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QUALITATIVE ANALYSIS OF PHYTOCHEMICALS OF FOUR MARINE ALGAL SPECIES OF A GENUS CAULERPA FROM MANDAPAM COASTAL REGIONS OF TAMIL NADU, INDIA

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ABSTRACT

The qualitative phytochemical screening was carried out for both the powder as well as petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of the marine green algae namely *Caulerpa scalpelliformis*, *Caulerpa serrulata*, *Caulerpa sertularioides*, and *Caulerpa racemosa*. *Caulerpa scalpelliformis* had shown nine phytochemical, The petroleum ether solvent extract eluted the maximum of seven phytochemicals in *Caulerpa serrulata*. The ethyl acetate solvent extract eluted the maximum of seven phytochemicals in *Caulerpa sertularioides*, the aqueous extract showed six phytochemicals namely carbohydrates, saponins, proteins, and amino acids, phenolic compounds, flavonoids and tannins from the alga *Caulerpa racemosa*.

KEYWORDS: *Caulerpa scalpelliformis*, *Caulerpa serrulata*, *Caulerpa sertularioides*, *Caulerpa racemosa*, Mandapam.

INTRODUCTION

Nearly 5, 000, 00 species available in the sea are a virtually untapped source of secondary metabolites. Seaweeds are the extraordinary sustainable resources of marine ecosystem and man has been using the seaweeds as food, feed, and medicine. It was measured that about

ninety percent of the plant species of marine are algae and about fifty percent of the global photosynthesis is being contributed from algae.[1] Kaliaperumal[2] reported that about 271 genera and 1153 species of marine algae have been found in the Indian coastal area. These algae form an important renewable resource in the marine environment and have been a part of human cultivation from time immemorial. Seaweeds have been used as a foodstuff in the Asian diet as it contains carotenoids, proteins, vitamins, dietary fibers, essential fatty acids, and minerals. Marine algae are utilized mainly for the industrial production of phycocolloids. Bio-stimulant and the antimicrobial properties of seaweeds are used in agriculture and in the development of novel antibiotics respectively. Scheuer[3] reported that seaweeds have valuable medicinal principles such as antibiotics, laxatives, anticoagulants, anti-ulcer products, neurotoxins and suspending agents in radiological preparations.

Seaweeds are traditionally consumed in many parts of the world as a daily diet. Fresh and dried marine algal seaweeds are being extensively consumed 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.[4,5] The previous literature reports on edible marine algae showed that they contain a significant amount of protein, vitamins, and minerals which are essential for human nutrition.[6,7] Marine algae contain more than 60 trace elements in concentration *i.e.* much higher than terrestrial plants.[8] They contain a wide range of organizational, functional elements which are beneficial for good health, to boost circulation as well as perfect skin humectants.[9-13] 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. In this present study, the preliminary phytochemicals from the selected four marine algal seaweeds were qualitatively tested.

MATERIALS AND METHODS

The marine green algae *Caulerpa scalpelliformis*, *Caulerpa serrulata*, *Caulerpa sertularioides*, and *Caulerpa racemosa* were collected by handpicking from the intertidal area of Mandapam coast, Gulf of Mannar region, Rameswaram, Tamil Nadu, India in the months from January 2017 to January 2018. Analytical grade chemicals were obtained from Hi-media, E. Merck, S.D. Fine chemicals, Qualigens and Sigma chemicals (USA). All solvents

and chemicals used are of analytical grade. The selected air - dried and coarsely powdered four seaweeds were subjected to qualitative analysis of phytochemicals.

Analysis of Phytochemicals

Metabolites from plants have recently gained the interest of researchers as the herbal medications have always been the most reliable source of treatment to mankind. Phytochemicals and several aromatic compounds are secondary metabolites; they are serving as defense mechanisms against predation by many microorganisms, insects and other herbivores.[14] The qualitative phytochemical screening was carried out for both the powder as well as petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of the marine green algae *Caulerpa scalpelliformis*, *Caulerpa serrulata*, *Caulerpa sertularioides*, and *Caulerpa racemosa* according to Harbone[15] and Kokate.[16]

The different qualitative chemical tests were performed for establishing the profile of the extract for its chemical composition; the following tests were performed on the powder and various solvent extracts of marine green algae *Caulerpa scalpelliformis*, *Caulerpa serrulata*, *Caulerpa sertularioides*, and *Caulerpa racemosa.*

Test for Alkaloids

Extract of each sample was separately stirred with a few ml of dilute hydrochloric acid and filtered. The extracts were tested with various alkaloidal reagents as follows:

- **Dragendorff's test**: To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added (Potassium bismuth iodide solution). A prominent orange precipitate indicated the presence of alkaloids.
- **Mayer's test**: To a few ml of filtrate, added 1 ml of Mayer's reagent (potassium mercuric iodide solution). White or cream-colored precipitate indicated the presence of alkaloids.
- **Hager's test**: To a few ml of filtrate, added 2 ml of Hager's reagent (saturated aqueous solution of picric acid); yellow-colored precipitate indicated the presence of alkaloids.
- **Wagner's test:** To a few ml of filtrate, added 2 ml of Wagner's reagent (iodine in potassium iodide), the formation of reddish-brown precipitate indicated the presence of alkaloids.

