Biomedical & Pharmacology Journal, December 2020. Vol. **13**(4), p. 1921-1926

Gas Chromatography – Mass Spectroscopy [GC - MS] Analysis and Phytochemical Screening for Bioactive Compounds in *Caulerpa peltata* (Green alga)

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https://dx.doi.org/10.13005/bpj/2069

(Received: 23 April 2020; accepted: 29 October 2020)

Seaweeds (Marine macroalgae) area large group of marine organisms containing important phytochemical constituents with various biological activities .They are potentially prolific sources of highly bioactive secondary metabolites, which manifest many of the therapeutic effects like anti-cancer, anti-oxidant, anti-inflammatory and anti-diabetic activities. Seaweeds are used by many Asian cultures for traditional medicine preparations. The *Caulerpa peltata* was collected from Rameshwaram coastal area it was shade dried, made in to powder using standardized procedure to get Caulerpa peltata Methanolic Extract (CPME). The phytochemicals and Gas Chromatography-Mass Spectrometry (GC-MS) analysis was done on prepared CPME for identifying the bioactive compounds. Phytochemical investigation suggests that the Caulerpa peltata exhibited the presence of phytochemicals like Alkaloids, Carbohydrates, Phytosterols, Saponins and Diterpenes,which may contribute to its biological activities. GC-MS analysis showed 28 variety of compounds,among which Dibutylphthalate, n-hexadecanoic acid, and 1,2-Benzene dicarboxylic acid was found in high percentage. The phytochemical studies and the compounds available in GC–MS showed that the *Caulerpa peltata* contain important bioactive compounds,which may have anti-microbial, anti-fungal and anti-cancer activity. Further research is needed for finding its use in development of new pharmaceutical agent and its safe consumption by human for various health benefits.

Keywords: CPME, *Caulerpa peltata*, GC-MS analysis, Phytochemicals.

Plant -derived compounds are fascinating the world due to their multifaceted therapeutic use in modern medicine. Medicinal plants are abundant bio-resource of drugs for traditional system of medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for developing synthetic drugs[1].

Seaweeds or marine macro algae are plants and ecologically, commercially valuable living marine resources that belong to the primitive groups of non-flowering plants without true root, stem and leaves in the division Thallophyta of Plant kingdom. Marine macro algae are classified into four groups based on pigments, stored food materials, morphological and anatomical characters into Chlorophyta (Green seaweeds), Phaeophyta (Brown seaweeds), Rhodophyta (Red seaweeds) and Cyanophyta (Blue green algae)[2]. Seaweeds

obtained from coastal region are the potential marine resource for many biochemical compounds. Pharmaceutical importance of seaweeds globally has led to ample research to extract out bioactive compounds from algae. Marine habitat is an abundant resource of functional materials such as polyunsaturated fatty acids, polysaccharides, essential minerals and vitamins, antioxidants, enzymes and bioactive peptides[3].

The nutritional value of a food depends on its chemical composition such as carbohydrates, proteins, lipids, sugars and also the minerals present in them. Seaweeds are the rich source of bioactive compounds like carotenoids, dietary fiber, protein, essential fatty acids, vitamins and mineral[4] and they contain biologically active substances such as lipids, proteins, polysaccharides and polyphenol[5]. The biochemical composition of seaweeds differs, which are affected by inflow of land sources, geographic areas as on of the year and temperature of water[6]

Recently, the importance of sea weeds as a source of novel bioactive substances is growing rapidly and researchers have revealed that their compounds exhibit various biological activities due to the secondary metabolites[7]. The bioactive compounds extracted from different Marine algae have antioxidant, antiviral, antifungal and antimicrobial activities[8].

Caulerpa peltata is marine green algae with small fleshy 'umbrellas' is sometimes seen on our southern shores growing on coral rubble, near reefs. These species have several benefits for which it is consumed as food and also as medicine for its antifungal properties & its potential to reduce blood pressure. Although, there are no comprehensive studies regarding the active components in this seaweed. Hence, the current research work has focused on the phytochemical profiling of *Caulerpa peltata* and the active biochemical compounds were further identified using gas chromatographic mass spectrometry analysis.

