

# Nutraceutical value added sea weed - *Gracilaria corticata* var. *cylindrica* from thoothukudi

## Abstract

The marine macroalgae represent one of the major components of coastal ecosystem and are of fundamental ecological importance as primary producers in the coastal regions of ocean waters. Of the total marine macrophytic algae, red algae with most ancient eukaryotic lineage are diverse in their habitats and cellular organizations, and comprise the highest number of species diversity over. The proximate composition, dietary fiber, element and amino acid contents of the red seaweed-*Gracilaria corticata* var. *cylindrica* and was collected from Thoothukudi to evaluate their potential nutritional value. The protein content was significantly higher ( $P < 0.05$ ). Lipid, ash, total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber were present in substantial level. Qualitative analysis revealed a pool of phytochemicals in various extracts and more in methanolic extract. Total phenol, flavonoids and terpenoids were present in remarkable levels. The result also indicated that the species contained high levels of K and Cl. The essential amino acids with the highest content. Thus, the overall study suggested that *Gracilaria* species could potentially be used as nutraceutical food. Further studies are planned to isolate, purify the major phytochemicals and its evaluation of biological potentialities.

**Keywords:** selective transfer, superficial layer, structural analysis, intensity x-rays, width of diffraction lines, crystalline network constant

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## Introduction

*Gracilaria* species are important seaweeds of India harvested regularly from the coastal belts of India for the extraction of many pharmaceutically important products. Excessive exploitation led to the depletion of the natural biota in this area. Currently, methods of cultivation may pave the way to meet the demand of the industry. Macroalgae, belongs to agrophytes synthesize many biologically active secondary metabolites, which includes carotenoids, terpenoids, xanthophylls, chlorophylls, phycobilins, polyunsaturated fatty acids, polysaccharides, vitamins, sterols, tocopherol and phycocyanins.<sup>1</sup> Seaweeds represent 23.4% of the tonnage and 9.7% of the value of the marine, brackish water, and freshwater aquaculture production, estimated at 59.4 million tonnes and \$ 70.3 billion in 2004.<sup>2,3</sup> They are used as food, fodder, feed and fertilizer<sup>4</sup> and many of the bioactive compounds produced by the macroalgae are known to have potential beneficial use in healthcare.<sup>5,6</sup> Many macroalgae in habitat in complex ecosystems exposed to adverse conditions and, in adapting to new environmental surroundings, they produce a wide variety of primary metabolites and phytochemicals which cannot be found in other plants. Marine-based bioactive molecules can be produced from a diverse sources, including marine plants, macro- and microalgae, microbes, and sponges, all of which contain their own unique set of biomolecules. Recently, Francavilla et al.<sup>7</sup> found that *Gracilaria* species harvested may be used as an interesting source of natural porous material with several biotechnological applications. Further, Buldaran et al.<sup>8</sup> found that microwave induced pyrolysis of this macroalga produced chemical rich bio-oils which are rich in aromatics, sugars and other high value chemicals. Polyphenolic compounds are usually found in edible brown, green and red seaweeds, whose antioxidant potentialities have been correlated to their phenolic contents. There are many reports on the antioxidant

activities of steroids terpenoids and saponins. The antioxidant activity of polysaccharide from *Bryopsis plumosa* was reported by Song et al.<sup>9</sup> Vinayak et al.<sup>10</sup> reported cytotoxic and antioxidant activities of crude methanol extract of the brown seaweed *Dictyopteris australis*. In the scenario, nutraceutical, pharmaceutical and cosmetically important biomaterials to be screened. Thus, the present study aims to analyze the biochemical composition of *Gracilaria corticata* var. *cylindrica* and was collected from Thoothukudi in order to evaluate their biological importance.

## Materials and methods

Fresh *Gracilaria corticata* var. *cylindrica* was collected from Thoothukudi.

### Proximate analysis

Standard protocol of AOAC<sup>11</sup> was employed to quantify the ash, lipid and moisture content of *G. corticata* var. *cylindrica*. The moisture content was determined by oven method at 105 °C until constant weight was attained. Crude protein content was analyzed by Lowry's method.<sup>12</sup> Ash content was done by incinerating the algae in a muffle furnace at 550 °C for 16 h and the content was determined gravimetrically. Crude lipid was isolated from seaweed powder with chloroform: methanol (2:1,v/v) in a Soxhlet extractor by the method of Bligh and Dryer<sup>13</sup> The crude lipid content was measured gravimetrically after oven-drying (80 °C) the extract overnight. The total, soluble and insoluble dietary fiber contents were analyzed as per the enzymatic-gravimetric protocol.<sup>11</sup>

### Mineral analysis

Elementary compositions of the sample was analyzed by atomic absorption spectrophotometry (AAS) for Ca, Mg, K and Na by

inductively coupled plasma optical emission spectrometry for Fe, Cu and Zn,<sup>11</sup> gravimetric method for<sup>14</sup> and chloride analyzer for Cl.

