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Original Article

Assessment of nutrient composition and antioxidant potential of Caulerpaceae seaweeds

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ABSTRACT

The proximate nutrient composition, mineral contents, enzymatic and non-enzymatic antioxidant potential of three *Caulerpa* species were investigated. All three species were high in ash (24.20–33.70%) and carbohydrate content (37.23–48.95%) on dry weight basis (DW). The lipid content ranged between 2.64 and 3.06% DW. The mineral contents varied marginally among the species but were in the order of Na > K > Ca > Mg. The Na/K ratio among the species varied from 1.80 to 2.55 and was lowest in *C. scalpelliformis*. A 10 g DW of *Caulerpa* powder contains 11–21% Fe, 52–60% Ca and 35–43% Mg, which is higher than the recommended daily allowance (RDA), compared with non-seafood. The percentage sum of PUFAs (C18:2, C18:3, C20:4 and C20:5) in total fatty acids was highest in both *C. scalpelliformis* (39.25%) and *C. veravelensis* (36.73%) while it was the lowest in *C. racemosa* (24.50%). The $n-6/n-3$ ratio among the species varied from 1.44 to 7.72 and remained within the prescribed WHO standards (<10). Further, the higher enzymatic dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) and non-enzymatic antioxidant potential of *Caulerpa* species found in the present study confirm their usefulness in terms of nutrients and antioxidants.

1. Introduction

An increasing human population, global climate change and the diversification of terrestrial food resources for energy needs in recent times have raised serious global food security concerns (Rosegrant and Cline, 2003). Further, the globalization of markets has also brought about an increasing globalization of foods, diminishing the boundaries of human races and geographical regions of the countries throughout the world. There has also been a quest to explore and utilize foods from non-conventional sources, of both terrestrial and marine origin, to enhance and supplement the nutritional quality of human foods. Also, this in turn eases off the growing burden on traditional foods. Marine macroalgae, commonly known as seaweeds, are one of the living renewable resources of the oceans with potential food applications. Consumption of seaweeds as sea vegetables in human diets has been the common practice in several Asian countries (Nisizawa, 2002). Presently, interest in supplementing the human foods with antioxidants particularly from natural sources has been on the raise as synthetic antioxidants have been suspected to be a possible cause for liver damage and carcinogenesis (Farag et al., 2003; Tang et al., 2001). Therefore, there is a need for isolation and characterization of antioxidants having least side effects from natural sources as an alternative to synthetic antioxidants.

The previous studies have demonstrated the potential of enzymatic superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) and non-enzymatic (polyphenols, glutathione, ascorbic acid and carotenoids) antioxidants scavenging the reactive oxygen species (ROS) thus relieving from the oxidative stresses and other associated health risks such as cancer, coronary heart diseases, neurodegenerative diseases and inflammation (Duan et al., 2006; Kuda and Ikemori, 2009; Nagai and Yukimoto, 2003). The recent findings have also revealed availability of useful metabolites with medicinal properties from some marine biota (Blunt et al., 2005; Mayer et al., 2009).

Recently, the genus *Caulerpa* has attracted the attention of researchers due to its important secondary metabolite caulerpenyne (CYN) that is reported to exhibit the antineoplastic, antibacterial and antiproliferative activities (Barbier et al., 2001; Cavas et al., 2006). Further, it has also been shown to inhibit the cell division of sea urchin eggs as well as cancer cell lines (Fischel et al., 1995; Lemee et al., 1993). Three species of *Caulerpa* namely *C. racemosa*, *C. scalpelliformis* and *C. veravelensis* have been found growing luxuriantly in the intertidal region during October–February along the Veraval coast of Gujarat (north-western coast of India). Among these three species, *C. racemosa* with a wide

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distribution over both tropical and subtropical regions of the world has been studied in greater details for its chemical and mineral composition (Hong et al., 2007) while *C. veravelensis*, and *C. scalpelliformis* remained unexplored.

The data on nutrients, minerals, fatty acids together with the enzymatic and non-enzymatic antioxidant properties of Indian *Caulerpa* species is either absent or scanty. In view of this, an attempt has been made in the present study to evaluate the possible utility of three *Caulerpa* species, namely *C. veravelensis*, *C. scalpelliformis* and *C. racemosa* in food and for other potential uses. In addition, iso-enzymes profile of SOD, APX and GR have also been analyzed to study the isomorphic pattern of respective enzymes in all these three *Caulerpa* species.

2. Materials and methods

2.1. Collection

The vegetative thalli of *Caulerpa veravelensis*, *C. scalpelliformis* and *C. racemosa* were collected from intertidal region during low tide periods in January 2009 from Veraval Coast (20°54′N, 70°22′E), Gujarat, India. Selected young thalli were then brought to the laboratory in a cool pack. The plants were then cleaned manually with brush in autoclaved seawater to remove epiphytic foreign materials and were then divided into two parts with each having 150 g FW. One part was freeze-dried and stored in airtight containers at −40 °C for the estimation of nutrient composition and non-enzymatic antioxidant potential while the other part was utilized directly for determining the antioxidant enzymes activities and pigment analysis.

