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QUALITATIVE AND QUANTITATIVE ANALYSIS OF SECONDARY METABOLITES FROM THE SEA WEEDS OF GULF OF MANNAR SOUTH INDIA

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ABSTRACT

Considering the significance and important of marine algae, especially sea weeds as food and raw material for industrial products. In the present study, sea weeds are collected from the Gulf of Mannar, nearly 18 Sea weeds were taken for qualitative and quantitative determination of secondary metabolites. The results shown that lowest to highest content of secondary metabolites from the 18 sea weeds. The results shown that the selected sea weeds contain rich source of secondary metabolites.

KEY WORDS

Sea weeds, Secondary metabolites, Industrial products, Marine algae

INTRODUCTION

The algal floras are broadly categorized into fresh water algae and marine algae. As a whole, an aggregate of 1119 species, subspecies, 100 sorts and 42 frames, altogether representing 1263 taxa of 4 algae (excluding Dinophyceae), belonging to 8 classes are distributed under 432 genera belonging to 115 families under 38 orders are reported to occur in Tamil Nadu. Rich seaweed beds occur around Visakhapatnam in the eastern coast, Mahabalipuram, Gulf of Mannar, Tiruchendur, Tuticorin, Kanyakumari and Kerala in the southern coast; Veraval and Gulf of Kutch in the western coast; Andaman and Nicobar Islands and Lakshadweep [**1-7**]. Seaweeds are considered to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae [**8-9**].

Marine macro algae are the most interesting group because of their broad spectrum of biological activities such as antimicrobial [**11**], antiviral [**12-14**], antifungal [**15**], anti-allergic [16], anti-coagulant [**17**], anti-cancer [**18**], anti-fouling and antioxidant activities. They produce a wide variety of chemically active metabolites in their surroundings as an aid to protect themselves against other settling organisms [**19**]. The present study is aim to analyze the secondary metabolites from the selected sea weeds from the gulf of Mannar Biosphere

MATERIALS AND METHODS

Study area

The Gulf of Mannar, the first Marine Biosphere Reserve (GOMMBR) in the South and South East Asia, running down south from Rameswaram to Kanyakumari in Tamil Nadu, India is situated between Longitudes 78008 E to 79030 E and along Latitudes from 8035 N to 9025 N. This Marine Biosphere Reserve encompasses a chain of 21 islands [20].

Collection of seaweeds

The green algal seaweeds were collected at a depth of 1-2 m from the coastal area of the Gulf of Mannar islands, in between Rameswaram to Kanyakumari, Tamilnadu, and South East Coast of India. Algae were collected during the low tide period in the early morning time, cleaned and removed the epiphytes and necrotic parts on them by using seawater and tape water and then rinsed with sterile water to remove any associated debris. The cleaned fresh material was air-dried [21]. The samples were identified and further confirmed as the marine green algae in chlorophyceae by using authentic floras and books [**22-23**]. They are namely ***Caulerpa chemnitzia*** (Esper) J.V.Lamououx, ***Caulerpa peldala*** J.V.Lamouroux**,** ***Caulerpa racemosa*** (Forsskål) J.Agardh**,** ***Caulerpa scalpelliformis*** (R.Brown ex Turner) C.Agardh, ***Caulerpa serrulata*** (Forsskål) J.Agardh, ***Caulerpa sertularioides*** (S.G.Gmelin) M.A.Howe, ***Caulerpa taxifolia*** (M.Vahl) C.Agardh**,** ***Caulerpa veravalensis*** Thivy & V.D.Chauhan, ***Chaetomorpha aerea*** (Dillwyn) Kützing, ***Chaetomorpha antennina*** (Bory de Saint-Vincent) Kützing**,** ***Codium adhaerens*** C.Agardh, ***Codium tomentosum*** Stackhouse, ***Enteromorpha compressa*** (Linnaeus) Nees, ***Enteromorpha intestinalis*** (Linnaeus) Nees, ***Halimeda macroloba*** Decaisne, ***Halimeda tuna*** (J.Ellis & Solander) J.V.Lamouroux, ***Ulva lactuca*** Linnaeus, **and** ***Ulva reticulata*** Forsskål,

Alkaloids (**Qualitative Test**)

For alkaloid identification, 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Total Alkaloids

About 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered, and the extract was concentrated in water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is the alkaloid, which was dried and weighed [24].

