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GC-MS Analysis of Bioactive Compounds of Seaweed Extracts Collected from Seashore of Manalmelkudi (Pudukkottai dist., Tamilnadu), responsible for Antifungal Activity

K. G. Anitha*, G. Arputha, G. Muthubala, R. Susithra, M. Mullaivendhan and R. Anandham

Agricultural College and Research Institute, Tamil Nadu Agricultural University, Kudumiyanmalai - 622 104, Pudukkottai, Tamil Nadu, India

**Corresponding author*

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A B S T R A C T

The antifungal activity of *Gracilaria cervicornis*, *Gracilaria gracilis*, *Endocladia muricata* is tested against *Macrophomina phaseolina* and *Lasiodiplodia theobromae.* The mean inhibion zones induced by seaweed extracts on the tested pathogens revealed that methanolic extract of *G. cervicornis* had highest zone than its acetone & aquous extracts followed by methanoilc extract of *G. gracilis* against *M. phaseolina* whereas no inhibition by *E. muricata.* The methanolic extract of *G. cervicornis* recorded inhibition zone of 20.7 mm against *M. phaseolina* whereas in *L. theobromae* it was 17.6 mm. But the acetone extract of *G. gracilis* showed 17.4 mm against *M. phaseolina* whereas it was 16.6 mm for *G. cervicornis.* The composition of bioactive compounds in the GC-MS chromatogram of these seaweed extracts were analysed and found that the phenols contributed major portion among the various fractions of the extract which contributes for the antimicrobial effect. The peaks for phenols with area % of 38.53 and height % of 28.58 for *G. cervicornis* and 32.15% and 19.56% respectively for *G. gracilis* are recorded.The chromatogram of *Endocladia muricata* showed no traces of phenols. *G. gracilis* is having appreciable amounts of fatty acids with notable height % viz., Hexadecanoic acid (3.45%), n-Hexadecanoic acid (5.10%), Furanacetic acid (2.3%) and in *G. cervicornis* Tridecanoicacid, n-Nonadecanoic acid, cyclo propane octanoic acid, Heneicosanoic acid which are also responsible for the exhibited antifungal activity.

Introduction

Marine macroalgae are characterized for their production of wide array of biocidal substances. It is recorded that more than 600 secondary metabolites have been isolated from seaweeds; among which most of them are toxic substances acting as chemical defense system for protection from their grazers. They normally produce such kinds of bioactive substances in a response to predation, competition for space and tide variations. Hence possibilities for exploring the antimicrobial compounds of seaweed origin

for control of plant pathogens are more. These antimicrobial compounds include polysaccharides, fatty acids, phenolics, carotenoids and terpenes. In this study, an attempt has been made to characterize the antifungal activity of *Gracilaria cervicornis, Gracilaria gracilis, Endocladia muricata* which were collected from seashore of Manalmelkudi, Pudukkottai District, Tamilnadu, India

Materials and Methods

Location of the sample

Seaweeds were collected from seashore of Manalmelkudi, Pudukkottai district (Latitude 10.0396^{o} N, Longitude 79.2318^{o} E). Totally 12 types of seaweeds were collected from 5 locations.

Processing of seaweed

The collected macroalgae were washed with fresh water to remove the extraneous substances and transported to laboratory by packing in polythene bags. In the lab they were again rinsed with saline solution, shade dried and powdered in a mixer grinder. The powder was immediately used for extraction.

Extraction of bioactive compounds

Each seaweed powder was mixed with two different hydrophilic solvents (methanol, acetone) in the ratio of 1:50 (w/v). The solutions were kept in an Orbital incubator shaker (Optics Technology) at 160 rpm for 48 hours. The extracts were filtered through Whatman No. 1 filter paper and concentrated by evaporation in vacuum. The residual extracts were stored at 0^{o}C.

Phytochemical analysis by GC-MS

A mass Spetrometry equipped with a data system in combination with GC was used for the analysis of seaweed extract. The methanolic extracts of the 3 screened seaweeds were injected (1µl) in GC-MS (Varian CP 2000) with Poropak Q column, FID detector, flow rate of 1.0 ml/min. and total run time of 20 minutes. The compounds were identified from the library search result of GC-MS.

Determination of antifungal activity

The antifungal activity was determined by agar well diffusion method (Suay *et al.*, 2000). The fungal pathogens used for this study were *Macrophomina phaseolina,* a root pathogen and *Lasiodiplodia theobromae,* a foliar pathogen. The agar plates inoculated with the test fungi were incubated for one hour before placing extracts and 80 µl of seaweed extract was placed in the wells and allowed to diffuse for 2 hours. Then the plates were incubated for 72 hours. The antifungal activity was determined by measuring the diameter of inhibition zone for each well and expressed in mm.

