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STUDIES ON GC-MS PROFILING OF SOME SEAWEEDS OF MAHIM BEACH DIST. PALGHAR, MAHARASHTRA USING VARIOUS SOLVENTS

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Keywords:

Bioactive compounds, Crude extract, GC-MS analysis, Seaweeds *etc.*

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ABSTRACT: Objective: To investigate the bioactive constituents of *Ulva lactuca, Sargassum prismaticum,* and *Sarconema filiforme* using gas chromatography-mass spectrometry (GC-MS) analysis. **Methods:** 10 gm sample of each seaweed was successfully extracted with HPLC grade methanol and pet. ether with continuous shaking in a dark room for 36 h. The concentrated extracts were subjected to GC MS analysis. **Results:** The GC MS analysis of methanol and pet ether extracts of *U. lactuca, S. prismaticum* and *S. filiforme* show various types of phytocompounds screened under different R.T. Major compounds were 9-Octadecenoic acid (Z)-, phenyl-methyl ester, Stannane,1,3-dithian-2-ylidenebis[tributyl,Octadecane, 3-ethyl-5-(2-ethylbutyl) in methanol extract and Decane, 6-ethyl-2-methyl-, Tricosane and Sulfurous acid, 2-ethylhexyl hexyl ester in pet. ether. These bioactive compounds are considered as biologically and pharmacologically important. **Conclusion:** The three different extracts contain diverse bioactive compounds that were identified and characterized spectroscopically. Hence, the identification of different biologically active compounds in the extracts of seaweeds justifies further biological and pharmacological studies.

INTRODUCTION: Seaweeds are one of the most significant existing marine resources in the world. These macroalgae have been a good source of food, fodder, manure, and medicine in the east as well as in the west, since centuries [1, 2]. Seaweeds have been classified into three major divisions, namely Chlorophyta (Green algae), Phaeophyta (Brown algae), and Rhodophyta (red algae) based on their photosynthetic pigments and reserve food [3]. Seaweeds are a significant marine resource as they are able to produce a great variety of secondary metabolites that could lead to the development of new functional ingredients [4].

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Seaweeds comprise of large number of structurally diverse bioactive compounds such as alkaloids, glycosides, saponins, tannins, flavonoids, steroids etc., which are of immense medicinal value and use in a broad spectrum of biological activities [5].

All over the world, seaweeds have been studied and found to have enormous therapeutic potential as they possess anti-oxidant, neuroprotective, anti-fungal, anti-viral anti-inflammatory, anticancer, anti-HIV, antimutagenic, scavenging free radicals and antimicrobial activities. Seaweeds are also good source of vitamins such as A, B_l, B_{12}, C, D, E, riboflavin, niacin, pantothenic acid and folic acid as well as minerals such as Ca, P, Na, and K [6]. Variation in nutrient content and bioactive compounds is observed in seaweeds in different seasonal cycles and with different environmental factors like ecological distribution, salinity, temperature, light, *etc.* [7, 8, 9, 10]

In spite of their significance towards mankind, seaweeds are relatively ignored in India. India has a total geographical area of about 329 million hectares with a vast coastline of over 7000 km [11, 12]. Along the coast of India, many of the rocky beaches, coral reefs, lagoons, estuaries, and mudflats provide ideal habitats for the growth of marine algae [13]. The marine algae resources are abundant around coastlines of Mumbai, Palghar, Raigad, Ratnagiri, and Sindhudurg in Maharashtra [14]. The distribution of seaweed resources along the Indian coast was first mapped by Thivy [15].

Large numbers of bioactive compounds have been isolated from the seaweeds so far; still, there is a need to explore and analyze many more seaweeds of different places which might show variation in biochemical content and could be used to produce various food supplements, industrial products, manure and compounds of pharmaceutical interest to cure different ailments.

