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Gas ghromatography and mass spectroscopic analysis of bioactive compounds in *Ulva reticulata* extract

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Abstract
The bioactive components of *Ulva reticulata* have been evaluated using GC/MS. The chemical compositions of the ethanolic extract of *Ulva reticulata* was investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of 15 compounds in the ethanolic extract of *Ulva reticulata* in which the prevailing compounds like Hoslundin, 2,4,6-Tris-(1-phenylethyl)-phenol, 1,2-Benzenedicarboxylic acid, Alpha.-Ergostenol, Stigmasterol and Gamma.-Sitosterol are reported to be biologically active. These findings support the traditional use of *Ulva reticulata* in various disorders.

Keywords: gas chromatography and mass spectroscopy, seaweeds, marine algae, *Ulva reticulata*, biologically compounds

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. Seaweeds or macroalgae are aquatic plants belonging to the plant kingdom *Thallophyta* and recognized as a potential source of bioactive natural products. Today, seaweeds are the raw materials for many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries (Mishra *et al*., 1993) [1]. Distribution of seaweed species in India are Gulf of Kutch, Gangeya, South West Coast of India; Mandapam, Kanyakumari, Muttam and Arokiapuram Karnataka, Kerala, Lakshadweep, Tamil Nadu, Andhra Pradesh, Gopalpur coast and brackish water lake Chilika of Orissa; West Bengal and Andaman & Nicobar Islands. India presently harvests only about 20,000 tonnes of macroalgae annually (Verma, 2012) [2].

Seaweeds are reservoirs of carotenoids, pigments, polyphenols, enzymes, diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B1, B12, C, D and E (Smit, 2004) [3] and have antibacterial, antialgal, antimacrofouling and antifungal properties which are effective in the prevention of biofouling and have other likely uses, as in therapeutics (Thoudam *et al*., 2011) [4]. Marine algae are one of the largest producers of biomass in the marine environment (Bhadury *et al*., 2004) [5] they produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against the other settling organisms. People have used algae as food fertilizer for hundreds of years. Recent uses of algae include biodiesel fuel, thickening agent for food, bacterial growth medium, and pollution control.
Within a decade, there was a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation identification and structure determination of phytochemicals. The aim of this study is to determine the bioactive compounds present in the given edible seaweed *Ulva reticulata* extract with the aid of GC-MS Technique, which may provide an insight in its use in conventional medicine.

Materials and Methods
Plant Materials
The collected Fresh edible green seaweed *Ulva reticulata* was cleaned well with sea water to remove all the extraneous matter such as epiphytes, particles of sand, pebbles and shells and brought to the laboratory in air tight container, then washed thoroughly with tap water followed by distilled water several times. Washed seaweed was blotted on blotting paper and spread at room temperature in shade to dry. The dried seaweed was ground to a fine powder using tissue blender and then the powdered sample was stored in refrigerator for further use.

Extract Preparation
20gm powdered sea weed was soaked in 50ml of Absolute alcohol overnight and then filtered through Whatmann filter paper No.41 along with 2gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate was then concentrated by bubbling nitrogen gas into the solution and reduced the volume to 1ml. The extract contains both polar and non-polar phytocomponents used for GC-MS analysis.

GC-MS Analysis

In recent years a significant number of novel metabolites with potent pharmacological properties have been reported from new world. GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5µI was employed (split ratio of 10:1) at injector temperature 270ºC; ion-source temperature 200ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min to 150ºC, then 8ºC/min to 250ºC, ending with a 20min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al*., 2013) [6].

Identification of Components

Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes, 2013) [7].

Results and Discussion

In the present study fifteen chemical constituents have been identified from the ethanolic extract of *Ulva reticulata* by gas chromatogram-mass spectrophotometry (GC-MS) analysis and the spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The active principle compounds with their retension time (RT), name of the compound, molecular formulae, molecular weight (MW) and area percentage are presented in Table 1 and Fig 1. The most prevailing compounds in *Ulva reticulata* were Hoslundin, 2, 4, 6-Tris-(1-phenylethyl)-phenol, 1,2-Benzenedicarboxylic acid, Alpha.-Ergostenol, Stigmasterol, Androstane-11, 17-dione, 3-[(trimethylsilyl)oxy]-17-[O-(phenylmethyl) oxime] (3α, 5α) and Gamma-Sitosterol.

