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STUDIES ON PHYTOCHEMICALS OF MARINE ALGAL SPECIES OF A GENUS CAULERPA FROM GULF OF MANNAR, COASTAL REGIONS, INDIA

[1]Nandakumaran T., [1]Anbalahan N., [2]Nivetha S., [2]Arunadevi S., [2]Revathi P. and Dr. G. Subramanian[2]*

[1]PG and Research Department of Botany, Kandaswami Kandar's College, Velur, Namakkal - 638 182.

[2]PG and Research Department of Botany, Arignar Anna Government Arts College, Namakkal – 637 002.

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***Corresponding Author**
Dr. G. Subramanian
PG and Research Department of Botany, Arignar Anna Government Arts College, Namakkal – 637 002.

ABSTRACT

This present study was carried out with four marine green algal species namely *Caulerpa scalpelliformis*, *Caulerpa serrulata*, *Caulerpa sertularioides*, *Caulerpa racemosa* for the measurement of phytochemicals quantitatively. The results of the phytochemicals carbohydrate, protein, lipid, and phenol were maximally 43.65% (*Caulerpa sertularioides*), 8.35% (*Caulerpa serrulata*), 6.7% (*Caulerpa serrulata*) and 16.77 mg GAE/g (*Caulerpa serrulata*) respectively.

INTRODUCTION

Seaweeds have been used as a foodstuff in Asian diet as it contains carotenoids, proteins, vitamins, dietary fibers, essential fatty acids, and minerals. Marine algae are exploited mainly for the industrial production of phycocolloids. Bio-stimulant and the antimicrobial properties of seaweeds are used in agriculture and the development of novel antibiotics respectively. Seaweeds have been reported to have valuable medicinal principles such as antibiotics, laxatives, anticoagulants, anti-ulcer products, neurotoxins and suspending agents in radiological preparations.[1] These algae form an important renewable resource in the marine environment and have been a part of human cultivation from time immemorial.

Seaweeds are traditionally consumed in many parts of the world as a daily diet. Fresh and

dried seaweeds are extensively consumed especially by people living in the coastal areas. Although nutrient contents vary with species, geographical location, season and temperature, the human consumption of green algae (5 %), brown algae (66.5 %) and red algae (33%) is high in Asia, mainly Japan, China, and Korea[2,3] Literature survey on certain edible seaweeds showed that they contain significant amount of protein, vitamins, and minerals which are essential for human nutrition.[4,5] Marine algae contain more than 60 trace elements in concentration *i.e.* much higher than terrestrial plants.[6] They contain a wide range of organizational, functional elements which are beneficial for good health, to boost circulation as well as perfect skin humectants.[7-11] The phytochemicals from marine algae are extensively used in various industries such as food, confectionery, and textile, pharmaceutical, dairy, and paper mostly as gelling, stabilizing and thickening agents. They are also used as food, feed, and fertilizer in many parts of the world.

The metabolic active compounds already isolated from seaweeds have helped in the development of new drugs against cancer, microbial infections and inflammation.[12] Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. Nowadays, there is an increase in the use of antibiotics due to heavy infections and the pathogenic bacteria becoming resistant because of the indiscriminate usage of antibiotics. It becomes a greater problem of giving treatment against resistant pathogenic bacteria. Moreover, the cost of the drugs is high and they cause an adverse effect like hypersensitivity reactions and depletion of beneficial microbes in the gut. Hence it became necessary for the development of new alternatives.[13] The increasing demand for antibiotics in the screening programs seeking therapeutic drugs from a marine natural source has led to interest, especially seaweed[14] and *Caulerpa is* used as green salad vegetable.[15]

MATERIALS AND METHODS

Four marine green algal seaweeds namely *Caulerpa scalpelliformis, C. serrulata, C. sertularioides,* and *C. racemosa* were collected by handpicking from the intertidal area of Mandapam coast, Gulf of Mannar, Rameswaram, Tamil Nadu, India. The seaweeds were shade dried and coarsely powdered and subjected to analysis of the phytochemicals quantitatively.