MATERIALS AND METHODS

Collection of Seaweed

Caulerpa peltata was collected from the vedalai coastal area of Rameshwaram, Tamil Nadu, India and Marine Biologist helped in identifying the sample. The herbarium of the *Caulerpa peltata* was prepared and stored.

Preparation of seaweed Material

After collection of the seaweed it was washed many times to remove epiphytes, sand particles, other species parts, etc. The collected sample was soaked in distilled water twice. After thorough washing, seaweed sample was shade dried, till it becomes moisture free.

Preparation of extracts

A dried sample of seaweed was ground into coarse powder in a mechanical grinder. The sample was subjected to maceration at 24- 25°C in 95% methanol for about 72 hours. The methanolic extract was derived after the process of distillation, evaporation, and drying it under reduced pressure[9].

Gas Chromatography-Mass Spectroscopy analysis

GC-MS analysis performed by using a Shimadzu QP-2010 Plus with Thermal Desorption System TD20.

Derivatization of plant extracts for GC-MS: In the ratio of 1:4, water and ethyl acetate was added to the separating funnel containing concentrated sample. Small amount of concentrated sample was taken in a separating funnel and shaken by adding in 1:4 ratio. The upper layer was collected and concentrated to 1.5ml in the rotary evaporator, which is taken in funnel to add 100µl N,O-Bis(trimethylsilyl) tri fluoro acetamide and trimethyl chlorosilane(BSTFA+TMCS) & 20µl of Pyridine and heat it at 60°Cf or half an hour. To this sample Aceto nitrile was added & filtered into a conical flask to which 50µl BSTFA+TMCS was added and heated again at 60°C in a water bath for 30 minutes. The CPME was filtered using 0. 45µ membrane filter to a vial[10].

Identification of phytoconstituents

The interpretation of Mass-Spectrum of GC-MS of the unknown compound was compared with the known components using database stored in National Institute Standard and Technology (NIST).

Qualitative analysis of phytochemical substance The sample was subjected to phytochemical analysis for detection of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, diterpenes, proteins & aminoacid using the standard qualitative procedures[11 -13].

RESULTS AND DISCUSSION

The extract was tested for various phytochemicals & constituents like alkaloids, saponins, phytosterols, diterpenes & carbohydrates were recorded in *C. peltata* are shown in the Table No.1. Alkaloids in seaweeds have antimicrobial, antidiarrheal & anthelmintic action[3]. In present study *Caulerpa peltata* methanolic extract contains alkaloids, which suggests that it can be used as medicinal drug.

Table 1. Phytochemicals presenting *C.peltata* methanolic extract

Phytochemicals	Chemical analysis & results
Alkaloids	Wagner's test++, Hager's test++ Dragendroff's test++
Saponins	Froth test++, Foam test++
Phytosterols	Liebermann Burchard's test++
Diterpenes	Copper acetate test++
Carbohydrates	Molisch's test++, Benedict's test++, Fehling's test++

Saponins are integral component of Chinese medicine because it has discrete properties responsible for most of the biological actions. Hence, seaweeds used in hypercholesterolemia, hyperglycemia, to reduce weight & as antioxidant, anticancer, anti-inflammatory agents[14]. Our study confirms presence of Saponins in methanol extract of *C.peltata*, suggesting that its benefit in suppressing infections & inflammation caused due to bacteria & fungi.

The extract of *C. peltata*, can be used as antimicrobial & antidiarrhoeal agents as it consists of both phytosterols & diterpenes[3]. As Steroids have been reported to have anti bacterial properties[15].