### Amino acid analysis

The samples were hydrolyzed with 6 N HCl containing 1% phenol in a heating block at 110 °C for 24 h by high-performance liquid chromatography (HPLC) using the wave length of 250 nm and an emission wavelength of 395 nm. Identification of the amino acids in the samples was carried out by comparison with retention times of the standards.

### Qualitative phytochemical analysis

**Preparation of plant extracts:** 5g of powdered material was subjected to hot continuous soxhlet extraction with different solvent such as petroleum ether, ethyl acetate, methanol and water for 8 h. The extract was stored in a refrigerator and used for the present study. Qualitative phytochemical analysis was carried following the protocol of Kokate<sup>15</sup> Alkaloids, carbohydrates, tannins and phenols, flavonoids, gums and mucilage, fixed oils and fats, saponins, pytosterol were qualitative analyzed.

### Polyphenol analysis

The total phenolic content of algal extracts was assessed according to the Folin-Ciocalteu method. Gallic acid was used as a standard to construct the calibration curve (20, 40, 60, 80 and 100mg·L<sup>-1</sup>). The total phenolic content of algal samples was expressed in mg·g<sup>-1</sup> of gallic acid equivalent (GAE).

Total flavonoid content was measured by the aluminum chloride colorimetric assay.<sup>16</sup> An aliquot (1ml) of extracts and standard solution of catechin (100 mg/ml) was added to 10 ml volumetric flask containing 4ml of distilled water. To this 0.3 ml 5 % NaNO<sub>2</sub> were added. After 5 min, 0.3ml 10 % AlCl<sub>3</sub> was added. Then after 1 min, 2ml of 1 M NaOH was added and the total volume was made up to 10ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of root extracts expressed as mg catechin equivalents (CE)/100 G fresh weights. All samples were analyzed in triplicates.

About 10 g of powdered was taken and soaked in alcohol for 24 h. It was filtered and filtrate extracted with petroleum ether; this ether extract was treated as total terpenoids.<sup>17</sup>

### Statistical analysis

All determinations were performed at least in triplicate. Statistical analysis was carried out by using the SPSS 10.0 version software for Windows. The analyzed data were expressed as mean with standard deviation (SD). Paired sample *t*-test was used to identify significant differences at *P*<0.05.

## Results and discussion

### Proximal chemical composition

The proximal composition of *G. corticata* var. *cylindrica* under present study reveals that the moisture content was 3.8 % of the dry weight (DW). The protein content was 4.7mg/g tissue (*P*<0.05). Previous reports showed that the red and green seaweeds possess a protein content within the wide range 10–47% (DW). Similarly, *Sargassum polycystum* (5.4% DW),<sup>18</sup> *G. domingensis* (6.2% DW) and

*G. birdiae* (7.1% DW), *Gelidium pristoides* (11.8% DW),<sup>19</sup> *Halymenia formosa* (21.2%),<sup>20</sup> *G. cervicornis* (23.0% DW)<sup>21</sup> and *Grateloupia turuturu* (22.9% DW),<sup>22</sup> *Porphyra tenera* (47% DW) and *Palmaria palmata* (35% DW).<sup>23</sup> These levels varied depending on algal species, season and environment.<sup>24</sup>

The lipid content was 2.4% DW. The amount was comparable with the previous reports (1–3% DW) (Fleurence, 1999), *G. coronopifolia* (2.1%), *G. salicornia* (2.4%), *G. parvispora* (2.8% DW),<sup>20</sup> *G. cervicornis* (0.43% DW) and *Sargassum vulgare* (0.45%).<sup>21</sup> The ash content was 18.6%. The amounts of ash obtained in the present study was in agree- ment with the report of Ruperez and Saura-Calixto,<sup>26</sup> Sánchez-Machado et al.<sup>27</sup> *G. domingensis* (23.8% DW), *G. birdiae* (22.5% DW), *Hypnea japonica* (22.1% DW) and *H. charoides* (22.8% DW).<sup>28</sup> Generally, the ash contents in marine seaweeds are higher than terrestrial plants (5–10% DW). The differences in ash contents depends up on physiology, environment, methodology and processing methods.<sup>26,29</sup>

Plant products contained large amounts of polysaccharides, which comprises high level of soluble and insoluble dietary fibers.<sup>30</sup> In this study, the amounts of soluble, insoluble (SDF, IDF), and total dietary fibers was 13.6 %, 37.9 % and 61.4% DW, respectively. The content of total dietary fiber in seaweed was higher than those in terrestrial plants such as wheat (44.5% DW), beans (36.5% DW) and onions (16.9% DW).<sup>31</sup> In addition, the ratios of SDF/IDF was well balanced (close to 1:2) as the suggested by Figuerola et al.<sup>32</sup> *Gracilaria* species has SDF as sulphated galactans, which is regarded influential in slowing digestion and absorption of nutrients, as well as reducing levels of blood cholesterol and glucose.<sup>33</sup> In contrast, IDF increases fecal bulk and decreases intestinal transit time.<sup>34</sup> They have several beneficial physiological effects on humans in preventing constipation, colon cancer, cardiovascular disease and obesity. Therefore, *G. corticata* var. *cylindrica* may be alternatively used as raw materials for high fiber food production or as ingredients in food industry.