Mahim beach, Dist. Palghar is a rocky beach. It shows ample collection of several seaweeds. Despite wealth of valuable raw products, seaweeds of this beach remained largely unexplored. *Ulva lactuca, Sargassum prismaticum,* and *Sarconema filiforme* were abundant at the Mahim beach and thus were selected for the present study of some bioactive compounds contained in them. *Ulva lactuca* belonging to the division Chlorophyta is a potential source of polysaccharides, proteins, pigments, cellulose, amino acids, lipids, fatty acids, and minerals, *etc*. [16, 17] It has the capacity to rapidly proliferate and stated to exhibit a wide range of applications in food and health industry. Significant therapeutic properties like antibacterial, antifungal, antioxidant etc., are reported from *Ulva* sp. collected all over the world. *Sarconema filiforme* belonging to division Rhodohyta has considerable pharmacological, antimicrobial, antifungal, and cytotoxic potentials [18].

Few sps. of *Sargassum* (Phaeophyta) are reported to be good source of phytochemical contents such as carotenoids, terpenes, phenolic compounds, fatty acids, lipids, and amino acids [19]. GC MS technique was selected for the characterization as it is the most appropriate technique to analyze and identify bioactive components of alcohols, esters, fatty acids, amino acids, steroids, long-chain hydro-carbons, essential oils, *etc*. Crude seaweed extract was made using solvents of different polarities.

Studies related to bioactive compounds of seaweeds of Mahim beach, Dist Palghar are reported for the first time.

MATERIALS AND METHODS:

Collection of Seaweeds: Fresh seaweed *Ulva lactuca, Sargassum prismaticum,* and *Sarconema filiforme* were collected from intertidal regions of Mahim beach, dist. Palghar during the month of December 2017. The collected seaweed sample was cleaned with the seawater until unwanted impurities and adhering sand particles were removed. It was then cleaned thoroughly with tap water to remove the salty surface materials. The seaweed sample was shade dried for 7-8 days. The dried seaweed samples were powdered using mixer and passed through 0.5 mm sieve. It was stored in refrigerator for further study.

Seaweed Extraction: Seaweed extracts were obtained using solvents of different polarities. The seaweed powdered sample (10 gm) was extracted successively with HPLC grade methanol and pet ether using the cold extraction method. The sample was kept in a dark room for 36 hrs with continuous shaking. The extract was collected and filtered using Whatman no.1. It was evaporated to dryness by a rotavap. The final residue obtained was stored at 4 ºC until further use. The volatile bioactive compounds present in methanol and pet. ether extracts of the seaweeds were identified by GC-MS characterization.

GC-MS Instruments: GC MS analysis of a different solvent extract of *Ulva lactuca, Sargassum prismaticum,* and *Sarconema filiforme* was carried out with Agilant 7890 system and gas chromatography interfaced to a mass spectrometer (GC-MS) employing the following conditions: HP 5 column [(5%-Phenyl)-methylpolysiloxane) (30mm × 0.25mm × 0.25µm] thickness, Helium gas (99.999%) was used as a carrier gas at a constant flow of 1ml/min, and injection volume of 1µl was employed (split ratio of 10:1) operating in electron impact mode at 70eV; injector temperature 250 ºC; Ion-source temperature 280 ºC. The oven temperature was programmed from 80 ºC (isothermal for 1 min), with an increase of 10 ºC

/min, to 200 ºC then 5 ºC/min to 280 ºC ending with 9 min, isothermal at 280 ºC. Mass spectra were taken at 70eV; a scan-interval of 0.5 seconds and fragments vary from 45-550 Da. Total GC running time was 35 min.

Identification of Chemical Constituents: The resulting peaks were analyzed with the database of the National Institute Standard and Technology (NIST) a library, which has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in it. The Name, Molecular weight and structure of the components of the seaweed sample was determined [20].