Results and Discussion

Among the 12 seaweeds collected 3 were identified based on the morphological characters & cross section under microscope (Marine seaweed Manual, 2018) and confirmed by Botanical Survey of India, Coimbatore as *Gracilaria cervicornis, Gracilaria gracilis* and *Endocladia muricata.*

Anatagonistic activity against fungal pathogens

In many studies it has been proved that hydrophyllic solvents provide better activity as many of the bioactive compounds are extracted by them rather than in lipophyllic solvents. Zineb *et al.*, (2004) has reported the total inhibition of *A. flavus* by the ethanolic

extract of brown marine algae, *Cystoseira tamariscifolia.* Cox *et al.*, (2010) suggested for usage of methanol for brown and red seaweed extraction. Hence methanol and acetone were used for extraction in this study.

The organic solvents extract showed activity against pathogens but aqueous extract showed minimum or no activity. In general *M. phaseolina* was fond to be sensitive to the extracts of both *G. gracilis* and *G. cervicornis* than *L. theobromae.* The mean inhibion zones induced by seaweed extracts on the tested pathogens revealed that methanolic extract of *G. cervicornis* had highest zone than its acetone & aqueous extracts followed by methanoilc extract of *G. gracilis* against *M. phaseolina.* The methanolic extract of *G. cervicornis* recorded inhibition zone of 20.7 mm against *M. phaseolina* whereas in *L. theobromae* it was 17.6 mm. But the acetone extract of *G. gracilis* showed 17.4 mm against *M. phaseolina* whereas it was 16.6 mm for *G. cervicornis* (Table 1). In the present study the antifungal activity of *Gracilaria* is proved in first of its kind against *M. phaseolina* and *L. theobromae*.

GC-MS analysis of bioactive compounds

During the run time of 20 minutes 50 peaks were obtained. It is stated that algae produce secondary metabolites up to 7% of dry weight which show antimicrobial activity. Among these about 60% are terpenes, 20% are fatty acids along with nitrogenous compounds (Paul and Fenical, 1987; Van Alstyne and Paul, 1988). In the present study as the methanolic extracts of *G. gracilis* and *G. cervicornis* have appreciable amount of antifungal activity. The composition of bioactive compounds in the GC-MS chromatogram of these seaweed extracts were analyzed and found that they contained a mixture of compounds. The retention time, peak area %, peak height % along with compound name, formula and molecular weight were fetched from the library data as given in table 2 & 3. Correlating the presence of certain phenolics, fatty acids and terpenes to the antifungal activity exhibited by seaweeds has been attempted.

In this study the phenols contributed major portion among the various fractions of the extract. The peaks for phenols (Fig 1a) was fetched at retention time of 17.31 with area % of 38.53 and height % of 28.58 for *G. cervicornis* and 32.15% and 19.56% respectively for *G. gracilis* (Fig 1b). The chromatogram of *Endocladia muricata* showed no traces of phenols.

Both the seaweeds showed the presence of diversified fatty acids viz., hexadecanoic acid (palmitic acid), Furanacetic acid, cyclopropaneoctanoic acid, heneicosanoic acid, tridecanoic acid, nonadecanoic acid etc.

In this study, GC-MS chromatogram has shown (Fig. 2) that the methanolic extract of *G. gracilis* is having appreciable amounts of fatty acids with notable height % viz., Hexadecanoic acid (3.45%), n- Hexadecanoic acid (5.10%), Furanacetic acid (2.3%) and in *G. cervicornis* Tridecanoic acid, n-Nonadecanoic acid, cyclo propane octanoic acid, Heneicosanoic acid. The compound ethyl isoallocholate is present in the extract of *G. cervicornis.* 3,7,11,17-Tetramethyl-2-hexadecen-1-ol and 17 1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4 which are generally proved for antimicrobial activity.

The presence of 3,7,11,17-Tetramethyl-2-hexadecen-1-ol, 17 1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4, Di-n-decylsulfone1, 2-Bis (trimethylsilyl) benzene compounds in chromatogram of *G. cervicornis* (Fig 3 a, b, c) with height % of 4.62, 6.0 and 3.8 respectively has been noted and in previous studies their antimicrobial activities have been proved

Table.1 Inhibition zone around *Macrophomina phaseolina* and *Lasiodiplodia theobromae* fungal pathogens by solvent extracts of seaweeds

Name of Seaweed	Methanol extract		Acetone extract		Aqueous Extract	
	Macrophomina phaseolina	*Lasiodiplodia theobromae*	*Macrophomina phaseolina*	*Lasiodiplodia theobromae*	*Macrophomina phaseolina*	*Lasiodiplodia theobromae*
Gracilaria gracilis	19.20±0.37	17.30±0.29	17.40±0.43	16.20±0.39	3.20±0.34	2.40±0.29
Gracilaria cervicornis	20.70±0.18	17.6±0.32	16.6±0.41	15.1±0.22	4.1±0.24	3.6±0.36
Endocladia muricata	0	0	0	0	0	0

