Seasonal Fluctuations in the Nutraceutical Value of Caulerpa taxifolia

(M. Vahl) C. Agardh- Killer Weed (Chlorophyceae)

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Abstract

The seasonal variation of the marine seaweed Caulerpa taxifolia (M. Vahl) C. Agardh a killer weed was analysed for total carbohydrates, lipids, proteins, photosynthetic pigments free amino acids, fatty acids vitamins and minerals. These results clearly depict the nutraceutical variations of the algae abundant in the shores of Kanyakumari, India during the month of April compared to that of December. Ethanolic extracts were screened for the presence of various phytochemicals like flavonoids, phenols, alkaloids, tannin, steroid and saponin using standard protocols. Though there have been several reports on biochemical composition of this seaweed across India, but there are very few studies focusing on the seasonal variations in chemical composition of this seaweeds.

Keywords: *Caulerpa; fluctuations; killer weed, nutraceutical; seasonal variation; shores of India.*

1. Introduction

Caulerpa taxifolia (M. Vahl) C. Agardh, (Chlorophyceae)is a marine seaweed, native to the Indian Ocean but common as ornamental in aquariums though a noxious killer weed or world's worst invasive algal species. They are peculiar as they consist of only one cell with many nuclei, coenocytic -making them among the biggest single cells in the world. The

recent increase in isolation of bioactive compounds from plants, has open doors to the poorly exploited marine ecosystem which appears to be a good candidate of natural resource in India [1] . As it has a vast coastline of 6100 km supporting a rich flora of marine plants such as seaweeds, mangroves and sea grasses [2]**.** Seaweeds are the most nutritious as they are rich in vitamins and minerals than any other food it also provides protein and provides carotenes, chlorophyll, enzymes, amino acids and fibre in large quantities. The distinctive salty taste of seaweeds is due to a balanced chelated combination of sodium, potassium, calcium, magnesium, phosphorus, iron and trace minerals. The levels of these minerals are ten to twenty times the total mineral content of land plants [3].

This paper deals with the seasonal fluctuations in the nutritional value of carbohydrates, total lipids, total proteins free amino acids and vitamins in additions to phytochemicals of this alga abundant in the shores of Kanyakumari, India during the month of April compared to that of December. Seaweeds generally show great variation in the nutrient contents, which could be related to several environmental factors such as water temperature, salinity and pressure. Furthermore it is related to the abundance of sunlight which is the main factor. Though there have been several reports on biochemical composition of this seaweed across India, but there are very few studies focusing on the seasonal variations in chemical composition of this seaweeds. This study aims at presenting the seasonal variation in the proximate composition of this killer weed collected during the summer month of April and winter month of December.

2. Materials and Methods

The experimental algae *Caulerpa taxifolia* (M. Vahl) C. Agardh, (Chlorophyceae) was collected in the month of April and December from the shores of Kanyakumari district, (lat.80ºN) Tamilnadu, India , identified and authenticated by R.Thevanathan, Reader and Head in Botany, Presidency College, Chennai. During this period the experimental algae were usually in the saprophytic phase. The collection also had juveniles and few gametophytic thalli.

Freshly collected algae were shade dried for about a week then the dried samples were powdered using a blender, packed in sterile polythene bags and stored at 4ºC until use. Herbaria were simultaneously prepared.

i) Total carbohydrates, proteins and lipids: The Carbohydrates as total sugars, were estimated following the procedure of[4]. The absorbance was read at 620 nm using HITACHI UV 2001 Spectrophotometer. A reagent blank was run simultaneously. Glucose (Analar) was used as the standard. [5]procedure was followed to estimate the Protein content. Freshly collected algae were used for the estimation of total soluble protein. The procedure outlined [6] was used with modifications to determine the total lipids in the sample.

ii) Photosynthetic pigments: The pigments chlorophyll “a” chlorophyll “c” and carotenoids were estimated following the procedures of [7] .

