Research Article

GC-MS Analysis of Phytocomponents of Seaweed, *Caulerpa racemosa*

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ABSTRACT

Caulerpa racemosa seaweed is widely distributed in tropical and subtropical areas across the world. The presence of diverse phytoconstituents has been reported from the various seaweed species. However, there has been not much information available on phytochemical components and their biological activity of *Caulerpa racemosa*. This study was designed to determine the phytocomponents in the whole seaweed ethanol extract of *Caulerpa racemosa* using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. *Caulerpa racemosa* was collected from the coastal area of Gujarat state of India. After collection, it was subjected to purification, drying and ethanol extraction. GC-MS analysis of ethanol extract of *Caulerpa racemosa* was performed using a Shimadzu QP 5050A mass spectrometer coupled with a Shimadzu 17A gas chromatograph and a DB-5 fused silica capillary column. This investigation was carried out to determine the possible chemical components from *Caulerpa racemosa* by GC-MS analysis. In the present study fourteen phytocomponents have been identified from ethanolic extract of *Caulerpa racemosa* by GC-MS analysis. This analysis revealed the presence of major constituents like pseudoephedrine, 5-butyl-2-methyl-δ1-pyrrolidine, 2-myristynoyl pantetheine, tetratetracontane, deoxyspergualin, hexyl octyl ether, etc. Most of the identified major compounds were, generally, reported as having various biological activities *viz.* cytoprotection, anti-diabetic, anti-inflammatory, antioxidant, etc. From the results, it is evident that *Caulerpa racemosa* contains various bioactive compounds and can be recommended as seaweed of phytopharmaceutical importance.

Keywords: Marine algae, *Caulerpa racemosa*, diabetes, gas chromatography-mass spectrometry, seaweed

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INTRODUCTION

Caulerpa racemosa (family- Caulerpaceae) a large, edible green alga or seaweed, is widely distributed in tropical and subtropical areas across the world [1]. It is popularly referred as "sea grapes" and is found in many areas of shallow sea (figure 1). A number of different forms and varieties of seaweed are available. Caulerpa comes from a family of bisindole natural products, and it exerts variety of biological activities such as antitumor [2], anti-inflammatory [3] and growth regulator [4].

Caulerpa racemosa also contains sulphated polysaccharides (SP), with anticoagulant and antiviral activity, and recently it has been shown that SP fractions from *Caulerpa racemosa* have significant antitumor activity [1]. However, there are no detailed studies concerning the active components reported to date in this seaweed, therefore the present study was conducted to analyze the phytochemicals of unexplored seaweed, *Caulerpa racemosa*, by using Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Figure 1: Image of *Caulerpa racemosa* [5]

MATERIALS AND METHODS

Collection of Seaweed:

Caulerpa racemosa seaweed was collected from the coastal area of Gujarat state of India. The collected seaweed sample was authenticated by a local commercial seaweed based organization. The herbarium of the seaweed *Caulerpa racemosa* was prepared and preserved.

Preparation of Plant Material:

After collection of the seaweed it was washed thoroughly in seawater and then in tap water. Washing with tap water was necessary to remove dirt, salt, sand, other species parts, etc. The collected sample lot was washed multiple times and soaked in distilled water twice. After thorough washing, seaweed sample was spread under shade with open space. Washed seaweed was subjected to shade drying to avoid direct exposure to sun light. Drying was carried out approximately for one week, till it becomes moisture free.

Preparation of extracts:

A dried sample of *Caulerpa racemosa* was pulverized to powder in a mechanical grinder. 50 gm of dried seaweed powder was extracted with 500 ml of absolute ethanol
(95%) for 72 h by maceration until the powder was fully immersed, incubated over night and filtered through a Whatman number 41 filter paper. The filtrate is then concentrated by bubbling nitrogen gas in to the solution. The extract employed in GC-MS for analysis of different compounds.

GC – MS analysis:

GC-MS analysis was performed using a Shimadzu QP 5050A mass spectrometer coupled with a Shimadzu 17A gas chromatograph fitted with a split-splitless injector and a DB-5 fused silica capillary column (30m X 0.25 mm i.d., 0.25 µm film thickness). Gas chromatography analysis was performed by Agilent 6890N with FID using HP-5 capillary column. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 250^{o} C, and the split ratio was 40:1. Oven temperature programming was done from 50 to 280^{o} C, at 10^{o} C/min, and it was kept at 280^{o} C for 5 min. Interface temperature was kept at 250^{o} C. Ionization mode was electron impact ionization and the scanning range was from 40 amu to 400 amu. Mass spectra were obtained at 0.5 sec interval.

Identification of phytoconstituents:

Interpretation on Mass-Spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having huge data with their fragmentation pattern. The spectrum and fragmentation pattern of the unknown compound was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were determined.