RESULTS: The volatile bioactive compounds present in methanol and pet. ether extract of *Ulva lactuca*, *Sargassum prismaticum*, and *Sarconema filiforme* were identified by GC-MS analysis. The methanol and pet. ether extracts of seaweeds indicated the presence of different types of phytocompounds **Table 1-2** screened under different retention times (R.T). A total of (9, 4 and 9 of methanol extract) and (4, 7, and 11 of pet. ether extract) were observed with retention times for *U. lactuca*, *S. prismaticum* and *S. filiforme* respectively, as shown in **Fig. 1–6**. Chemical constituents were identified using spectral database NIST 11 software installed in the GC–MS. The compounds prediction is based on Pubchem [20]. The compound name with R.T, molecular formula, molecular weight and concentration % in the methanol and petether extract of seaweeds *Ulva lactuca*, *Sargassum prismaticum* and *Sarconema filiforme* are presented in **Table 1-2**. 9-Octadecenoic acid (Z)-, phenylmethyl ester are most abundant (R.T 31.81, 31.52%) in methanol *U. lactuca* extract followed by Stannane, 1,3-dithian-2-ylidenebis[tributyl- (R.T 14.82, 70.59%) in *S. prismaticum* and Octadecane, 3-ethyl-5-(2-ethylbutyl)- (R.T 31.62, 36.50%) present in *S. filiforme*. Crude extract of petetherindicates most abundant compound as Decane, 6-ethyl-2-methyl- (R.T 31.86, 41.07%), Tricosane (R.T 31.66, 25.84 %) and Sulfurous acid, 2-ethylhexyl hexyl ester (R.T 19.42, 31.96%) respectively.

TABLE 1: COMPOUNDS IDENTIFIED IN THE METHANOL EXTRACT OF *ULVA LACTUCA, SARGASSUM PRISMATICUM* AND *SARCONEMA FILIFORME* BY GC-MS ANALYSIS

Extract	RT (min)	Name of compound	Molecular formula	Molecular weight (g/mol)	Peak %
Ulva methanol	15.65	Decanoic acid 2-methyl-	$C_{11}H_{22}O_2$	186	3.288906
	18.93	11,14,17-eicosatrienoic acid,methyl ester	$C_{21}H_{36}O_2$	320	8.680821
	22.67	1-Nonyne,/-methyl	$C_{10}H_{18}$	138	1.552746
	22.97	9-Oxabicyclo[4.3.0]non-2-ene-4,5-dicarboxylic,8-hydroxy-1,2,7,7-tetramethyl-,dimethyl ester	$C_{16}H_{24}O_6$	312	14.59502
	23.25	Silane, (1,3,5-benzenetriyltris(methylene)tris(trimethyl-	$C_{15}H_{36}Si_3$	294	18.11925
	23.49	2,2'-Thiobis[4-methyl-6-(1, 1, 3, 3-tetramethylbutyl)phenol]	$C_{30}H_{46}O_2$	470	9.687301
	23.86	(t-butyl-dimethylsilyl)[2-methyl-2-(4-methyl-pent-3-enyl)-cyclopropyl]-methanol	$C_{17}H_{34}OSi$	282.537	1.73179
	31.26	d-Mannitol, 1-decylsulfonyl-	$C_{16}H_{34}O_7S$	370.501	10.82201
	31.81	9-Octadecenoic acid(Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	372	31.52216
Sargassum methanol	14.15	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	230.392	5.847037
	14.82	Stannane,1,3-dithian-2-ylidenebis[tributyl	$C_{28}H_{60}S_2S_{n2}$	700	70.59108
	29.32	Probucol	$C_{31}H_{48}O_2S_2$	516.843	5.945797
	31.85	Tetradecane, 1-iodo-	$C_{14}H_{29}I$	324.2845	17.61608
Sarconema methanol	22.75	1-Nitrododecane	$C_{12}H_{25}NO_2$	215.337	0.034011947
	23.07	Eseroline,octylcarbamate(ester)	$C_{22}H_{35}N_3O_2$	373	0.654931203
	23.39	1,2-Benzenedicarboxylic acid, ditridecyl ester;	$C_{34}H_{58}O_4$	530.8219	1.159361947
	23.74	Sulfurous acid, 2-propyl tridecyl ester	$C_{16}H_{34}O_3S$	306	1.281223925
	24.78	1-Bromoeicosane	$C_{20}H_{41}Br$	361.452	6.102793402
	26.90	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	19.3288882
	27.95	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7070	13.65299758
	28.7	Cholest-5-en-3-ol, 24-propylidene-, (3 β)-	$C_{30}H_{50}O$	426	21.27953871
	31.62	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7070	36.50625308

