extract (2.1 ± 0.03) % dry weight.

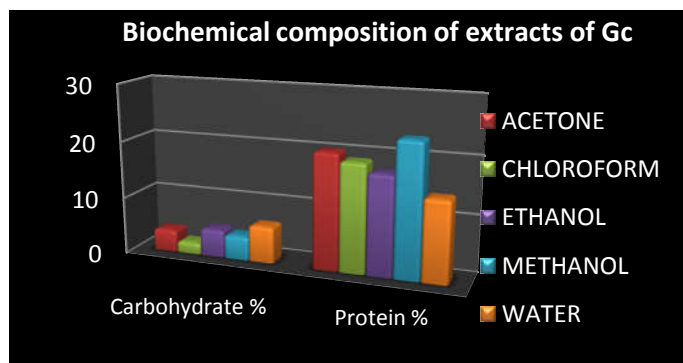


Fig.3. Protein and Carbohydrate contents of various extracts of *G.corticata*

Bird *et al.* (1990) had reported the higher carbohydrate content appropriate for bioconversion to biofuel present in Gracilariaceae. The chemical composition of two tropical seaweeds was reported to contain higher protein content of red algae *Gracilaria* spp. than the brown algae and besides higher concentration of protein in red seaweed was found when compared with some higher plants was studied by Soriano *et al.*, 2006. Omer *et al.*, 2013 had studied the higher carbohydrates concentration in particular to *G. corticata* (52.93%) from the Red Sea. Dawczynski *et al.*, 2007 had shown that the concentration of carbohydrates and proteins was found to vary with species thus showing greatest annotations on variations in temporal and seasonal. The present investigation fetches out ample data on the biochemical composition, CHN constituents, mineral composition and photosynthetic pigments of *G. corticata*.

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Conflict of interest

Authors declare no conflict of interest.

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