Anthraquinones (**Qualitative Test**)

For anthraquinone identification, 1 ml of plant extract few drops of 10% ammonia solution was added, appearance of pink color precipitate indicates the presence of anthraquinones.

Cardiac Glycosides (**Qualitative Test**)

For cardiac glycoside identification, 0.5 ml of extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

Coumarin (**Qualitative Test**)

For coumarins identification, 1 ml of extract, 1 ml of 10% NaOH was added. Formation of yellow colour indicates presence of coumarins.

Flavonoids (**Qualitative Test**)

For flavonoid identification, 2 ml of plant extract, 1 ml of 2 N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Total Flavonoids

A modified protocol was employed [25]. A 0.1 ml aliquot of methanolic extract, appropriately diluted, was mixed with 0.4 ml distilled water in a 1.5 mL microcentrifuge tube, 0.03 ml of 5% $NaNO_2$ was added and the mixture was allowed to react for 5 min. Following this, 0.03 ml of 10% $AlCl_3$ was added and the mixture stood for a further 5 min. Finally, the reaction mixture was treated with 0.2 ml of 1 M Na_2CO_3 and 0.24 ml distilled water, and the absorbance at 510 nm was obtained against a blank prepared similarly, by replacing extract with distilled water. Total flavonoid content was calculated from a calibration curve using catechin as standard and expressed as mg catechin equivalents (CTE) per 100 g plant [26].

Glycosides (**Qualitative Test**)

For glycoside identification, 2 ml of plant extract, 3 ml of chloroform and 10% ammonia solution were added. Formation of pink color indicates presence of glycosides.

Total Lignin (**Qualitative Test**)

0.5 ml of extract was added with 1% of aqueous Potassium permanganate solution and few drops of Ammonium chloride solution and a drop of Ammonium hydroxide. Red rose color is the indicator of the presence of lignin.

Phenols (**Qualitative Test**)

For phenol identification, 1 ml of the extract, 2 ml of distilled water followed by few drops of 10% ferric

chloride was added. Formation of blue or green color indicates presence of phenols.

Total Phenols

About 500 mg of air-dried powdered sample was taken in a 100 ml flask, to which 50 ml of 1% (v/v) HCl in methanol was added. The samples were shaken on a reciprocating shaker for 24 hours, at room temperature. The contents were centrifuged at 10000×g for 5 minutes. The supernatant was collected separately and used for further analysis. The amounts of total phenolic contents of extracts of seaweeds were determined by the spectrophotometric method with slight medication. A diluted plant extract (1 ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3. Total flavonoids Determination solution was mixed in to the test sample. The solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The Total Phenolic Content was expressed as milligrams of Gallic acid equivalents (GAE) per gram of dried sample [27-28].

Phlobatannins(Qualitative Test)

For phlobatannin identification, 1 ml of plant extract few drops of 2% HCl was added. Appearance of red color precipitate indicates the presence of phlobatannins.

Phytosteroids(Qualitative Test)

For steroid and phytosteroid identification, 1 ml of plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid. Appearance of bluish brown ring indicates the presence of phytosteroids.

Quinines (Qualitative Test)

For quinine identification, 1 ml of extract, 1 ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Saponins (Qualitative Test)

For saponin identification, 2 ml of plant extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.

Total Saponins

The samples were ground and 20 g of each were put into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered, and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated with 60 ml of n-butanol. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight, the Saponin content was calculated as percentage [29-31].