Bioactive compounds extensively researched in carbohydrate are sulfated polysaccharides which possess good antibacterial, anti-viral activity[16]. In our study *C.peltata* extract tested positive for carbohydrates suggesting that

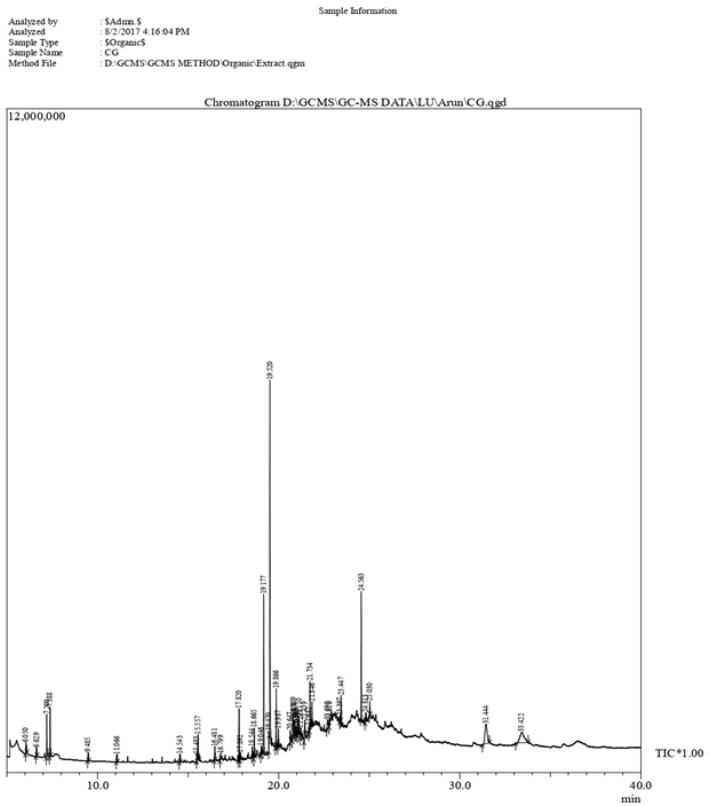


Fig. 1. Chromatogram analysis of *C.peltata*

it can be used in medical field as antibacterial & antiviral agents. The algal dietary fiber consists soluble polysaccharides having salient actions such as antioxidant, anticoagulant, antimutagenic and antitumour effect and have an key role in the modification of lipid metabolism in human body[17].

The results observed by Rajasulochana P *et al*, Venkatesh R *et al*, Jayasree NB *et al*, on phytochemical compounds[18-20] help our study on the usefulness of marine algae in traditional medicine and added a note on the phytochemical history. Due the presence of phytoconstituents in the seaweeds, they are beneficial in obtaining drugs for treating various ailments in humans. These phytoconstituents possess antibacterial[21], antiviral[22], antifungal[23], anticoagulant, antitumor[24] and anti-inflammatory activities[25].

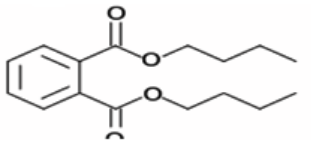
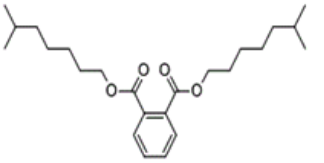
GC-MS analysis was conducted in crude extract of *Caulerpa peltata*. The current study 28 different biochemical compounds have been identified from methanolic extract of the algae *Caulerpa peltata*. The compounds exhibited a wide range in their nature. Largest peak area was observed for the compound Dibutyl phthalate & other phytochemicals like n-hexadecanoic acid &1,2-Benzene dicarboxylic acid also have a large area. The compound with highest molecular weight was Hexacosyl pentafluoropropionate (Figure No.1 & Table No.2). Major phytochemical's with their chemical structures mentioned in Table No.3.