RESULTS AND DISCUSSION

GC-MS analysis was carried out in crude extracts of seaweed *Caulerpa racemosa*. In the present study fourteen chemical constituents have been identified from

ethanolic extract of the alga *Caulerpa racemosa* by GC-MS analysis. The total ion chromatogram of alcoholic extract showing the GC-MS profile of the compounds identified is given in the figures 2 & table 1. The m/z of prominent peaks of fractions was compared with the standard one. The probable peak and its fragmentation pattern are given in Table 2. The presence of fourteen different phytocomponents has been observed, which belonging to broad groups of compounds such as long chain aliphatic compounds, acids, esters, ethers, amines and alcohols. Aromatic compounds with hetero-atoms such as oxygen, nitrogen are also prominently seen. The base peaks in the spectrum m/z = 118, m/z = 92, m/z = 58, m/z = 68, m/z = 56, m/z = 57, m/z = 148, m/z = 84, m/z = 71, m/z = 57 are due to the presence of disiliazane, aromatic and aliphatic amines, aliphatic ethers and hydrocarbons, cyclic compounds containing hetero-atoms such as O, N, aromatic carboxylic acids.

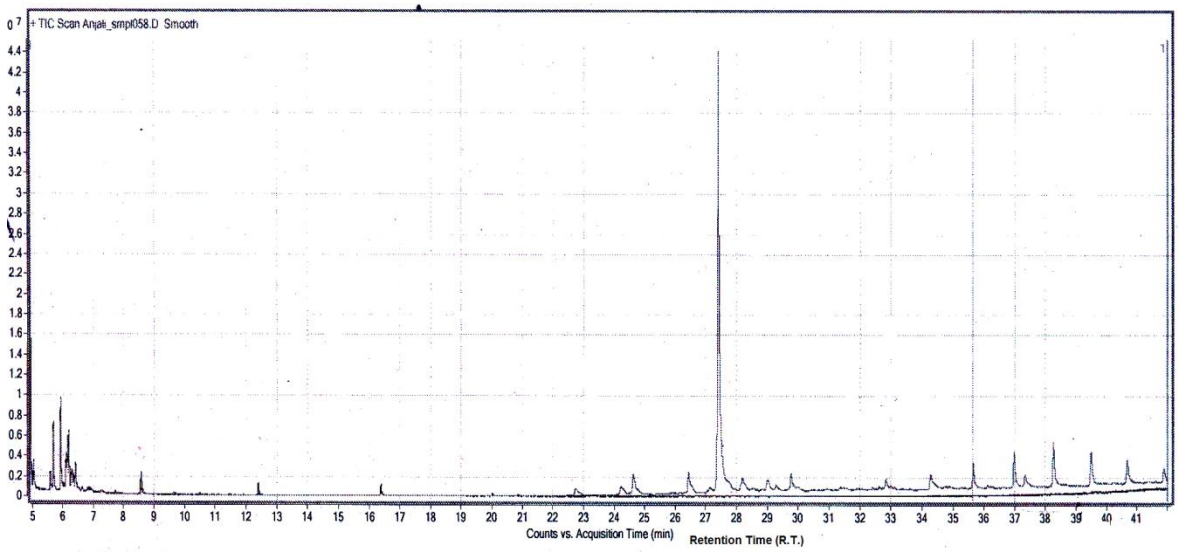


Figure 2: GC-MS chromatogram of ethanolic extract of *Caulerpa racemosa*

This analysis revealed the presence of major constituents like pseudoephedrine, 5-butyl-2-methyl-δ1-pyrrolidine,2-myristyno-yl pantetheine, tetratetracontane, deoxy-spergualin, hexyl octyl ether, etc. Most of the identified major compounds were, generally, reported as having various biological activities (**Table 1**). Recently pseudoephedrine has been reported to elicit a potent anti-inflammatory activity against acute liver failure model in rats, and this comprehensive anti-inflammatory effect may result from the inhibition of TNF-α production [6]. Pyrrolidine class of compound has shown the protective effect on islet β-cells from oxidative damage and improves insulin production in a diabetic rat model [7]. The antioxidant and cytoprotective activities have also been reported for tetratetracontane [8] and deoxyspergualin [9], respectively. In a post-marketing surveillance study, Donati *et al* [10] have recommended pantethine therapy for the treatment of lipid abnormalities also in patients at risk such as those with diabetes mellitus. Further, hexyl octyl ether class of compound has been reported to possess cytoprotective activity [11]. The various biological activities reported for these phytocomponents are supported by the findings from animal study conducted by us to evaluate pharmacological activity of ethanolic extract of *Caulerpa racemosa* (unpublished data).