The knowledge of the phytocomponents in a medicinal plant is very essential to analyse their therapeautic value and such precise qualitative analysis can be obtained by Gas Chromatography coupled with Mass Spectrometry (GC-MS) (Cyriac and Eswaran, 2015) [8]. This study has revealed the presence of 15 compounds that possess various biological properties. The biological activity of *Ulva reticulata* extract is represented in Table 2 (Dr. Dukes. 2013) [7]. Fig 2 shows the structure of selective compounds.

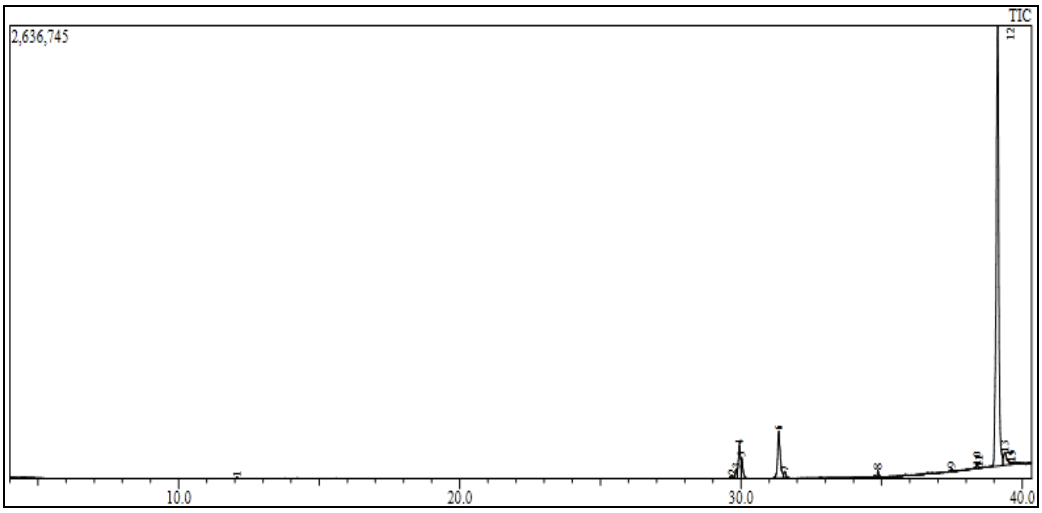

Fig 1: GC-MS Chromatogram of the ethanolic extract of *Ulva reticulata*

Table 1: Identification of bioactive compounds in ethanolic extract of *Ulva reticulata* using GC-MS

Peak	R. time	Area %	Height %	Molecular formula	Molecular weight	Molecular Name
1	12.083	0.11	0.25	$C_{18}H_{44}B_2N_2OSi_2$	382	2,3,4,4-Tretrapropyl-1-(trimethylsilyl)-1-(trimethylsilyloxy)-1,3-diaza-2,4-diborabutane
2	29.693	0.69	0.54	$C_{23}H_{18}O_7$	406	Hoslundin
3	29.835	1.22	1.65	$C_{30}H_{30}O$	406	2,4,6-Tris-(1-phenylethyl)-phenol
4	29.943	4.96	5.76	$C_{26}H_{48}O_2Si$	420	Silane, diethyl(2-phenylethoxy)tetradecyloxy
5	30.025	2.45	3.58	$C_{28}H_{38}O_2$	406	2-T-Butyl -3-[2',7'-di-t-butylfluoren-4'-yl] propanoic acid
6	31.341	8.7	7.85	$C_{30}H_{30}O$	406	2,4,6-Tris-(1-phenylethyl)-phenol
7	31.555	0.82	1.04	$C_{28}H_{38}O_2$	406	2-t-Butyl -3-[2',7'-di-t-butylfluoren-4'-yl] propanoic acid
8	34.867	0.49	1.11	$C_{24}H_{38}O_4$	390	1,2-Benzenedicarboxylic acid
9	37.487	0.28	0.47	$C_{20}H_{36}O_4$	340	2-Butenedioic acid (e)-, bis(2-ethylhexyl) ester
10	38.391	0.49	0.66	$C_{28}H_{48}O$	400	Alpha.-Ergostenol
11	38.465	0.08	0.26	$C_{22}H_{18}N_2O_2$	342	Pyrrolo[3,4-b]carbazole-1,3(2H,3AH)-dione, 4,5-dihydro-4,5-dimethyl-
12	39.126	76.59	74.05	$C_{29}H_{48}O$	412	Stigmasterol