2.2. Analytical methods for determining chemical composition

The water content of the fresh material was determined by oven drying the sample at 60 °C for constant weight and subtracting the dry weight values from the wet weights. Total ash content was determined gravimetrically after heating at 550 °C for 18 h in a muffle furnace (Heraeus Thermicon). The total carbon and nitrogen content was determined by combusting the freeze dried samples using CHN Elemental Analyzer (PerkinElmer Model 2400, USA), calibrated using reference standard Acetanilide (Table 1). It was seen that the results agreed with the certified ones. Carbohydrate content of freeze dried samples was determined by the phenolic sulphuric acid colorimetric method outlined by Dubois et al. (1956) using glycogen as a standard. The percentage crude proteins were calculated by multiplying the nitrogen content with a factor of 6.25. The photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) were extracted following the method of Dawes et al. (1999) and were estimated according to the formulas of Lichtentaler and Wellburn (1985) described below:

$$\text{Chl } a = 11.75A_{662} - 2.350A_{645}$$
$$\text{Chl } b = 18.61A_{645} - 3.960A_{662}$$
$$\text{Carotenoids} = (1000A_{470} - 2.270\text{Chl } a - 81.4\text{Chl } b)/227$$

The energy content of the samples was determined by multiplying the values obtained for protein, carbohydrate and lipid by 23.86, 17.16 and 36.42 kJ g^{-1}, respectively (Brett and Groves, 1979).

2.3. Crude lipid extraction and fatty acid analysis

Lipids were extracted following the method of Bligh and Dyer (1959). Ground tissue was extracted with chloroform:methanol (1:2), and the residue was re-extracted three times with small portions of chloroform:methanol (1:1). Extracts were pooled, filtered and mixed with $CHCl_3$ and water for phase separation. Organic phase was collected and evaporated to dryness *in vacuo* and the total lipid content was determined gravimetrically. Fatty acids were converted to respective methyl esters by trans-methylation of extracted lipid samples using 1% NaOH in MeOH and heated for 15 min at 55 °C, followed by adding 2 mL of 5% methanolic HCl and again heated for 15 min at 55 °C. Fatty acid methyl esters (FAMEs) were extracted in hexane and the organic phase was evaporated to dryness under reduced pressure. FAMEs were analyzed by GC-2010 coupled with GCMS-QP2010. Non-adecanoic acid was used as internal standard. FAMEs peaks were identified by comparison with their respective retention times obtained from standard mixture (FAME Mix C4-C24, Sigma) by GC–MS post-run analysis and quantified by area normalization. For the validation of this method, standard FAMEs mixture was analyzed on intra/inter-day basis to ensure the instrument response and data accuracy (Table 2) and a five point calibration was carried out to ensure the linearity.

2.4. Mineral analysis

The mineral contents of samples were determined by following the method of Santoso et al. (2006). Freeze dried samples (200 mg) were weighed in a Kjeldahl flask and 20 mL of concentrated HNO_3 was added to the sample and left to stand overnight. Concentrated $HClO_4$ (5 mL) and H_2SO_4 (0.5 mL) were added to the flask and then heated until no white smoke was emitted. The samples thus digested were dissolved in 100 mL of 2% HCl and filtered with 0.22 μm membrane filter. Mineralogical analysis was carried out using inductively coupled plasma atomic emission spectroscopy (ICP-AES, PerkinElmer, Optima 2000, USA), using ICP Multi element reference standard solution VIII (Product No. 1.09492.0100, Merk, Germany) with a concentration of 10 mg/L for each of the elements analyzed. In addition, the average recoveries for the reference standards were recorded as: Na (98.93), K (97.59), Ca (99.78), Mg (104.12), Fe (97.26), Cu (98.84), Zn (99.82), Mn (102.17), Ni (98.95), Mo (101.16), As (99.89) and Se (97.55).

2.5. Extraction, estimation of antioxidative enzymes activities and water soluble antioxidants

For assay of SOD, CAT, APX and GR, 250 mg fresh sample was weighed, ground in liquid N_2 and extracted in 0.5 mL of respective extraction buffers (Wu and Lee, 2008). The extraction buffer for

Table 1
Analysis of certified reference material Acetanilide: theoretical values, measured values and recovery (mean ± S.D., $n = 5$).

Element	Acetanilide				
	Theoretical value	Experimental value			
		Intra-day variation		Inter-day variation	
		Measured value	Recovery (%)	Measured value	Recovery (%)
C (carbon)	71.09 ± 0.44%	70.35 ± 0.26%	98.95 ± 0.37	70.15 ± 0.13%	98.67 ± 0.19
N (nitrogen)	10.36 ± 0.07	10.10 ± 0.08	97.49 ± 0.77	10.05 ± 0.03	96.98 ± 0.24