Steroids (Qualitative Test)

For steroid and phytosteroid identification, 1 ml of plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids

Tannins (Qualitative Test)

For tannin identification, 1 ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Total Tannins

A 50 µl aliquot of each extract was mixed with 1.5 ml of 4% vanillin (prepared with methanol) and then 750 µl of concentrated HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. Absorbance against blank was read at 500nm. Catechin was used to prepare the standard curve and results were expressed as mg catechin equivalents (CE)/g extracts [31]

Terpenoids (Qualitative Test)

For terpenoids identification, 0.5 ml of extract, 2 ml of chloroform along with concentrated sulphuric acid. Red brown color at the interface indicates the presence.

STATISTICAL ANALYSIS

The Percentage of Secondary metabolites in respective methods were expressed as mean + Standard deviation, which were calculated by using the method of Grap pad prism (Version 7).

RESULTS AND DISCUSSION

From the 18 selected sea weeds, the qualitative determination of alkaloids, anthroquinone, cardiac glycosides, coumarine and flavonoids identified in green algal seaweeds by using four different solvents such as methanol, ethanol, aqueous and acetone (Table 1).

The qualitative determination of glycosides, lignins, phenols, phlobatanins and phytosteroids from the green algal sea weeds by using four different solvent extractions such as methanol ethanol, aqueous and acetone. The results are represented (Table 2).

The qualitative determination of quinine, saponins tannins of green algal sea weeds with four different solvent extractions such as methanol, ethanol, aqueous and acetone. The results are tabulated (Table 3). In quantitative determination of alkaloids, flavonoids, phenols, saponins and tannins studied from the 18 different algal sea weeds Alkaloids rich in *Caulerpa scalpelliformis*, flavonoid content highest in *Caulerpa peldata,* phenol content was recorded in maximum at *C. peldata,* saponins recorded in *H. macroloba* and tannin shows highest result in *Caulerpa sertularioides*. The result are shown in ± standard deviation with four number of replications (Table 4).

Ultraviolet radiation increases secondary metabolites in alpine algae and cyanobacteria [32]. Influence of primary metabolites such as carbohydrates, aminoacids and nucleosides still not reported, but sometimes essential substrates for secondary metabolic pathway [33]. Phytotoxic impact of secondary metabolites involves alteration of assimilation of composition. Algae as a rich source of fiber, minerals, antioxidants, vitamins, pigments, steroids, lectins, halogenated compounds, polyketides, polysaccharides, mycosporine-like aminoacids, proteins, polyunsaturated fatty acids and other lipids [34]. Marine organisms both plants and animal species are potentially important sources of highly bio-active secondary metabolites that might represent useful leads in the development of new drugs in pharmaceuticals [35]. In our study, a total of 18 different algal species contains secondary metabolites. Among seaweeds, especially numerous macro algae have potent cytotoxic activities [36-37] and algal consumption has been suggested as a chemo-preventive agent against several cancers [38]. The obtained result shows that the algal species contains alkaloids, flavonoids, phenol, saponin and tannins.

Table 1. Qualitative Determination of alkaloids, anthroquinones, cardiac glycosides, coumarine and flavonoids of green algal seaweeds with four different solvents extraction

SNo.	Sea-weeds	Alkaloids				Anthraquinones				Cardiac Glycocides				Coumarine				Flavonoids			
		M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac
1	*C.chemnitzia*	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
2	*C.peldala*	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
3	*C.racemosa*	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
4	*C.Scalpelliformis*	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
5	*C.serrulata*	-	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
6	*C.sertularioides*	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
7	*C.taxifolia*	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
8	*C.veravalensis*	+	+	-	+	+	+	+	+	-	-	-	+	+	-	-	-	+	+	+	+
9	*Che.aerea*	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
10	*Che.antennina*	+	+	-	+	-	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
11	*Co.adhaerens*	+	+	-	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+
12	*Co.tomentosum*	+	+	-	+	-	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+
13	*E. compressa*	+	-	+	+	-	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+
14	*E. intestinalis*	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	+	+	+	+
15	*H. macroloba*	+	-	-	+	-	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
16	*H. tuna*	-	+	-	+	-	+	+	+	-	-	-	+	+	-	-	-	+	+	+	+
17	*U. lactuca*	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	+	+	+	+
18	*U. reticulata*	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+
	Number of species	16	15	12	18	4	18	18	18	10	13	11	18	16	14	0	0	18	18	18	18