TABLE 2: COMPOUNDS IDENTIFIED IN THE PET. ETHER EXTRACT OF *ULVA LACTUCA, SARGASSUM PRISMATICUM* AND *SARCONEMA FILIFORME* BY GC-MS ANALYSIS

Extract	RT (min)	Name of compound	Molecular formula	Molecular weight (g/mol)	Peak %
Ulva Pet. ether	19.44	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	$C_{19}H_{30}O_3$	306.446	36.37524
	19.99	Tetradecane, 1-iodo-	$C_{14}H_{29}I$	324.29	1.34168
	31.40	L-Norvaline,N-(2methoxyethoxycarboyl)-,isohexyl ester	$C_{15}H_{29}NO_5$	303	21.20727
	31.86	Decane, 6-ethyl-2-methyl-	$C_{13}H_{28}$	184	41.07581
Sargassum Pet. ether	9.94	Sulfurous acid, 2-ethylhexyl hexyl ester	$C_{14}H_{30}O_3S$	278.451	12.60196
	12.35	1-Hentetracontanol	$C_{41}H_{84}O$	593.122	4.817065
	19.33	L-Norvaline, N-(2-methoxyethoxycarbo yl)-, undecyl ester	$C_{20}H_{39}NO_5$	373.52736	0.580089
	19.45	L-Norvaline, N-(2-methoxyethoxycarbo yl)-, undecyl ester	$C_{20}H_{39}NO_5$	373.52736	29.55033
	19.97	6-Fluoro-2-trifluoromethylbenzoic acid, 2-formyl-4,6-dichlorophenyl ester	$C_{15}H_6Cl_2F_4O_3$	381.106	1.319213
	31.66	Tricosane	$C_{23}H_{48}$	324.637	25.84063
	31.86	1-Octadecanesulfonyl chloride	$C_{18}H_{37}ClO_2S$	353.002	25.29071
Sarconema Pet. ether	9.3	Decane, 2,9-dimethyl-	$C_{12}H_{26}$	170.3348	6.073613
	12.03	6-Fluoro-2-trifluoromethylbenzoic acid, 2-formyl-4,6-dichlorophenyl ester	$C_{15}H_6Cl_2F_4O_3$	381.106	0.478575
	12.25	Sulfurous acid, 2-ethylhexyl hexyl ester	$C_{14}H_{30}O_3S$	278.451	20.24306
	19.18	Decane, 1-bromo-2-methyl-	$C_{11}H_{23}Br$	235.209	1.647081
	19.42	Sulfurous acid, 2-ethylhexyl isohexyl ester	$C_{14}H_{30}O_3S$	278.451	31.96578
	19.79	l-norvaline,n-(2methoxyethoxycarboyl)-,octyl ester	$C_{17}H_{33}NO_5$	331	1.844492
	19.97	l-Valine, n-pentafluoropropionyl-,undecyl ester	$C_{19}H_{32}F_5NO_3$	417.4543	1.48032
	27.23	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7070	3.714758
	28.51	Di-n-decylsulfone	$C_{20}H_{42}O_2S$	346.614	4.630403
	31.54	Heptacosane	$C_{27}H_{56}$	380.745	8.254636
	31.85	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7070	19.66728