ANALYSIS OF PHYTOCHEMICALS

Carbohydrate Estimation

Carbohydrates are the important components of storage and structural materials in the seaweeds. They exist as free sugars and polysaccharide. They are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethylfurfural which forms a green coloured product with phenol and has an absorption maximum at 490 nm. The total carbohydrate content of the powdered marine green alga *Caulerpa scalpelliformis*, brown algae *Caulerpa serrulata, Caulerpa sertularioides,* and red alga *Caulerpa racemosa* were estimated by phenol-sulphuric acid method.[16]

Reagents

(i) 2.5 N HCl

(ii) Solid sodium carbonate

(iii) Stock glucose solution: weighed accurately 10 mg of glucose and dissolved in distilled water and made up to 10 ml in the standard flask (concentration 1 mg/1ml).

(iv) Phenol 5 %: Redistilled (reagent grade) phenol (50 g) dissolved in water and diluted to one liter.

(v) Sulphuric acid 96% reagent grade.

(vi) Working standard: Dilute 1ml of stock solution to 10 ml with distilled water in standard flask.1 ml of this solution containing 100 µg of glucose.

(vii) Preparation of sample: About 100 mg of the crude powdered seaweeds were hydrolyzed by boiling with 2.5 N HCl for 3 hours and then cooled to room temperature. This mixture was then neutralized using solid sodium carbonate until the effervescence ceases and the volume was made up to 100 ml and centrifuged. The supernatant was separated and used for estimation.

Procedure

The various concentrations of the working standard were pipetted out and 0.1 ml of supernatant in triplicates into a series of test tubes. All the test tubes were made up with a volume of 1 ml distilled water. A tube with 1 ml of distilled water served as the blank. 1 ml of phenol solution was added followed by 5 ml of 96% sulphuric acid to each test tube and shaken well for 10 minutes. The test tubes were placed in a water bath at 25-30º C for 20 minutes. The green color developed was read at 490 nm using the spectrometer. A standard

graph of glucose was plotted, from which the carbohydrate content of the four seaweed powders was determined.

Protein Estimation

The seaweeds are a good source of proteins rich in amino acids. The amino acids present in proteins react with Folin-Ciocalteau agent, which contains phosphomolybdic acid and tungstate, to produce a blue colored complex, which absorbs maximally at 620 nm.[17]

Reagents

(i) 2 % w/v sodium carbonate in 0.1 N sodiumhydroxide (Reagent-A)

(ii) 0.5 % copper sulphate in 1 % w/v potassium sodium tartarate (Reagent-B).

(iii) Alkaline copper sulphate : Mix 50 ml of reagent A and 1 ml of reagent -B before use (Reagent C)

(iv) Folin-ciocalteau reagent (Reagent D), commercially available (1:2)

(v) Stock protein solution: Weighed accurately 50 mg of bovine serum albumin (fraction V) and dissolved in distilled water and made up to 50 ml in a standard flask.

(V) Working standard: Diluted 10 ml of the stock solution to 50 ml with distilled water in a standard flask. One ml of this solution contains 200 µg of proteins.

Procedure

The various concentrations of the working standard and 5 mg of dried seaweed powder in triplicates were pipetted out into test tubes. The volumes of all the test tubes were made to 1 ml with distilled water. A tube with 1 ml of water serves as the blank. 5 ml of the reagent-C was added to each tube including the blank. Mixed well and allowed to stand for 10 minutes. 0.5 ml of reagent-D was added mixed well and incubated at room temperature in the dark for 30 min. Blue colour developed was read at 660 nm. A standard graph of protein was plotted, from which the protein content of the crude powdered seaweeds was determined.

Lipids Estimation

The lipid content of marine green alga *Caulerpa scalpelliformis*, *Caulerpa serrulata*, *Caulerpa sertularioides*, and *Caulerpa racemosa* were estimated by using chloroform-methanol mixture as described by Folch *et al.*[18]

Reagents: Chloroform- methanol (2:1).