13	39.381	2.87	2.12	$C_{29}H_{50}O$	414	Gamma.-Sitosterol
14	39.565	0.15	0.41	$C_8H_7N_3S$	177	2-Thiazolamine, 4-(2-pyridinyl)-
15	39.63	0.11	0.26	$C_{29}H_{43}NO_3Si$	481	Androstane-11, 17-dione, 3-[(trimethylsilyl) oxy]-, 17-[o-(phenylmethyl)oxime], (3α,5α.)

Table 2: Biological activity of phytocomponents identified in the ethanolic extract of *Ulva reticulata* by GC MS

Compound	Biological activity
Hoslundin	Significant action on Gonorrhooea, cystitis, hookworm, cough, fevers, colds, wounds bilharzias and also anti-malarial
2,4,6-Tris-(1-phenylethyl)-phenol	Antibacterial and antioxidant
1,2-Benzenedicarboxylic acid	Antimicrobial, Antifouling
Alpha.-Ergostenol	Antimicrobial, Inhibition of Sterol Biosynthesis
Stigmasterol	Antihepatotoxic, Antiviral, Antioxidant, Cancer preventive, Hypocholesterolemic.
Gamma-Sitosterol	Anti-diabetic, Anti-angeogenic, Anticancer, antimicrobial, anti-inflammatory, antidiarrhoeal and antiviral
Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl) oxime], (3α,5α)	Anticancer, Antitumour and Antimicrobial Activity

Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

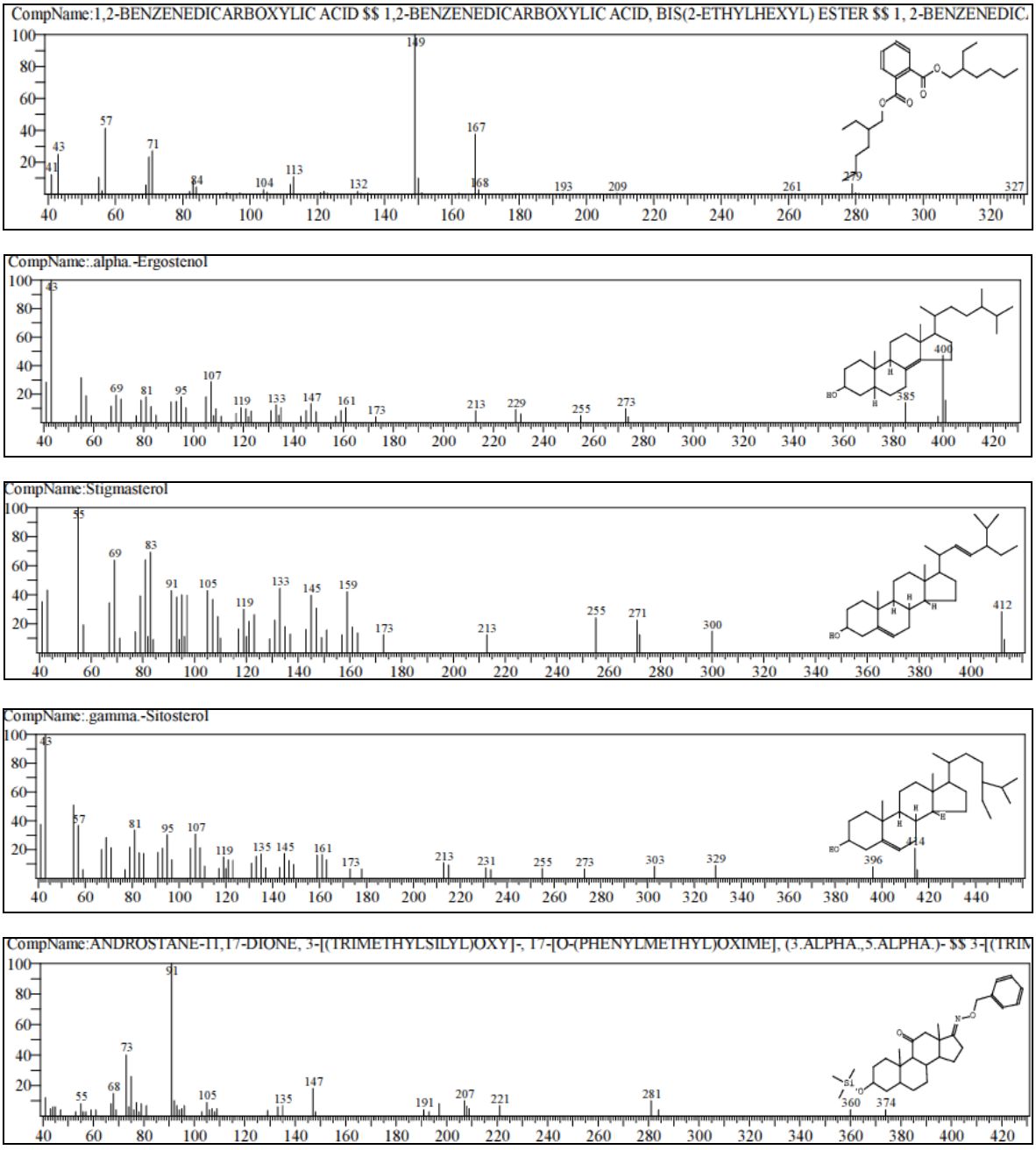

Fig 2: Structure of the selected biological active compounds

The study indicated that seven compounds having significant biological functions which was supported by variety of literature. Most of the compounds had antimicrobial, antioxidant, antiinflammatory, antitumour and cancer preventive properties. It was in agreement with the results of Mayer and Lehmann (2001) [9], Mayer and Hamann (2004) [10], Smit (2004) [11], Flora and Maria Victorial Rani (2013) [12], Gihan *et al*., (2014-2015) [13].

Usha *et al*., (2019) [14] investigated the active phytoconstituents present in the Cladophora glomerata (Green marine algae) using GC-MS, 42 compounds were identified in the methanolic extract of *Cladophora glomerata* and the major components are dibutyl Phthalate, hexadecanoic acid, methyl ester, 1, 2-benzene-di-carboxylic acid, octatriacontyl trifluoroacetate, cholesterol etc and found to have antimicrobial, antioxidant, anti-inflammatory, antitumor, anti-androgenic and cancer preventive properties which corroborates with our study.

Kavitha and Palani (2016) [15] reported the phytochemical screening of seaweed *Chlorococcum humicola* and GC-MS analysis has been done to analyze and identify different constituents present. The results of GC-MS study indicated the presence of 14 different compounds. The compounds exhibited a wide range of activities in their nature.

Swapna *et al*., (2020) [16] revealed the richness of bioactive compounds in *Caulerpa peltata* another green marine algae which further supports our study. Methanolic extract was subjected to GC-MS analysis revealed 28 chemical constituents. Seaweed exhibits potentially bioactive major constituents like Dibutylphthalate, n-hexadecanoic acid, and 1, 2-Benzene dicarboxylic acid. The phytochemical analysis presented the total carbohydrate, alkaloids, saponins, phytosterols, diterpenes in the seaweed. Further phytochemical and the compounds available in GC-MS showed that the *Caulerpa peltata* contains important bioactive compounds which may have anti-microbial, anti-fungal and anticancer activity.

The findings of the present study confirm that green algae *Ulva reticulata is* a rich and varied source of pharmacologically active natural products and nutraceuticals. Further research is needed for finding its use in development of new pharmaceutical agent from marine algae to alleviate various diseases and its safe consumption by human for various health benefits.

Conclusion

The investigation concluded that the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of pharmacologically active compounds and further investigation needs to elute novel active compounds from the medicinal plant which may create a new way to treat many incurable diseases

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