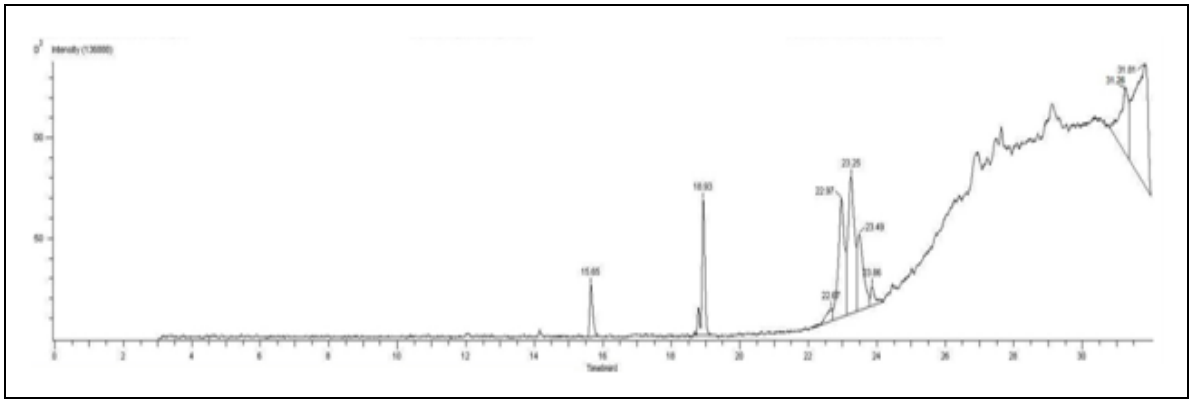

FIG. 1: GC–MS CHROMATOGRAM OF THE CRUDE METHANOL EXTRACT OF *ULVA LACTUCA*

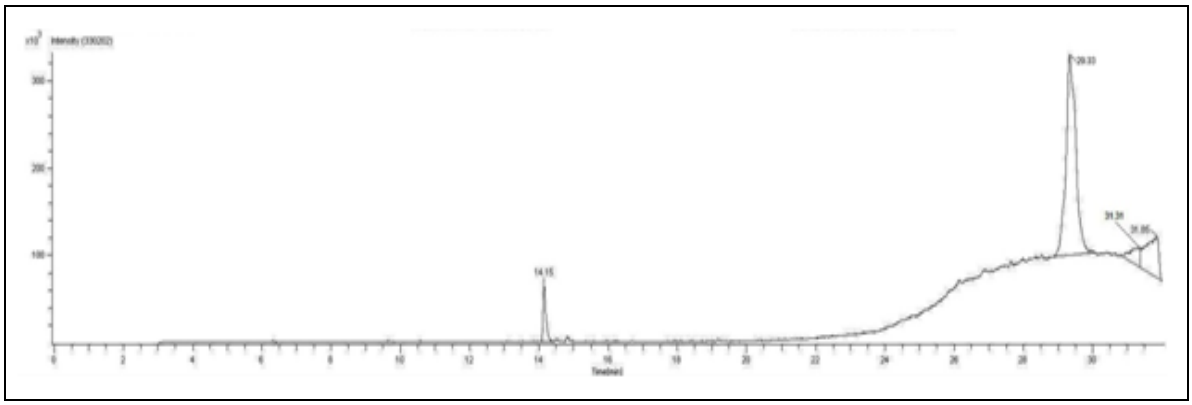

FIG. 2: GC–MS CHROMATOGRAM OF THE CRUDE METHANOL EXTRACT OF *SARGASSUM PRISMATICUM*

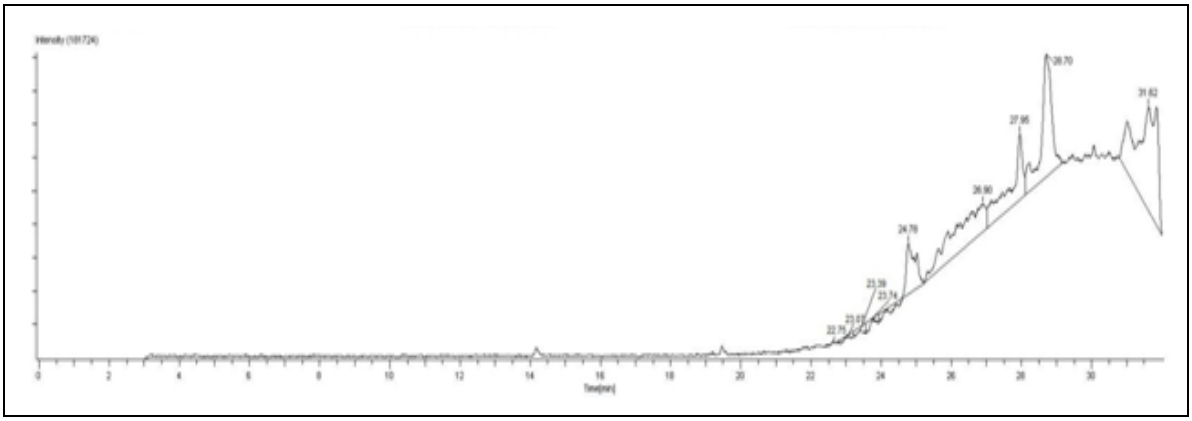

FIG. 3: GC–MS CHROMATOGRAM OF THE CRUDE METHANOL EXTRACT OF *SARCONEMA FILIFORME*

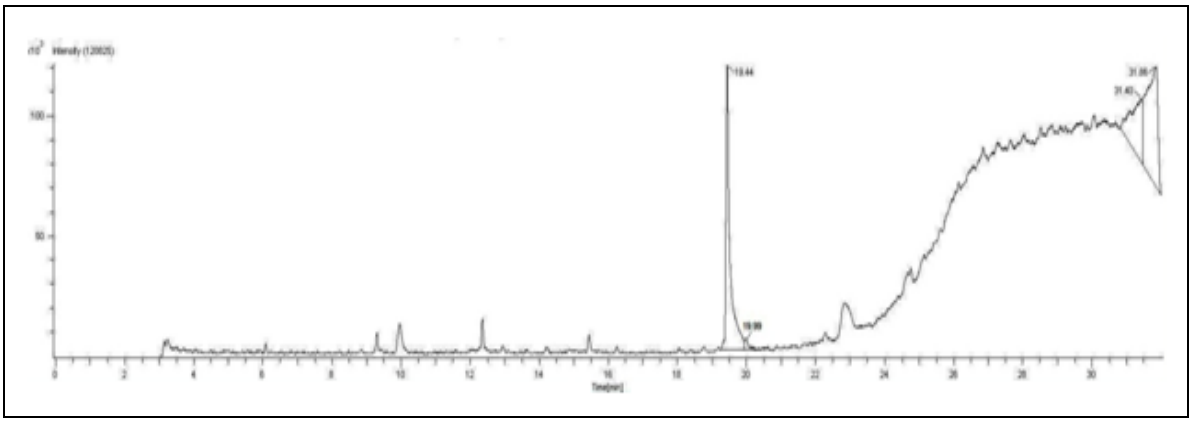

FIG. 4: GC–MS CHROMATOGRAM OF THE CRUDE PET. ETHER EXTRACT OF *ULVA LACTUCA*

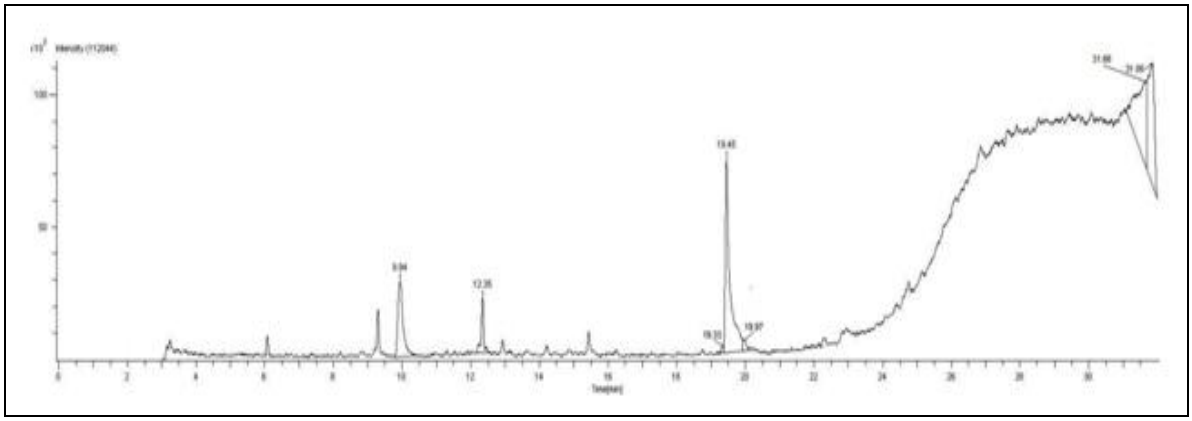

FIG. 5: GC–MS CHROMATOGRAM OF THE CRUDE PET. ETHER EXTRACT OF *SARGASSUM PRISMATICUM*

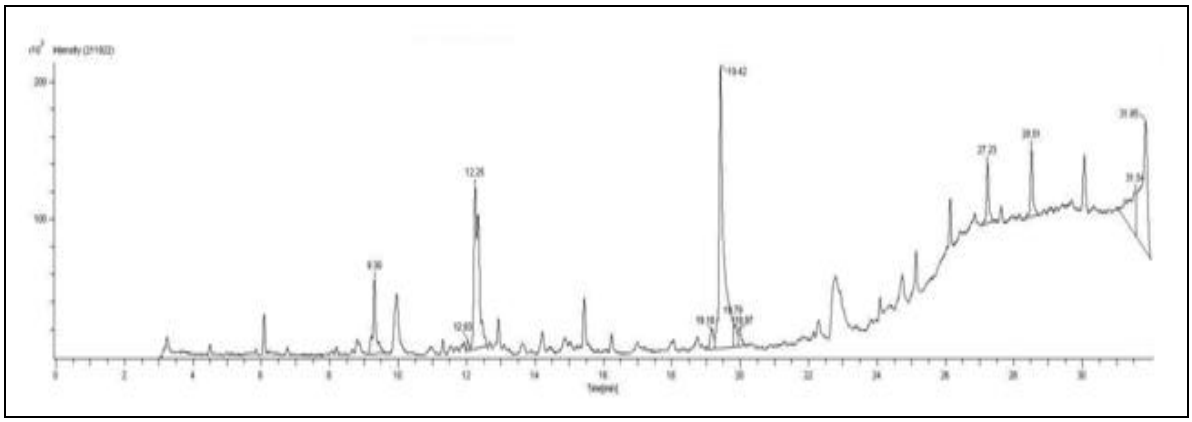

FIG. 6: GC–MS CHROMATOGRAM OF THE CRUDE PET. ETHER EXTRACT OF *SARCONEMA FILIFORME*