### Mineral contents

The mean macro elements (Ca (225.3), P (300.2), K (7432), Mg (450), Na (312) and Cl (1456) and trace elements (Cu (0.18) and Zn (0.83) contents ranged from 225.3–7432mg/100g DW and 0.18–60.83mg/100g DW, respectively i.e., the seaweed species showed remarkable levels of K and Cl but poor in Na. Furthermore, their Na/K ratio was low and therefore the seaweeds can help balance Na/K ratio diets and reduce hypertension risk as described in the studies of the red and brown seaweeds by Rupérez. As for the trace elements, copper and zinc contents were found within small range and also below the maximum level allowed in seaweeds for human consumption in Japan and France (10mg/100 g).<sup>35</sup>

Thus, the present study suggests the possibility of using the seaweed species as food supplements to improve the nutritive value for the human diet and animal feed.

### Amino acid composition

The essential amino acids (EAA) included are methionine, leucine, isoleucine, lysine, phenyl ala- nine, tyrosine, arginine, threonine and valine. However, the analytical method used could not determine tryptophan and cysteine. The levels of different essential amino acids ranged from 3.43 to 8.96 mg/100mg DW and sound levels of arginine (0.74), leucine (0.64) and threonine (0.57). Meanwhile, the non-EAA like aspartic acid (0.88), glutamic acid (0.62), serine (0.57),

proline, glycine 0.61) and alanine (0.59) mg/100mg DW. Aspartic and glutamic acids, which are responsible for the aroma and taste, are present in substantial levels.

### Qualitative analysis

The different solvent extracts of the algae show presence of compound for carbohydrates, alkaloids, phenols, tannins, saponins and flavonoids. Methanol extract of the plant sample show these compounds at remarkable levels (Table 1).

### Phenols, flavonoids and terpenoid levels

Total phenol content (TPC) was 23 mg/g GAE, flavonoids 8.2mg/g and terpenoid 2.3mg/g. Different plants show diverse total phenolic contents and may be due to many influencing factors, such as algal species, geographical origin or the area of cultivation, seasonal, physiological, and environmental variations. The proven sources of

phenolic compounds, *i.e.*, cocoa, red wine, and green tea constitute extraordinary exceptions; their phenolic contents of 611, 340 and 165 mg·g<sup>-1</sup> GAE, respectively. Similarly, black tea (124mg·g<sup>-1</sup> GAE) had values comparable to the highest measured data.<sup>36</sup> Generally, the phenolic contents in fresh algae was correlated with different extraction conditions used like ethanol extract of *Eisenia bicyclis* contained 319mg·g<sup>-1</sup> GAE,<sup>37</sup> aqueous extract of *Hizikia fusiformis* 4.1 mg·g<sup>-1</sup> GAE,<sup>38</sup> methanol-chloroform extract of *Laminaria japonica* 0.3mg·g<sup>-1</sup> GAE,<sup>39</sup> aqueous extract of *Undaria pinnatifida* 3.8mg·g<sup>-1</sup> GAE,<sup>40</sup> ethanol extract of *Palmaria palmata* 10.3mg·g<sup>-1</sup> GAE,<sup>41</sup> aqueous extract of *Porphyra tenera* 10.1mg·g<sup>-1</sup> GAE<sup>42</sup> and *Spirulina platensis* 19.5mg·g<sup>-1</sup> GAE.<sup>43</sup> The differences between phenolic contents could also be caused by the characteristics of the samples because all mentioned studies were realized with fresh algal samples contrary to the processed algal products analyzed in this work.

**Table 1** Qualitative analysis of biological compounds in *G. corticata* var. *cylindrica*

Compound Tested	Reagent's	Petroleum ether	Ethyl acetate	Methanol	Water
Carbohydrates	Fehling's	-	-	+	+
	Molish's	-	-	+	+
	Dragendraff's	+	+	-	-
Alkaloids	Wagner's	+	+	-	-
	Hager's	+	+	-	-
	Mayer's	+	+	-	-
Tannins & Phenols	10% Lead acetate	+	+	+++	++
Flavonoids	NaOH +HCl	+	+	++	+
Gum & Mucilage	Alcoholic Precipitation	-	+	-	-
Fixed oils & Fats	Spot Test	+	-	-	-
Saponins	Foam Test	+	-	-	+
Phytosterol	LB Test	+	-	-	-

### Conclusion

The seaweed *G. corticata* var. *cylindrica* analyzed in this study had appreciable protein content, ash and dietary fiber, as well as minerals and essential amino acids. Therefore, the *Gracilaria* species appear to be potential sources or ingredients in functional food products and animal feed. Further studies are warranted to screen the secondary metabolites, its purification and evaluation of its biological potentialities.

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None

### Conflict of interest

Authors declare there is no conflict of interest towards this manuscript.

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