Procedure

10 mg of dried powder sample were taken in two test tubes and to that added 5 ml of chloroform-methanol (2:1) mixture. The mixture was incubated at room temperature for 24 hrs after closing the mouth of the test tube with aluminum foil. After incubation, the mixture was filtered and the filtrate was collected in a small pre-weighed beaker and kept on a hot plate. The chloroform-methanol mixture was evaporated leaving a residue at the bottom of the beaker. The beaker with the residue was weighed. The difference between the beaker with residue and the empty beaker gives the weight of the lipid present in the sample.

Phenol Estimation

The type of phenolic content is influenced by seasons, species and place. It is assumed that the antioxidant properties of phenolics are related to the number of phenol rings that makes them more effective hydrogen donors and quenchers. The phenolic hydroxyl groups present in the phenolic compounds of the seaweed reacts with Folin reagent to produce a colored product which absorbs at 750 nm.

Reagents

(i). Folins -Ciocalteau reagent (1:2)

(ii) 7% w/v sodium carbonate

(iii) Gallic acid

Procedure

The total phenolic was determined using Folin-Ciocalteau reagent according to the method of Singleton and Rossi[19] using Gallic acid as the standard. A standard stock solution of 10 mg/ml of gallic acid was prepared in distilled water. Various concentration ranging from 200-1000 µg/ml were prepared. Various concentrations of gallic acid and 100 µl the seaweeds (1gm of dry sample in 10 ml of acetone) were incubated with 1ml of Folin-Ciocalteau reagent (1:2 with water) at room temperature for 5 minutes and then 1 ml of 7% sodium carbonate solution was added to the reaction mixture and incubated at room temperature for 90 minutes. The color developed was read at 750 nm. Gallic acid was used as the reference standard and a standard graph was plotted, from which the phenol content of the brown and red algae was determined and expressed as milligram gallic acid equivalent mg/g dry weight of seaweed material.

RESULTS AND DISCUSSION

Quantitative Phytochemical Analysis

The biochemical composition of seaweeds *Caulerpa sertularioides, Caulerpa serrulata, Caulerpa racemosa and Caulerpa scalpelliformis* were studied on a dry weight basis. Macronutrients such as total carbohydrate, total protein, and total phenols were quantified using spectrophotometric method and the total lipid by gravimetric method. The results are given in Table 1 and Figures 1-2.

Studies on the chemical composition of seaweeds have shown that these are good sources of total phenols, proteins, lipids, and carbohydrates. These compounds have been reported to possess strong antibacterial, anti-viral and anti-cancer properties.[19]

The total carbohydrate content of *Caulerpa sertularioides* was found to be 43.65% w/w, *Caulerpa serrulata* was found to be 42.55% w/w, *Caulerpa racemosa* was found to be 32.23% w/w and *Caulerpa scalpelliformis* was found 31.33% w/w. (Table -1and Figure - 1). Higher carbohydrate content in green algae may be due to the presence of polysaccharides.[20] Bioactive compounds extensively researched in carbohydrate are sulfated polysaccharides which possess good antibacterial, anti-viral activity.[21]