Test for Carbohydrates

- **Molisch's Test**: To the extract, one ml of alcoholic solution of □-naphthol were added, the mixture was shaken well and 1ml of concentrated sulphuric acid was added slowly

along the sides of the test tube and allowed to stand. Purple or reddish-violet color at the junction of the two liquids indicated the presence of carbohydrates.

- **Fehling's test:** One ml of filtrate was boiled on a water bath with 1 ml each of Fehling solutions A and B. A red precipitate indicated the presence of sugars.
- **Benedict's test**: To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The filtrate mixture was heated in a boiling water bath for 5 minutes. A characteristic reddish-brown colored precipitate showed the presence of sugars.

Test for Saponins

Each of the extracts was separately stirred in a test tube, foaming which persisted on warming was taken as evidence for the presence of saponins.

Test for Glycosides

A small portion of the extract was hydrolyzed with dilute hydrochloric acid for a few hours in a water bath and then the hydrolysate was tested with Legal's reagent, borntrager's reagent and Baljet reagent to detect the presence of various sugars.

Test for Proteins and Amino Acids

The extract was dissolved in 10 ml of distilled water and filtered through Whatman No.1 filter paper and the filtrate was subjected to the following test:

- **Biuret Test**: An aliquot of 2 ml of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by an excess of potassium hydroxide pellets. The pink color in the ethanolic layer indicated the presence of proteins.
- **Millon's Test**: To 2 ml of filtrate and few drops of Millon's reagent were added. A white precipitate indicated the presence of proteins.
- **Ninhydrin Test**: Two drops of ninhydrin solution (10 mg ninhydrin in 200 ml of acetone) was added to 2 ml of aqueous filtrate. A characteristic purple color showed that the presence of amino acids.

Test for Steroids

Libermann-Burchard test: A small portion of the extract was dissolved in a few drops of chloroform, 2 ml of acetic anhydride were added, warmed and cooled under the tap and one or two drops of concentrated sulphuric acid was added along the sides of the test tube. The appearance of the bluish-green color indicated the presence of steroids.

Salkowski test: The extract was dissolved in chloroform and an equal volume of concentrated sulphuric acid was added. Bluish red to cherry red in the chloroform layer and green fluorescence in the acid layer indicated the steroidal components in the tested extract.

Test for Tannins and Phenolic Compounds

Ferric chloride test: The extract was dissolved in 5 ml of distilled water. A few drops of neutral 5% ferric chloride solution added in the extract. A dark green color showed that the presence of phenolic compounds.

Gelatin test: The extract was dissolved in 5 ml of distilled water and 2 ml of 1% solution of gelation containing 10% sodium chloride was added to it. White precipitate indicated the presence of phenolic compounds.

Lead acetate test: The extract was dissolved in distilled water and to this; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presences of phenolic compounds.

Test for Flavonoids

Shinoda test: The extract was treated with a small piece of magnesium ribbon; this was followed by the dropwise addition of concentrated hydrochloric acid**.** A color ranging from orange to red indicates flavones, red to crimson indicated flavonols, crimson to magenta indicates flavanones.

Test for Terpenoids

Noller's Test: Two or three granules of tin metal were added in 2 ml thionyl chloride solution. Then the extract was added into the test tube and warmed, the formation of pink-colored indicated the presence of triterpenoids.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The powder samples of marine green algae showed the presence of alkaloids, carbohydrates, saponins, glycosides, proteins, and amino acids, steroids, phenolic compounds, flavonoids, terpenoids, tannins which added to its potentiality as a bioactive principle. Results of preliminary phytochemical analysis of various extracts of marine green algae namely *Caulerpa scalpelliformis*, *Caulerpa serrulata*, *Caulerpa sertularioides*, and *Caulerpa racemosa* were given in Tables- 1- 4.

Table 1: Preliminary phytochemical screening *Caulerpa scalpelliformis*.

Chemical Test	Powder	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloids	+	+	+	+	+	-
Carbohydrates	+	-	-	+	+	+
Saponins	+	+	+	+	+	+
Glycosides	+	-	+	+	+	-
Proteins	+	-	-	+	-	+
Steroids	+	+	+	+	+	-
Phenolic compounds	+	-	+	-	+	-
Flavonoids	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	-
Tannins	+	+	+	+	+	+
Total No. of phytochemical presence in different Solvent.	**10**	**6**	**7**	**9**	**9**	**5**

Note : (+) indicates present (-) indicates absent

The present investigation brings out adequate data on the phytochemical constituents present in all the four marine algal seaweeds.

Five solvents were used to detect the preliminary phytochemical screening of the four selected marine algal seaweeds. Ten different phytochemicals were analyzed in algal seaweeds. In *Caulerpa scalpelliformis*, ethyl acetate and ethanol showed the maximum of nine phytochemical among the ten and petroleum ether showed 6 phytochemicals in *Caulerpa scalpelliformis* dry powder. (Table-1).