DISCUSSION: Marine algae have been rich source of carotenoids, polyunsaturated fatty acids [21], polysaccharides [22], flavonoids, phenolic acids [23], sterols [24] Phlorotannins [25] *etc*. Present GC–MS analysis of crude extracts of methanol and pet. ether of *Ulva lactuca*, *Sargassum prismaticum* and *Sarconema filiforme* revealed various compounds. Major compounds were 9-Octadecenoic acid(Z)-, phenylmethyl ester, Stannane, 1,3-dithian-2-ylidenebis [tributyl, Octadecane, 3-ethyl-5-(2-ethylbutyl) in methanol extract and Decane, 6-ethyl-2-methyl-, Tricosane and Sulfurous acid, 2-ethylhexyl hexyl ester in pet. ether. Some other important bioactive compounds mentioned in **Table 1-2** are rich source of structurally novel and biologically active metabolites and can be used as potential natural antioxidants in different food and pharmaceutical products [26]. Babu *et al.*, (2014) reported 17 different components in ethanolic extract of *Ulva lactuca.* Major components Phytol (10.51%), Hexadecanoic acid, ethyl ester (20.24%) and (E)-9- Octadecenoic acid ethyl ester (9.59%) are considered to have significant medicinal properties. Kumar *et al.*, (2013) found 12 compounds having antibacterial activity in methanolic extract of *Sargassum tenerrimum* by GC MS analysis. Red algae *Halimeda opuntia* and *Sarconema filiforme* are described as potent algal species with considerable antimicrobial, anti-plasmid, and cytotoxic activity [29].

CONCLUSION: The present study helps to predict the formula and structure of bioactive compounds in *Ulva lactuca*, *Sargassum prismaticum,* and *Sarconema filiforme.* Further work related to verifying their efficacy may lead to the improvement of drug formulation.

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CONFLICTS OF INTEREST: Nil

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