(-) indicates Not Detected & (+) indicates detected

Table. 2. Qualitative determination of glycosides, lignins, phenols, phlobatanins and phytosteroids of green algal seaweeds with four different solvents extraction

SNo.	Sea-weeds	Glycosides				Lignins				phenols				Phlobatanins				Phytosterioids			
		M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac
1	*C.chemnitzia*	-	+	-	+	-	+	-	+	+	+	-	+	-	+	+	-	-	+	+	-
2	*C.peldala*	-	+	-	+	-	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-
3	*C.racemosa*	-	+	-	+	-	+	-	+	+	+	-	+	-	+	+	-	-	+	+	-
4	*C.Scalpelliformis*	-	+	-	+	-	+	-	+	+	+	-	+	+	+	+	-	-	+	+	-
5	*C.serrulata*	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-
6	*C.sertularioides*	-	+	-	+	-	+	-	+	+	+	-	+	+	+	+	-	-	+	+	-
7	*C.taxifolia*	-	+	-	+	-	+	-	+	+	+	-	+	-	+	+	-	-	+	+	-
8	*C.veravalensis*	+	+	-	+	-	+	-	+	+	+	-	+	-	+	+	-	-	+	+	-
9	*Che.aerea*	-	+	-	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	-
10	*Che.antennina*	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-
11	*Co.adhaerens*	+	+	-	+	-	-	-	-	+	+	-	+	-	+	+	-	-	+	+	-
12	*Co.tomentosum*	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-
13	*E. compressa*	-	+	-	+	-	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-
14	*E. intestinalis*	-	+	-	+	-	-	-	-	+	+	-	+	-	+	+	-	-	+	+	-
15	*H. macroloba*	+	+	-	+	-	-	-	+	+	+	-	+	+	+	+	-	-	+	+	-
16	*H. tuna*	-	+	-	+	-	+	-	+	+	+	+	+	-	+	+	-	-	-	-	-
17	*U. lactuca*	-	+	-	+	-	+	-	+	+	+	-	+	-	+	+	-	-	+	+	-
18	*U. reticulata*	-	+	-	+	-	+	-	+	+	+	-	+	+	+	+	-	-	+	+	-
	Number of species	4	18	0	18	0	15	0	16	18	18	5	18	9	18	18	0	0	15	15	0

(-) indicates Not Detected & (+) indicates detected

Table 3. Qualitative Determination of Quinine, Saponins, Steroids, Tannins and Terpenoids of Green Algal Seaweeds with 4 Different Solvents Extraction

S.No	Seaweeds	Quinine				Saponins				Steroids				Tannins				Terpenoids			
		M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac
1	*C.chemnitzia*	-	+	+	-	+	+	+	-	-	+	-	-	-	+	-	-	+	+	-	+
2	*C.peldala*	-	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	+
3	*C.racemosa*	-	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	-	+	-	+
4	*C.scalpellilformis*	-	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	-
5	*C.serulata*	-	-	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	+
6	*C.sertularioides*	-	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	+
7	*C.taxifolia*	-	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	+
8	*C.veravalensis*	-	+	+	-	+	-	+	-	-	+	-	-	+	+	+	-	+	+	-	+
9	*Che.aerea*	-	-	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	-	-	+
10	*Che.antennina*	-	-	-	-	+	+	+	-	-	+	-	-	-	+	+	-	+	+	+	+
11	*Co.adhaerens*	-	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	+
12	*Co.tomentosum*	-	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	+
13	*E.compressa*	-	+	+	-	+	+	+	-	-	+	-	-	+	+	-	-	-	+	+	+
14	*E.intestinallis*	-	-	-	-	+	+	+	-	-	+	-	-	+	+	+	-	-	+	-	-
15	*H.macroloba*	-	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	+
16	*H.tuna*	-	+	+	-	-	-	+	-	-	+	-	-	-	+	+	-	+	+	+	-
17	*U.lactuca*	-	+	+	-	+	+	+	-	-	+	-	-	-	+	+	-	+	+	-	+
18	*U.reticulata*	-	-	+	-	+	+	+	-	-	+	-	-	-	+	-	-	-	+	-	+
	Number of species Detected	0	13	16	0	17	16	18	0	0	18	0	0	13	18	15	0	14	17	3	15