iii) Total free Amino acid content :Freshly collected frozen tissues of algae was estimated by ninhydrin method [8] . To suitable aliquots of the algal extract, water was added to make the total volume [2]o 4.0 mL. To this, 1.0 mL of ninhydrin reagent was added, mixed and kept in a boiling water bath for 15 minutes. The tubes were then removed, cooled and 1.0 mL of 50% ethanol was added.The pink colour developed was measured at 550 nm in a Spectronic 21 photocolorimeter. A composite mixture of alanine, aspartic acid, tryptophan, proline and lysine (in equal weights) was used as the standard.

iv) Vitamin content : Dry, powdered sample of algae was estimated by HPLC [9]. HPLC system (SCHIMADZU) equipped with UV detector was used under the following analytical conditions for the estimation of nicotinic acid, vitamin B1, vitamin B6 and vitamin B2.For vitamin A the algal powder was saponified with ethanolic KOH for 30 minutes and transferred to a separating funnel and repeatedly extracted with n-hexane. The final pooled extract was evaporated to dryness under reduced pressure in a rota evaporator and vitamin A level was determined by HPLC.

v) Fatty acids : The fatty acids were determined and quantified by NEON II gas chromatography analysis outlined by [10].

vi) Mineral analysis or elemental analysis: Shade dried sample was carried out by procedure outlined by Perkin Elmer atomic absorption spectrophotometry, 1981. Standards for the above elements were prepared according to PERKIN – ELMER'S manual.

vii) Phytochemicals :Dry samples of the alga with 75% Ethanol(ethanolic extract) was used to detect the presence of phytochemicals like flavonoids, phenols, alkaloids, tannin, steroid and saponin using standard protocol [9] and [11].

3. Observations

i) Total carbohydrates, lipids and proteins

The levels of total carbohydrates, lipids and proteins were high in April as compared to December (Table 1). The levels of carbohydrate and lipids in April were nearly twice that of December However, the total lipid content was 36% more in December than that in April.

ii) Photosynthetic pigments

Photosynthetic pigment levels in the experimental algae were more or less similar in April as compared to that of December (Table 2). Hence, the ratio of chl.a to chl.b was slightly higher in April (1 : 1.2) as against 1 : 1.4 of December.

iii) Total amino acids

Eighteen amino acids namely, aspartic acid, glutamic acid, asparagine, serine, glutamine, glycine, threonine, arginine, alanine, cystine, tyrosine, histidine, valine, methionine, isoleucine, phenylalanine, leucine and lysine were found to be present in the shade dried, powdered samples of the experimental algae . The total pool size in April was 32.0% smaller than that of December. Lysine, phenylalanine and aspartic acid were the major constituents (Table 3). The levels of asparagine, phenylalanine, leucine, valine and glutamic acid were 59.20. 50.58, 49.53, 45.33 and 42.21 $\mu g\ g^{-1}$ dry wt respectively.

iv) Vitamins

The vitamins nicotinic acid, B6, B1 and B2 were detected in the shade dried, powdered samples of the experimental algae (Table 4). In both algae, vitamin B6 was present in huge quantities as compared to the other three. Nicotinic acid and vitamin B2 levels were always high in December as compared to the month of April.

v) Fatty acids

The relative levels of fatty acids in the experimental algae are shown in Table 5. Gas chromatographic analysis of the shade dried powdered samples showed the presence of fifteen fatty acids namely, caprylic acid, lauric acid, tridecanoic acid, myristic acid, pentadecanoic acid, cis-pentadecanoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, cis-heptadecanoic acid, stearic acid, oleic acid, linoleic acid, γ-linoleic acid and α-linoleic acid. In both the months palmitic acid was the major constituent forming the bulk of total fatty acid content. Palmitic acid alone constituted 93% of total fatty acid content Lauric acid, palmitoleic acid and linoleic acid were the major constituents among these fatty acids.

vi) Minerals

The minerals copper, zinc, iron, manganese, chromium, lead, calcium, nickel, cadmium, magnesium, sodium, potassium and cobalt were estimated in the shade dried powdered samples of the experimental algae.Calcium was the major constituent next major constituent in these alga was iron. Table 6.

viii) Phytochemical components

Preliminary phytochemical analysis of the killer weed revealed the presence of different bioactive chemical components in crude extracts with ethanol(75%) showed the presence of various chemical groups including flavonoids, phenols, alkaloids. No tannin, steroid and saponin were detected in this alga.Table 7.