The most abundant phytocompounds compounds, Dibutyl phthalate acts as antimetabolic agent[26], n-hexadecanoic acid has various biological activities antioxidant, hypolipidaemic, nematocide, lubricant & haemolytic inhibitor[27],where as 1,2-Benzene dicarboxylic acid has antimicrobial & antifouling activity[28]. Hence *C. peltata* should be extensively studied so that it can be used in

Table 2. Phytocomponents in methanolic extract of *Caulerpa peltata*

Sl No.	Reaction Time	Area %	Mol. weight	Compound name	Molecular Formula
1	7.163	1.33	115	Acetamide, N,N-diethyl-	$C_6H_{13}NO$
2	7.382	1.57	87	Acetamide, N-Ethyl-	C_4H_9NO
3	13.563	0.58	204	Caryophyllene	$C_{15}H_{24}$
4	14.543	0.67	206	Phenol, 3,5-bis(1,1dimethylethyl)-	$C_{14}H_{22}O$
5	15.533	2.52	222	Diethyl Phthalate	$C_{12}H_{14}O_4$
6	16.482	0.48	226	8-Pentadecanone	$C_{15}H_{30}O$
7	17.444	0.78	228	Tetradecanoicacid	$C_{14}H_{28}O_2$
8	17.819	1.23	238	1-Heptadecene	$C_{17}H_{34}$
9	18.278	0.91	278	Neophytadiene	$C_{20}H_{38}$
10	18.542	0.62	278	1,2-benzenedicarboxylic acid, Bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$
11	18.666	1.33	226	8-Pentadecanone	$C_{15}H_{30}O$
12	19.175	2.73	270	Hexadecanoic acid, methylester	$C_{17}H_{34}O_2$
13	19.519	42.02	278	Dibutyl phthalate	$C_{16}H_{22}O_4$
14	19.552	17.24	256	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
15	19.865	1.65	266	1-Nonadecene	$C_{19}H_{38}$
16	19.986	0.84	340	Eicosyl acetate	$C_{22}H_{44}O_2$
17	20.646	0.58	282	10-Nonadecanone	$C_{19}H_{38}O$
18	20.813	1.33	268	Oxirane, hexadecyl-	$C_{18}H_{36}O$
19	20.872	0.41	296	Methyl Dihydromal Valate	$C_{19}H_{36}O_2$
20	21.225	0.96	282	Heptadecene-(8)-CarbonicAcid-(1)	$C_{18}H_{34}O_2$
21	21.438	3.36	284	Octadecanoic acid	$C_{18}H_{34}O_2$
22	21.733	1.49	410	Octacosanol	$C_{28}H_{58}O$
23	21.843	1.16	340	Eicosyl acetate	$C_{22}H_{44}O_2$
24	23.446	1.00	354	n-Tetracosanol-1	$C_{24}H_{50}O$
25	24.562	10.22	390	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$
26	25.030	0.51	396	1-Heptacosanol	$C_{27}H_{56}O$
27	27.861	0.92	528	Hexacosyl pentafluoropropionate	$C_{29}H_{53}F_5O_2$
28	31.520	1.55	386	Cholesterol	$C_{27}H_{46}$

Table 3. Major phytochemical compounds in the methanolic extract of *C. peltata* & their chemical structure

S. No	RT	Compound Name	Mol. formula	Mol. Wt	Peak area	Structure
1	19.552	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	17.24	
2	19.519	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	42.02	
3	25.562	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	10.22	

pharmaceutical industry for their medicinal value.

CONCLUSION

The current study confirmed 28 distinct phytochemical substances present in the extract of *Caulerpa peltata* which could be the bioactive constituents. The alga *C. peltata* are valuable reservoir of bioactive compounds of medicinal interest which needs detailed studies so that it may be used in drug formulation by the pharmaceutical industries.

ACKNOWLEDGMENT

We Acknowledge Dr S Bragdeeswaran, Associate Professor CAS in Marine biology, Annamalai University, who really gave invaluable support & contribution in the study.

Conflict of Interest

Authors declare there is no conflict of interest

Funding Source

There is no funding source

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