(-) indicates Not Detected & (+) indicates detected

Table 4. Qualitative and Quantitative determination of Alkaloids, Flavonoids, Phenols, Saponins and Tannins of Green Algal Seaweeds

S.No	Seaweeds	Alkaloids mg/g of dw	Flavonoids mg QE/g fw	Phenol mg GAE /g dw	Saponins mg/ gm fw	Tannins mg CE/g dw
1	***C. chemnitzia***	1.025±0.002	1.16 ± 0.87	21.46 ± 0.08	1.006 ± 0.08	45.27 ± 0.05
2	***C. peldala***	2.321± 0.005	11.37±0.48	32.01±0.06	1.501±0.06	27.33 ± 0.68
3	***C. racemosa***	5.287±0.007	2.59 ± 0.05	31.23 ± 0.02	1.003 ± 0.02	9.25±0.09
4	***C. scalpelliformis***	5.678± 0.05	0.39 ± 0.05	5.13 ± 0.02	0.003 ± 0.02	14.32±1.06
5	***C. serrulata***	4.213± 0.003	0.63±0.03	6.16 ± 0.02	0.106 ± 0.00	35.17 ± 1.91
6	***C. sertularioides***	3.331±0.003	2.71 ± 0.02	2.14±0.03	1.14±0.03	72.84 ± 0.24
7	***C. taxifolia***	2.336± 0.002	0.79 ± 0.05	11.33 ± 0.02	0.303 ± 0.02	20.31±0.07
8	***C.veravalensis***	2.365± 0.003	0.79 ± 0.05	17.53 ± 0.02	0.33 ± 0.02	19.36±0.21
9	***Che. aerea***	3.215± 0.005	0.59 ± 0.05	9.23 ± 0.02	0.003 ± 0.02	27.36±0.34
10	***Che. antennina***	1.256± 0.003	3.56 ± 1.74	13.05 ± 0.01	4.15 ± 0.01	50.20 ± 0.55
11	***Co. adhaerens***	2.315± 0.004	0.59 ± 0.05	17.23 ± 0.02	0.023 ± 0.02	12.03±0.02
12	***Co. tomentosum***	1.235± 0.002	6.97 ± 0.47	1.16 ± 0.03	1.13 ± 0.03	17.0 ± 1.50
13	***E. compressa***	1.279± 0.05	1.59 ± 0.05	0.53 ± 0.02	1.53 ± 0.02	21.03±0.03
14	***E. intestinalis***	2.365± 0.005	5.58 ± 0.24	0.18 ± 0.01	0.018 ± 0.01	9.42 ± 1.50
15	***H. macroloba***	1.325± 0.001	0.59 ± 0.05	29.23 ± 0.02	5.023 ± 0.02	23.01±0.25
16	***H. tuna***	2.365± 0.005	0.72 ± 0.17	31.24 ± 0.01	0.024 ± 0.01	45.23 ± 1.34
17	***U. lactuca***	2.365± 0.027	0.62 ± 0.02	18.14 ± 0.02	4.004 ± 0.02	40.33±1.03
18	***U. reticulata***	1.325± 0.002	3.59 ± 0.05	22.43 ± 0.02	3.003 ± 0.02	31.02±0.23

± Standard deviation, 4 replicates

CONCLUSION

From the present study it is concluded that the difference in resources of sea weeds especially the green algae in southern coastal region of Gulf of Mannar Islands from Rameshwaram to Kanyakumari. Being plants with unique structure and biochemical composition of secondary metabolites in sea weeds.

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