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PHYTOCHEMICAL PROFILING AND GC-MS ANALYSIS OF *CAULERPA RACEMOSA*

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ABSTRACT: Aeons ago, since the inception of humans on earth, medicinal plants are being used to treat various ailments due to their high therapeutic potential. *Caulerpa racemosa* is a seaweed that is believed to possess high medicinal property especially in treating diabetes and rheumatoid arthritis. The present work was designed to investigate the preliminary phytochemical constituents and Gas chromatography - Mass spectrometry analysis of solvent extracts of the seaweed. The phytochemical profiling of the solvent extract of the seaweed revealed the presence of terpenoids, saponins, glycosides and cardiac glycosides. The chloroform extract of *Caulerpa racemosa* analysed using GC-MS affirmed the presence of 19 compound. The mass spectra of the compounds found in the chloroform extract was interrelated with the National Institute of the Standards and Technology (NIST) library. The bioactive compounds were identified by correlating their peak areas and the retention times with literature and interpreting the mass spectra.

KEYWORDS: *Caulerpa racemosa*, GC-MS, phytochemical, seaweed.

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1.INTRODUCTION

About 75% of the Earth's surface is covered by oceans, which has a wide diversity of marine organisms. These marine organisms provide a rich source of natural products that include Polyunsaturated Fatty Acids (PUFA), polysaccharides, essential minerals, vitamins, antioxidants, enzymes, peptides and several other bioactive compounds [1]. Marine organisms are the primary sources of natural products with pharmacological and biological activities [2]. Marine macroalgae

constitute a rich source of diversified secondary metabolites known as bioactive compounds. These are considered as unique and novel compounds that possess various biological activities [3]. These secondary metabolites are secreted in response to the situation of oxidation and extreme marine environmental conditions [4]. Among the macroalgae, seaweed is considered as the most accessible marine resource of the coastal zone. These seaweeds exhibit potential therapeutic importance; thus, well known as pharmaceutical active principles [5]. Extensive research has been made and reported regarding the medicinal value of the seaweeds belonging to Phaeophyceae, Rhodophyceae and Chlorophyceae from all over the world [6]. Marine macroalgae have greater ecological and commercial importance across the world especially in Asian countries. They are potential sources of drug development, valuable food resource and dietary fibres [7]. A greater diversity in biochemical composition of seaweeds suggests the exploration of various compounds with a wide range of physiological and biochemical characteristics, which may or may not be present in other taxonomic groups [8]. The bioactive compounds of seaweeds such as polyketides, isoprenoids (terpenes, carotenoids, steroids), alkaloids, phlorotannins and shikimates (flavonoids) have influenced various biochemists owing to their diversity when compared to higher plants [9]. Seaweeds are submerged and floating plants that are primitive, non-flowering without true root, stem and leaves that grow in intertidal, shallow marine meadows and also in deep sea areas up to 180 meter depth. Moreover, seaweeds are also found in estuaries, backwaters and lagoons on solid substrates such as rocks, dead corals, pebbles, shells, mangroves and other plant materials [10,11]. Seaweeds are comprised of major secondary metabolites such as alkaloids, glycosides, flavonoids, saponins, tannins, steroids, and related active metabolites with potential pharmaceutical applications [12]. *Caulerpa racemosa* is an edible green seaweed under chlorophyta belonging to Caulerpaceae family in the order of Bryophydales; widely distributed in tropical areas, rocks or in association with other seaweeds. *Caulerpa* spp. had fortified sterols and palmitic acid, which were also found in brown algae, green algae, soft corals, molluscus, sponges, ascidians and marine derived microorganisms [13-17]. Various research reports conferred that *C.racemosa* contains secondary metabolites, which can be used in numerous biological applications such as anti-inflammatory, antiviral and cytotoxic properties [18-22]. Phytochemical profiling of seaweeds are the basis for drug designing and development against many clinical complications. Phytochemisty of plants are gaining importance in recent years across the world. The present research work has focused on the phytochemical profiling of *C.racemosa* and the active biochemical compounds were further identified using gas chromatographic mass spectrometry analysis.