(Values (mm) of inhibition zones are mean ± SD; sample (n)= 7)

Table.2 Phytochemical composition of methanolic extract of *Gracilaria cervicornis* by GC-MS

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	3.321	3.290	3.355	14520	0.33	8160	0.83	1.78	3,3-Dimethoxy-2-butanone
2	3.485	3.445	3.525	25593	0.59	7454	0.76	3.43	3,3-Dimethoxy-2-butanone
3	3.570	3.525	3.585	22845	0.52	8188	0.84	2.79	1,3-Dioxolane-4-methanol, 2-ethyl-
4	3.708	3.680	3.750	38346	0.88	11383	1.16	3.37	Ethylbenzene
5	3.800	3.750	3.825	35722	0.82	11634	1.19	3.07	Benzene, 1,3-dimethyl-
6	5.221	5.200	5.245	15016	0.34	8952	0.91	1.68	Decane
7	6.461	6.390	6.495	18232	0.42	8452	0.86	2.16	Decane, 2-methyl-
8	11.610	11.565	11.675	63818	1.46	19076	1.95	3.35	Diethyl Phthalate
9	12.490	12.445	12.545	90895	2.08	25109	2.56	3.62	Heptadecane
10	12.560	12.545	12.575	16142	0.37	10309	1.05	1.57	Ethyl iso-allocholate
11	13.570	13.540	13.580	52070	1.19	30234	3.08	1.72	Neophytadiene
12	13.623	13.580	13.665	183625	4.21	45259	4.62	4.06	Cholesterol
13	13.753	13.665	13.860	419879	9.62	58832	6.00	7.14	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,1
14	13.910	13.860	13.930	65543	1.50	21011	2.14	3.12	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
15	13.940	13.930	13.970	16944	0.39	15129	1.54	1.12	5-Cholene, 3,24-dihydroxy-
16	14.230	14.170	14.275	28333	0.65	11045	1.13	2.57	Tridecanoic acid, 12-methyl-, methyl ester
17	15.100	15.050	15.120	20712	0.47	7603	0.78	2.72	l-(+)-Ascorbic acid 2,6-dihexadecanoate
18	15.155	15.120	15.170	20627	0.47	11226	1.15	1.84	n-Nonadecanoic acid, pentamethyldisilyl ester
19	15.185	15.170	15.205	21873	0.50	13094	1.34	1.67	Card-20(22)-enolide, 2,3,14-trihydroxy-, (2.alpha.,3.beta.,5.alpha.)-
20	15.245	15.230	15.265	11893	0.27	8003	0.82	1.49	Diethyl 3-chloro-2-hydroxypropylmalonate
21	15.275	15.265	15.345	29759	0.68	9680	0.99	3.07	Propyleneglycol monoleate
22	15.366	15.345	15.380	13368	0.31	9319	0.95	1.43	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-buten
23	15.395	15.380	15.525	66206	1.52	7103	0.72	9.32	Benzylpiperazine, TMS derivative
24	15.552	15.525	15.585	66082	1.51	25606	2.61	2.58	Phytol
25	15.820	15.735	15.865	57512	1.32	9120	0.93	6.31	4,7,7-Trimethylbicyclo[2.2.1]heptan-2,3-dione, 2-oxime (syn)
26	15.900	15.865	15.935	32140	0.74	9996	1.02	3.22	Ethyl hexatriacontyl ether
27	15.951	15.935	15.975	28548	0.65	14728	1.50	1.94	d-Mannitol, 1-O-(22-hydroxydocosyl)-
28	16.124	15.975	16.220	358176	8.21	37231	3.80	9.62	9-Octadecenamide, (Z)-
29	16.290	16.220	16.330	155125	3.56	28311	2.89	5.48	Di-n-decylsulfone
30	16.347	16.330	16.370	49743	1.14	23841	2.43	2.09	1,2-Bis(trimethylsilyl)benzene
31	16.440	16.370	16.450	72423	1.66	15082	1.54	4.80	6-Methyltricosane
32	16.467	16.450	16.480	21637	0.50	13397	1.37	1.62	2.beta.,4.beta.,16.alpha.-Tribromoallopregn-16-ene-3,20-dione
33	16.495	16.480	16.515	18182	0.42	10177	1.04	1.79	9,12-Tetradecadien-1-ol, (Z,E)-, TMS derivative
34	16.570	16.515	16.585	33432	0.77	9470	0.97	3.53	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
35	16.625	16.585	16.655	27413	0.63	8801	0.90	3.11	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-,[3
36	16.685	16.655	16.765	40068	0.92	9101	0.93	4.40	5.beta.,6.beta.-Epoxy-17-oxo-6.alpha.-pentyl-4-nor-3,5-secoandrost
37	16.780	16.765	16.895	30870	0.71	7379	0.75	4.18	1,1,3,3-Tetraallyl-1,3-disilacyclobutane
38	16.906	16.895	16.930	10996	0.25	8344	0.85	1.32	1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl)amino-
39	17.319	17.110	17.425	1680730	38.53	280087	28.58	6.00	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)
40	17.445	17.425	17.460	11057	0.25	7936	0.81	1.39	Cyclopentene, 3,3-dimethyl-4-methylene-1,2-bis(trimethylsilyl oxym
41	17.663	17.645	17.775	66129	1.52	12190	1.24	5.42	Cyclopropaneoctanoic acid, 2-[(2-pentylcyclopropyl)methyl]-, methy
42	17.855	17.775	17.870	24246	0.56	8437	0.86	2.87	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-
43	18.105	17.870	18.150	79500	1.82	10793	1.10	7.37	Henicosanoic acid, 20-oxo-, methyl ester
44	18.200	18.150	18.220	13511	0.31	7259	0.74	1.86	2-Bromolauric acid
45	18.320	18.300	18.415	21787	0.50	7131	0.73	3.06	d-Mannitol, 1-decylsulfonyl-
46	18.488	18.415	18.510	41519	0.95	15023	1.53	2.76	Di-n-octyl phthalate
47	18.570	18.510	18.595	31091	0.71	9640	0.98	3.23	.beta.-n-Butylether of 11-epi-dihydroartemisin
48	18.749	18.690	18.775	22351	0.51	7538	0.77	2.97	1,1,3,3-Tetraallyl-1,3-disilacyclobutane
49	19.079	18.920	19.110	39582	0.91	7284	0.74	5.43	Butyl 9-octadecenoate or 9-18:1
50	19.960	19.885	19.995	36591	0.84	9997	1.02	3.66	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-
				4362402	100.00	980154	100.00		