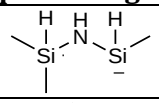
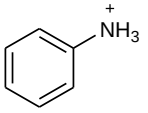
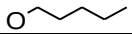
CONCLUSION

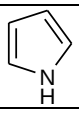
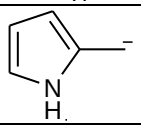
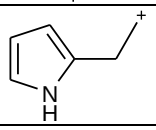
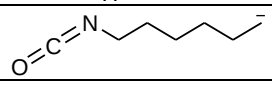
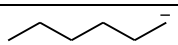
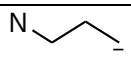
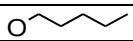
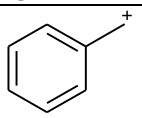
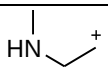
Isolation of individual phytochemical constituents from *Caulerpa racemosa* and subjecting them to meticulous biological screening can give fruitful results. From the results, it could be concluded that *Caulerpa racemosa* contains various bioactive compounds. Therefore, it is recommended as seaweed of phytopharmaceutical importance.

Table 1: Phytocomponents identified in ethanolic extract of *Caulerpa racemosa*

Sr No	Name of the compound	RT	MW	Biological activity	Reference
1	1,1,3,3, - Tetramethyldisilazane	9.651	133	Not reported	-
2	5-Allyl-4-[1-(p-aminophenyl)ethylidenehydrazono]-6-methyl-2-phenylpyrimidine	10.941	357	Not reported	-
3	Hexyl octyl ether	22.728	214	Cytoprotection	11
4	5-Butyl-2-methyl-δ1-pyrrolidine	24.225	139	Protect pancreactic islet β-cells from oxidative damage	7
5	Hexane, 1,6-diisocyanato-	24.623	168	Not reported	-
6	Octane, 1,1'-oxybis-	26.426	242	Not reported	-
7	Pseudoephedrine, (+)-	27.396	165	Anti-inflammatory	6
8	N -[3-[N-Aziridyl]propylidene]-Tetahydrofurfurylamine	29.771	182	Not reported	-
9	2-Myristynoyl pantetheine	34.272	484	Used to treat lipid abnormalities and diabetes mellitus	10
10	N -[3-[N-Aziridyl]propylidene]-Tetrahydrofurfurylamine	35.653	182	Not reported	-
11	Cyclohexylamine, N-methyl-4,4-dimethyl	36.977	141	Not reported	-
12	6-Phthalazine carboxylic acid, 1,2,3,4-Tetrahydro-1,4-dioxo-	37.329	206	Not reported	-
13	Tetratetracontane	38.262	618	Antioxidant	8
14	Dodecane, 5,8-diethyl-	39.493	226	Not reported	-
15	N-[2- [1-Piperazyl]ethyl]- N'-[2-thiophosphatoethyl]-1,3-propanamine	40.677	326	Not reported	-
16	Deoxyspergualin	41.886	387	Cytoprotection Immunomodulation	9

Table 2: GC-MS analysis fragmentation pattern of ethanolic extract of *Caulerpa racemosa*

No.	MO. Ion peak	Base peak	M/Z	Proposed fragment
1	133	118	118	
			59	
2	206	92	77	
			92	
3	148	58	88	
			71	
			58	

4	139	68	68	
			82	
			96	
5	168	56	126	
			84	
			56	
6	242	57	57	
			71	
			85	
7	148	148	92	
			57	
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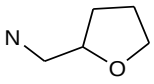
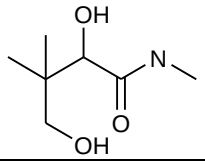
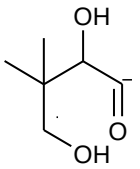
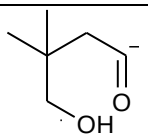
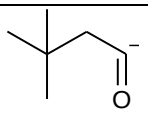
			99	
9	484	84	160	
			133	
			113	
			96	
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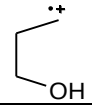
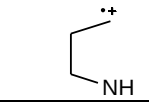
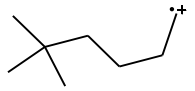
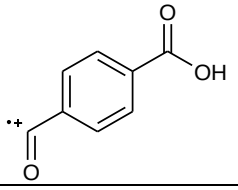
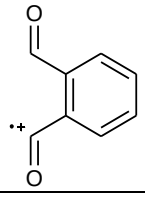
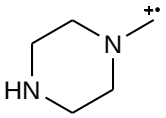
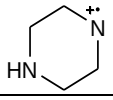
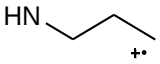
			56	

11	141	57	57	
			113	
12	206	148	148	
				
13	618	57	57	
			71	
			85	
14	Comparison not available			
15	326	56	99	
			85	
			56	
16	Comparison not available			

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