2. MATERIALS AND METHODS

2.1 Collection of seaweed material

The macroalgae *Caulerpa racemosa* was collected from the Mandapam region, Ramanathapuram district, Tamilnadu. Once collected, they were washed well in fresh water to remove the sea water

and other epiphytes attached. After subsequent washing, the seaweed was shade dried for a month and powdered in a blender and stored for further use.

2.2 Preparation of seaweed solvent extracts

Powdered seaweed material was ground using a pulverizer; soaked in dichloromethane and chloroform, respectively for 48 hrs at room temperature and the solvent was then filtered with Whatman filter paper (125mm). This was repeated 3-4 times until the extract turned colorless. The extracts were collected and stored in refrigerator at 4^{o}C for further phytochemical analysis.

2.3 Qualitative analysis of phytochemical constituents

Phytochemical screening was performed using standard procedures [23].

2.3.1 Test for terpenoids (Salkowski test)

5 ml of extracts were mixed with 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. Formation of a reddish brown color at the interface shows the positive result for the presence of terpenoids.

2.3.2 Test for flavonoids

Alkaline Reagent Test - Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test - Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids**.**

2.3.3 Test for saponins (Foam Test)

Foam test - To 5 ml of extracts, added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Froth Test - Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

2.3.4 Test for tannins

About 0.5 g of dried seaweed sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

2.3.5 Test for alkaloids

Mayer’s test - 1.2 ml of the extracts were taken in a test tube, 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer’s reagent (1.36 g of mercuric chloride was dissolved in 60 ml of distilled water and 5 g of potassium iodide in 10 ml of water. The two solutions were mixed and diluted to 100 ml with distilled water. Formation of yellowish buff coloured precipitate confirms the presence of alkaloids.

Hager's Test - Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow coloured precipitate confirms the presence of alkaloids.

2.3.6 Test for Steroids (Salkowski Test)

To 2 ml of extracts, 2 ml of chloroform and few drops of concentrated sulphuric acid were added. Reddish brown ring confirms the presence of steroids.

2.3.7 Test for Glycosides (Liebermann's Test)

To 2 ml of extracts, 2 ml of chloroform and 2 ml of acetic acid were added. Violet to blue to green color is regarded as positive for the presence of glycosides.

2.3.8 Test for Phlobatannins(Precipitate Test)

To 2 ml of extracts, 2 ml of 1% hydrochloric acid was added and boiled. Red precipitate is regarded as positive for the presence of Phlobatannins.

2.3.9 Test for Proteins

Xanthoproteic Test - To 1 ml of extracts, 1 ml of concentrated sulphuric acid was added and boiled. White precipitate was regarded as positive for the presence of proteins.

Ninhydrin Test - To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

2.3.10 Test for Coumarins

To 2 ml of extract, 3 ml of 10 % sodium hydroxide was added. Yellow colour was regarded as positive for the presence of coumarins.

2.3.11 Test for Cardiac Glycosides (Keller-Killani Test)

5 ml of extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of H_2SO_4. A brown ring at the interface indicates the deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the layer

2.3.12 Test for carbohydrates

Fehling's test - The filtrates were treated with 1 ml of Fehling's A and B and heated in a boiling water bath for 5-10min. Appearance of reddish orange precipitate shows the presence of carbohydrates.

Benedict's Test - Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

2.4 Determination of Total Phenolic Content

The total phenolic content of the seaweed extract was determined spectrophotometrically by Folin-Ciocalteu method [24]. Briefly, 0.5 mL of chloroform extract solution (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml) and 2.5 mL of 1:10 Folin-Ciocalteau reagent were mixed and then 2 mL of sodium carbonate (75 g/L) were added. After 15 min of incubation at 45°C, the absorbance at 765 nm was measured. The total phenolic concentration was calculated from

catechol calibration curve. Data were expressed as catechol equivalents (CE)/g of extract averaged from 3 measurements. Total Phenolic content was calculated as follows;

$$TPC= C*V/M$$

Where, TPC = total phenolic content, milligram per gram of sample extract, in CE (catechol equivalent); C = the concentration of catechol established from the calibration curve, mg/mL; V = the volume of extract, milliliter; M = the weight of sample extract (g).