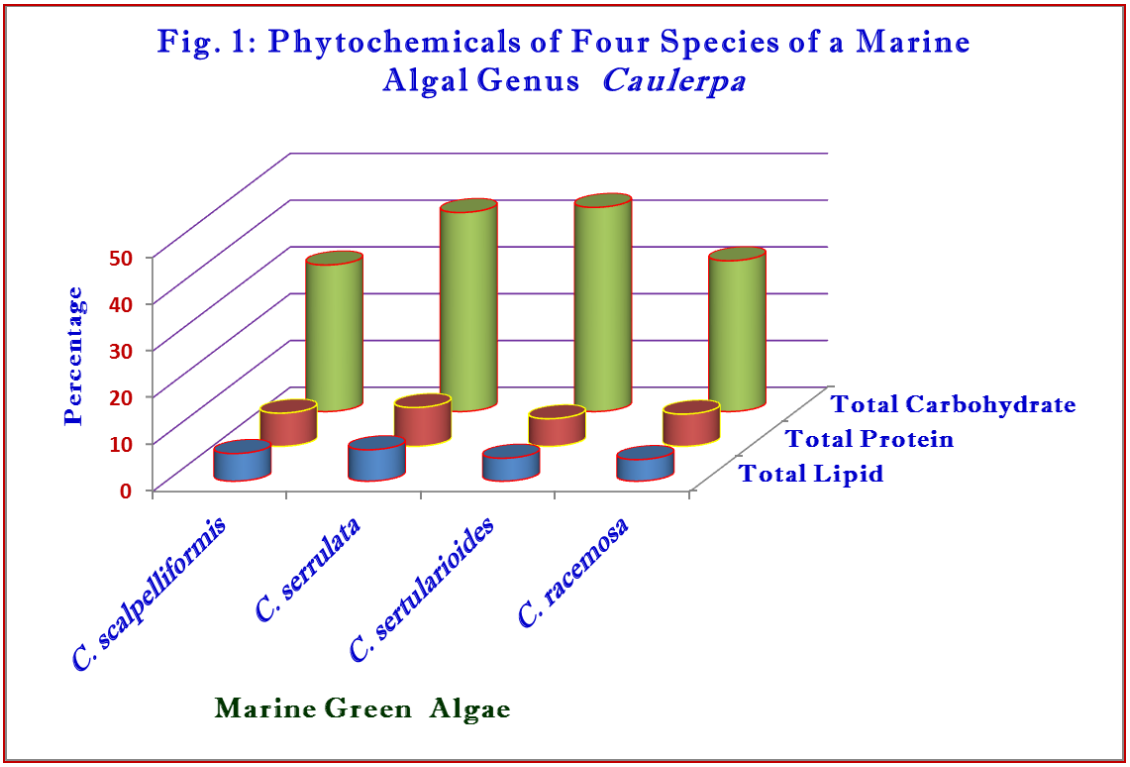
Table – 1: Quantitative phytochemical analysis of the selected seaweeds.

S.No.	Phytochemical Content(% w/w)	*Caulerpa scalpelliformis*	*Caulerpa serrulata*	*Caulerpa sertularioides*	*Caulerpa racemosa*
1	Total Carbohydrate	31.33	42.55	43.65	32.23
2	Total Protein	7.1	8.3	5.9	6.9
3	Total Lipid	5.9	6.7	4.9	4.6
4	Total Phenols (mg GAE / g)	5.2	16.77	11.98	11.44

The total protein content of *Caulerpa serrulata* was found to be 8.3% w/w, *Caulerpa racemosa* was found to be 6.9% w/w, *Caulerpa scalpelliformis* was found to be 7.1%w/w and *Caulerpa sertularioides* was found to be 5.9% w/w. (Table -1and Figure - 1). Seaweed protein is a source of all amino acids, especially glycine, alanine, arginine, proline, glutamic, and aspartic acids. The four green seaweeds *Caulerpa serrulata, C. racemosa, Caulerpa scalpelliformis* and *Caulerpa sertularioides* showed moderate protein content so it may be used as an alternative source of protein.