FIGURES

Experimental alga in the month of April and December

TABLES

Table 1: Total carbohydrate, lipid and protein of *Caulerpa taxifolia.*

Experimental alga month of collection	Total carbohydrate mg g^{-1} dry wt (mean ± S.E.)	Total lipid mg g^{-1} dry wt (mean ± S.E.)	Total protein mg g^{-1} dry wt (mean ± S.E.)
April	52.0 ± 0.577	98.0 ± 2.309	11.95 ± 0.058
December	40.9 ± 0.52	124.0 ± 1.155	12.6 ± 0.162

Table 2: Pigments of *Caulerpa taxifolia.*

Experimental alga month of collection	Chlorophyll a mg g^{-1} fresh wt (mean ± S.E.)	Chlorophyll b mg g^{-1} fresh wt (mean ± S.E.)	Carotenoids mg g^{-1} fresh wt (mean ± S.E.)
April	5.178 ± 0.03	6.075 ± 0.009	11.201 ± 0.005
December	3.245 ± 0.003	4.286 ± 0.003	15.6 ± 0.006

Table 3: Amino acid content of *Caulerpa taxifolia.*

Amino acid	Month of April µg g^{-1} dry wt (mean ± S.E.)	Month of December µg g^{-1} dry wt (mean ± S.E.)
Aspartic acid	71.666 ± 0.289	27.083 ± 0.254
Glutamic acid	39.199 ± 0.115	42.212 ± 0.058
Asparagine	54.526 ± 0.231	59.200 ± 0.058
Serine	24.069 ± 0.18	26.000 ± 0.017
Glutamine	35.680 ± 0.046	14.698 ± 0.041
Glycine	19.650 ± 0.115	33.103 ± 0.369
Threonine	15.237 ± 0.021	17.931 ± 0.001
Arginine	23.984 ± 0.020	24.562 ± 0.030
Alanine	14.963 ± 0.105	25.140 ± 0.012
Cystine	35.471 ± 0.115	26.995 ± 0.012
Tyrosine	25.450 ± 0.026	18.671 ± 0.115
Histidine	28.951 ± 0.717	44.898 ± 0.046
Valine	13.497 ± 0.004	45.332 ± 0.018
Methionine	24.385 ± 0.049	29.826 ± 0.015
Isoleucine	13.999 ± 0.057	74.393 ± 0.005
Phenylalanine	80.127 ± 0.058	50.585 ± 0.049
Leucine	30.890 ± 0.111	49.535 ± 0.003
Lysine	118.210 ± 1.732	5.022 ± 0.013

Table 4: Vitamin content of *Caulerpa taxifolia.*

Vitamin	**April** **µg g^{-1} dry wt** **(mean Ъ S.E.)**	**December** **µg g^{-1} dry wt** **(mean Ъ S.E.)**
Nicotinic acid	42 ± 1.732	68 ± 1.732
Vitamin B 6	181 ± 2.887	115 ± 0.577
Vitamin B 1	59 ± 1.732	46 ± 2.309
Vitamin B 2	32 ± 1.433	75 ± 1.732