Table 2: Preliminary phytochemical screening of *Caulerpa serrulata.*

Chemical Test	Powder	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloids	+	+	+	+	-	-
Carbohydrates	+	-	-	-	+	+
Saponins	+	+	+	+	-	+
Glycosides	+	+	-	+	+	-
Proteins & Amino acids	+	-	+	-	+	+
Steroids	+	+	-	-	-	-
Phenolic compounds	+	+	+	+	+	+

Flavonoids	+	-	+	-	+	-
Terpenoids	+	+	+	-	-	-
Tannins	+	+	-	+	+	+
Total No. of phytochemical presence in different Solvent.	**10**	**7**	**6**	**5**	**6**	**5**

Note: (+) indicates present (-) indicates absent

The petroleum ether solvent extract eluted the maximum of seven phytochemicals *Caulerpa serrulata* and the minimum of five phytochemicals elution in *Caulerpa serrulata* with the three solvents such as ethyl acetate, and aqueous extracts (Table-2).

The ethyl acetate solvent extract eluted the maximum of seven phytochemicals in *Caulerpa sertularioides*, and the minimum of six phytochemicals elution in *Caulerpa sertularioides* with the three solvents such as petroleum ether, chloroform, and aqueous extracts (Table-3).

Table 3: Preliminary phytochemical screening of *Caulerpa sertularioides.*

Chemical Test	Powder	Petroleum ether extract	Chloroform extract	Ethyl Acetate extract	Ethanol extract	Aqueous extract
Alkaloids	+	-	+	+	+	-
Carbohydrates	+	-	-	-	-	+
Saponins	+	+	+	+	-	+
Glycosides	+	-	+	+	-	-
Proteins & Amino acids	+	-	-	-	-	+
Steroids	+	+	+	+	-	-
Phenolic compounds	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Terpenoids	+	+	-	-	+	-
Tannins	+	+	-	+	+	+
Total No. of phytochemical presence in different Solvent.	**10**	**6**	**6**	**7**	**5**	**6**

Note : (+) indicates present (-) indicates absent

Green alga *Caulerpa racemosa* powder had been used to detect the phytochemicals availability by using different solvents such as petroleum ether, chloroform, ethyl acetate, ethanol, and aqueous. Petroleum ether had detected 6 phytochemicals from the *Caulerpa*

racemosa species such as saponins, steroids, phenolic compounds, flavonoids, terpenoids, and tannins. The chloroform extract showed the phytochemical presence such as alkaloids, saponins, glycosides, steroids, phenolic compounds, and flavonoids. The ethyl acetate extract had detected seven phytochemicals from the algal powder such as alkaloids, saponins, glycosides, steroids, phenolic compounds, flavonoids, and tannins. The ethanol extract showed five phytochemicals presence in the algal powder namely alkaloids, phenolic compounds, flavonoids, terpenoids, and tannins and the last solvent aqueous extract showed six phytochemicals namely carbohydrates, saponins, proteins, and amino acids, phenolic compounds, flavonoids and tannins from the alga *Caulerpa racemosa.*

Table 4: Preliminary phytochemical screening of *Caulerpa racemosa.*

Chemical Test	Powder	Petroleum ether extract	Chloroform extract	Ethyl Acetate extract	Ethanol extract	Aqueous extract
Alkaloids	+	+	+	+	+	-
Carbohydrates	+	-	+	+	-	+
Saponins	+	+	+	+	+	+
Glycosides	+	-	-	-	+	-
Proteins & Amino acids	+	-	+	+	+	+
Steroids	+	+	-	-	+	-
Phenolic compounds	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Terpenoids	+	+	-	-	-	-
Tannins	+	+	-	+	+	+
Total No. of phytochemical presence in different Solvent.	**10**	**7**	**6**	**7**	**8**	**6**

Note: (+) indicates present (-) indicates absent

The ethanol solvent extract eluted the maximum of eight phytochemicals in *Caulerpa racemosa* and the minimum of six phytochemicals elution in *Caulerpa racemosa* with the one solvent namely aqueous extracts and seven phytochemicals in *Caulerpa racemosa* with two solvents such as petroleum ether and ethyl acetate (Table-4).

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[17], antiviral[18], antifungal[19], anticoagulant, antitumor[20] and anti-

inflammatory[21] activities.

CONCLUSION

In this present study, four marine green algal seaweeds were selected namely *Caulerpa scalpelliformis, Caulerpa serrulata, Caulerpa sertularioides*, and *Caulerpa racemosa.* They were collected by handpicking from an intertidal area of Mandapam coast, Gulf of Mannar region, Rameswaram, Tamil Nadu, India on November 2017 to January 2018. Air-dried coarsely powdered marine algal seaweeds were subjected to qualitative analysis of macronutrients and microelements. Results showed the presence of phytochemicals namely alkaloids, carbohydrates, saponins, glycosides, proteins, and amino acids, steroids, phenolic compounds, flavonoids, terpenoids, tannins which added to its potentiality as a bioactive principle.

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