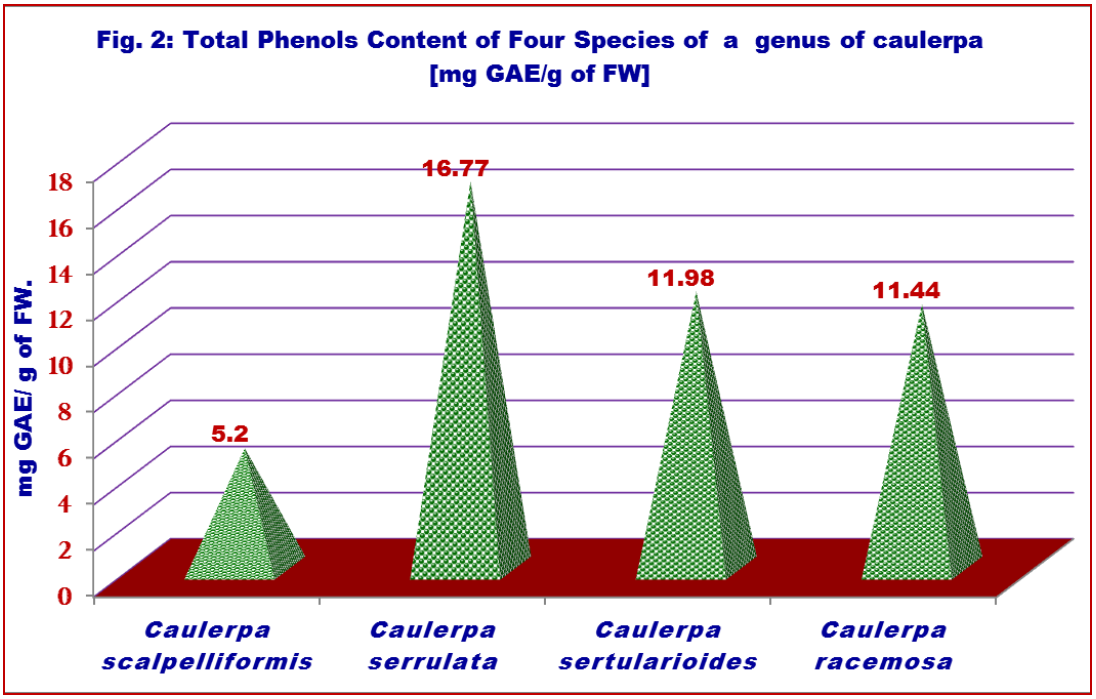
The total lipid content of *Caulerpa serrulata* was found to be 6.7%w/w, *Caulerpa scalpelliformis* was found to be 5.9% w/w, *Caulerpa racemosa* was found to be 4.6% w/w and *Caulerpa sertularioides* was found to be 4.9% w/w. (Table -1and Figure - 1). Burtin[22] reported that the lipid content of seaweeds represents only 1-5 %, thus its contribution as a food energy source.

The total phenol content of *Caulerpa serrulata* was found to be 16.77 mg GAE/ g, *Caulerpa sertularioides* was found to be 11.98 mg GAE/g., *Caulerpa racemosa* was found to be 11.44 mg GAE/g., and *Caulerpa scalpelliformis* was found to be 5.2 mg GAE/g. (Table -1and Figure - 2).



It has been earlier reported phenolic compounds possess antioxidative properties and they can also act as metal chelators which prevent the catalytic function of metal in the process of initiating radicals in the Fenton reaction.[23] The total phenol content of *Caulerpa serrulata* was found to be high (16.77 mg GAE/ g) thus could be potential rich sources of natural antioxidants. Antioxidant compounds play an important role in various diseases such as atherosclerosis, chronic inflammation, cardiovascular disorders, cancer and aging processes.[24]

The presence of phytochemicals makes the seaweeds useful for treating different ailments and have the potential of providing useful drugs of human use. These phytochemical possess antibacterial[25], antiviral[26], antifungal[27], anticoagulant, antitumor[28] and anti-inflammatory[29] activities.



CONCLUSION

Four marine algal seaweeds were taken from green algae; *Caulerpa scalpelliformis*, *Caulerpa serrulata*, *Caulerpa sertularioides*, and *Caulerpa racemosa.* They were collected by handpicking from the intertidal area of Mandapam coast, Gulf of Mannar region, Rameswaram, Tamil Nadu, The seaweeds were shade dried and coarsely powdered. Air-dried coarsely powdered marine algal seaweeds were subjected to quantitative analysis of macro-nutrients. The total carbohydrate content of *Caulerpa sertularioides* was found to be 43.65 % w/w, *Caulerpa serrulata* was found to be 42.55 % w/w. *Caulerpa racemosa* was found to be 32.23 % w/w and *Caulerpa scalpelliformis* was found to be 31.33% w/w. The total protein content of *Caulerpa serrulata* was found to be 8.3% w/w. The total lipid content of *Caulerpa serrulata* was found to be 6.7%w/w. The total phenol content of *Caulerpa serrulata* was maximally found to be 16.77 mg GAE/ g. The selected algal species of *Caulerpa* have an adequate quantity of nutritive value for human foods and they also are useful for treating different ailments and have the potential of providing useful drugs of human use.

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