Table 5: Fatty acid Profile of *Caulerpa taxifolia*

Fatty acid	**Month of April** **µg g^{-1} dry wt (mean Ъ S.E.)**	**Month of December** **µg g^{-1} dry wt (mean Ъ S.E.)**
Caprylic acid	28.0 ± 1.155	22.0 ± 0.866
Lauric acid	73.0 ± 3.464	67.0 ± 0.577
Tridecanoic acid	33.0 ± 0.577	27.0 ± 0.289
Myristic acid	12.0 ± 0.289	11.067 ± 0.346
Pentadecanoic acid	11.7 ± 0.404	11.6 ± 0.176
Cis– 10– Pentadecanoic acid	7.1 ± 0.058	7.3 ± 0.173
Palmitoleic acid	42.0 ± 1.155	34.1 ± 0.577
Heptadecanoic acid	29.0 ± 1.155	27.3 ± 0.173
Cis-10-Heptadecanoic acid	34.0 ± 1.732	42.0 ± 0.577
Stearic acid	24.0 ± 1.155	29.4 ± 0.866
Oleic acid	27.0 ± 1.732	24.0 ± 0.808
Linoleic acid	47.0 ± 1.155	47.7 ± 0.981
¡ Linoleic acid	8.6 ± 0.289	10.0 ± 0.173
µ Linoleic acid	10.0 ± 0.346	8.6 ± 0.346
Palmitic acid	5008 ± 12.124	5001 ± 15.011

Table 6: Mineral Content of *Caulerpa taxifolia*

Mineral	**Month of April** **(µg g^{-1} dry wt) (mean ± S.E.)**	**Month of December** **(µg g^{-1} dry wt) (mean ± S.E.)**
Copper	9.1 ± 0.017	8.641 ± 0.173
Zinc	19.96 ± 0.115	19.92 ± 0.115
Manganese	53.05 ± 0.058	8.75 ± 0.144
Chromium	5.15 ± 0.087	16.6 ± 0.115
Lead	2.15 ± 0.144	0.4 ± 0.006
Nickel	22.4 ± 0.023	25.2 ± 0.115
Cadmium	8.5 ± 0.115	5.9 ± 0.058
Magnesium	5.126 ± 0.058	9.6 ± 0.116

Sodium	32.67 ± 0.173	39.11 ± 0.058
Potassium	20.6 ± 0.035	29.65 ± 0.029
Cobalt	9.35 ± 0.115	3.47 ± 0.058
Calcium	6261.4 ± 34.641	2053.4 ± 5.774
Iron	728.55 ± 0.231	858.5 ± 0.26

Table 7: Qualitative phytochemical properties of *Caulerpa taxifolia.*

S.No	**Phytochemiclas in ethanolic extract of Caulerpa**	**The month of April**	**The month of December**
1	Alkaloids	++	+
2	Steroids	-	-
3	Tannin	-	-
4	Saponin	-	-
5	Flavonoids	+	++
6	Phenols	-	++

*Shade dried powdered material. (+) presence and (-) absence

4. Discussion

The search for novel antibiotics for therapeutic exploitations is continuous and algae have found new applications in many fields that include food preservation, agriculture and biotechnology.

The present observations on the proximate composition of the experimental alga was nearly similar to that observed by [12] . Analysis of the mineral content of the alga showed the presence of copper, zinc, iron, manganese, chromium, lead, calcium, nickel, cadmium, magnesium, sodium, potassium and cobalt in their tissues. Interestingly, calcium was the major constituent accounting to nearly 66 to 87% of the total mineral content [13].

Seasonal variations in the level of minerals in many green algae have also been shown [14]

The observed levels of lysine in *Caulerpa taxifolia* is more than that reported for *Spirulina* and less than that reported for another fresh water algae *Pithophora oedogonia* [15]. The experimental alga has high levels of asparagine in addition to having arginine and cystine indicating the possible role of these amino compounds in the storage and transport of assimilated nitrogen [16].

In recent years, nutritional value of long chain polyunsaturated fatty acids (LCPUFA) has been recognized and considerable attention is being focused on algae as a source of these fatty acids. Many microalgae have been shown to be a good source of LCPUFA Hence, there is a slight variation in the nutraceutical value of the experimental alga *Caulerpa taxifolia* as collected in the months of April and December.

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