2.5 Quantification of total terpenoids

About 2g of dried seaweed powder was weighed and soaked in 50 ml of 95% ethanol for 24h. The extract was filtered and the filtrate was extracted with petroleum ether (60 to 80 deg. Celsius) and concentrated to dryness. The dried ether extract was treated as total terpenoids [25].

2.6 GC-MS analysis

GC-MS analysis of the chloroform extract was carried out by following the method of Hema et al. GC-MS was performed using Perkin elmer clarus SQ8C mass spectrometer coupled with a Shimadzu 17A gas chromatograph fitted with a split-splitless injector and a DB-5 capillary standard non-polar coloumn (30mts, 0.25mm i.d., 0.25µm film thickness). The injection volume was 1µl. Mass spectra were obtained at 0.5 sec intervals.

2.7 Identification of bioactive compounds

Interpretation of the spectrum obtained using GC-MS analysis was performed by comparing with the database of National Institute of Standard and Technology(NIST) having more than 62000 patterns.

3. RESULTS AND DISCUSSION

3.1 Phytochemical profiling

The qualitative analysis of the two different extracts (chloroform and dichloromethane) of *Caulerpa racemosa* showed the presence of phytochemical constituents such as terpenoids, saponins, glycosides, cardiac glycosides and phenols. These phytochemicals were present in both the solvent extracts of *Caulerpa racemosa* but higher in the chloroform extract. The qualitative report is tabulated in Table.1. From the preliminary results, the chloroform extract was further analyzed for total phenolic and terpenoid contents. Total phenolic content of the seaweed extract was calculated to be 7.84mg per g of catechol equivalent. Total terpenoid content of the chloroform extract of *Caulerpa racemosa* was found to be 11.5 mg per g.

Table.1. Qualitative phytochemical analysis of *Caulerpa racemosa* solvent extracts

Sr.No.	Phytoconstituents	Test performed	Presence/ Absence	
			$CHCl_3$	DCM
1	Terpenoids	Salkowski test	+	+
2	Flavonoids	Alkaline Reagent Test	-	-
		Lead acetate Test	-	-
3	Saponins	Foam Test	+	+
		Froth Test	+	+
4	Tannins		-	-
5	Alkaloids	Mayer's test	-	-
		Hager's Test	-	-
6	Steroids	Salkowski Test	-	-
7	Glycosides	Liebermann's Test	++	+
8	Phlobatannins	Precipitate Test	-	-
9	Proteins	Xanthoproteic Test	-	-
		Ninhydrin Test	-	-
10	Coumarins		-	-
11	Cardiac Glycosides	Keller-Killani Test	++	+
12	Carbohydrates	Fehling's test	-	-
		Benedict's Test	-	-
13	Phenols		++	+

+: present; ++: highly present; -: absent

The phytochemical analysis of the solvent extracts of *Caulerpa racemosa* showed the presence of terpenoids, saponins, glycosides, cardiac glycosides and phenols. On contrary, flavonoids, tannins, alkaloids, steroids and carbohydrates were absent. Terpenoids and saponins are well known possess anti-inflammatory and hypoglycemic activities [26]. For instance, terpenoids were found to be capable of reducing blood glucose levels in animal studies reported in literature [27]. Saponins have also demonstrated hemolytic effects on red blood cells and cholesterol binding properties; specifically, dietary saponins could reduce plasma cholesterol level [28]. Phenols are believed to possess strong antioxidant activities. These phytochemicals, also known as secondary metabolites have been reported to manifest various biological and therapeutic properties; hence, the seaweed chosen for the study, *C.racemosa* is expected to divulge several medicinal properties [29-32].

3.2Bioactive compound identification by GC-MS analysis

GC-MS analysis of chloroform extract of *Caulerpa racemosa* revealed 19 chemical compounds that are represented in Figure 2 and Table 2. A broad range of compounds such as amines, alcohol, esters and ethers could be observed in the chromatogram. The active principles with their retention time (RT), molecular weight (MW) and molecular formula are presented in Table 2.

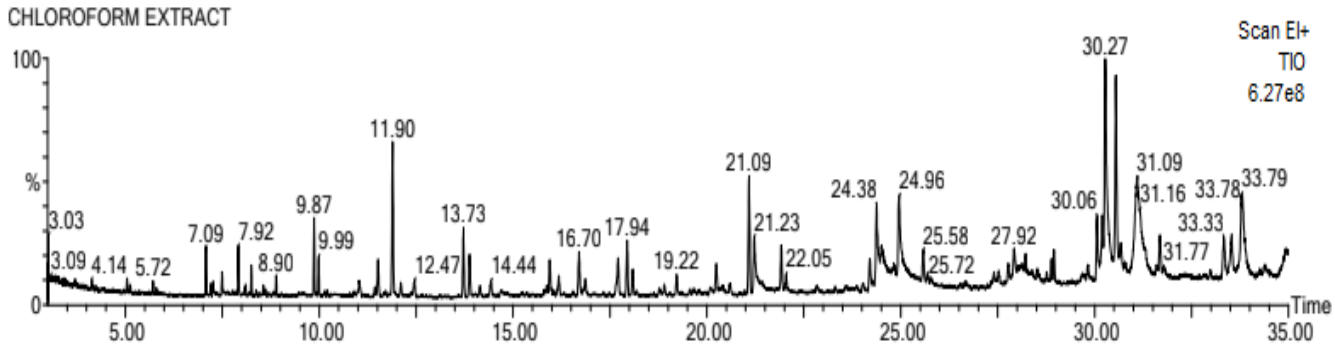

Figure 2: GC-MS chromatogram of chloroform extract of *Caulerpa racemosa*

The preliminary qualitative and quantitative analysis of phytochemical constituents present in *C.racemosa* has aided further to acquire much more precise information as depicted in GC-MS spectra provide in Fig.2. The GC-MS analysis of chloroform extract of *Caulerpa racemosa* revealed 19 chemical compounds. Similar results were observed by various researchers and the compounds were found to have various biological applications such as hypocholesterolemic, antiarthritic, anticoronary, antiandrogenic, antiinflammatory, anticancer and antioxidant activities [33-36].

Table 2: Bioactive compounds in chloroform extract of *Caulerpa racemosa*

Sr. No.	Name of the Compound	Retention Time	Molecular Weight	Formula
1	Cyclotetradecane	9.871	196.378	$C_{14}H_{28}$
2	2,4-di tert-butylphenol	11.897	206.329	$C_{14}H_{22}O$
3	1-nonadecene	13.728	266.513	$C_{19}H_{38}$
4	Hexadecane	13.883	226.448	$C_{16}H_{34}$
5	Heptacosane	15.949	380.745	$C_{27}H_{56}$
6	6-hydroxy-4,4,7a-trimethyl-5,6,7,7 a-tetrahydrobenzofuran-2(4H)-one	16.704	196.243	$C_{11}H_{16}O_3$
7	2,4-Diphenyl-4-methyl-1-pentene	17.714	236.35	$C_{18}H_{20}$
8	3-Octadecene	17.944	252.486	$C_{18}H_{36}$
9	Nonadecane	20.245	268.529	$C_{19}H_{40}$
10	Phthalic acid	21.091	166.132	$C_8H_6O_4$

11	n-Hexadecanoic acid	21.226	256.43	$C_{16}H_{32}O_2$
12	1-Docosene	21.921	308.594	$C_{22}H_{44}$
13	9,12-Octadecadienoic acid	24.382	280.452	$C_{18}H_{32}O_2$
14	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	24.507	268.391	$C_{16}H_{28}O_3$
15	Octadecanoic acid	24.962	284.484	$C_{18}H_{36}O_2$
16	1-Docosene	25.582	308.594	$C_{22}H_{44}$
17	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	27.773	262.468	$C_{16}H_{26}OSi$
18	Octadecane	27.918	254.502	$C_{18}H_{38}$
19	9,19-Cyclolanostane-3,7-diol	28.123	444.744	$C_{30}H_{52}O_2$

4. CONCLUSION

The present research report concluded that *Caulerpa racemosa* contains active phytochemical compounds such as terpenoids, saponins, glycosides, cardiac glycosides and phenols, as identified through preliminary qualitative and quantitative analysis and were further confirmed by GC-MS analysis. The presence of various bioactive compounds identified through this study, rationalise the use of seaweeds for various ailments in traditional therapy. However, isolation of the individual components and investigation of their pharmacological activity are still under exploration.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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