Table.3 Phytochemical composition of methanolic extract of *Grcilaria gracilis* by GC-MS

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	3.323	3.245	3.430	50603	0.95	8028	0.55	6.30	Pentanoic acid, 3-methyl-
2	3.470	3.430	3.490	15741	0.29	7463	0.51	2.11	3-(5,5-Dimethyl-4-methylidene-2-oxo-1,3-oxazolidin-3-yl)propaneni
3	3.505	3.490	3.525	16047	0.30	10071	0.69	1.59	3-(1,3-Dihydroxyisopropyl)-1,5,8,11-tetraoxacyclotridecane
4	3.546	3.525	3.575	23952	0.45	10998	0.75	2.18	Carbamic acid, (.alpha.-methylbenzyl)-, 1-ethyl-1-methylbutyl ester
5	3.714	3.680	3.755	47849	0.90	17150	1.17	2.79	Ethylbenzene
6	3.795	3.755	3.860	30833	0.58	9007	0.62	3.42	p-Xylene
7	4.042	4.020	4.075	16661	0.31	8175	0.56	2.04	Benzene, 1,3-dimethyl-
8	9.580	9.530	9.610	21422	0.40	9789	0.67	2.19	2-(Ethylenedioxy)ethylamine,N-methyl-N-[4-(1-pyrrolidinyl)-2-buty
9	11.591	11.545	11.740	955240	17.88	302074	20.69	3.16	Diethyl Phthalate
10	11.800	11.740	11.815	38385	0.72	8637	0.59	4.44	1-Oxaspiro[2.5]octan-4-one, 2,2,6-trimethyl-, cis-
11	11.840	11.815	11.905	35187	0.66	8828	0.60	3.99	2,2,5,5-Tetramethyl-4-ethyl-3-imidazoline-3-oxide-1-oxyl
12	11.916	11.905	11.945	9573	0.18	8940	0.61	1.07	4,7,7-Trimethylbicyclo[2.2.1]heptan-2-one O-allyloxime
13	12.478	12.420	12.565	214337	4.01	76657	5.25	2.80	Heptadecane
14	13.565	13.535	13.590	57061	1.07	30730	2.10	1.86	Neophytadiene
15	13.608	13.590	13.635	24570	0.46	14802	1.01	1.66	2-Pentadecanone, 6,10,14-trimethyl-
16	13.794	13.770	13.835	29955	0.56	12402	0.85	2.42	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
17	13.898	13.845	13.935	39943	0.75	14896	1.02	2.68	Neophytadiene
18	14.089	14.040	14.120	37539	0.70	13149	0.90	2.85	Cetrimonium Bromide
19	14.208	14.120	14.275	138664	2.60	50437	3.45	2.75	Hexadecanoic acid, methyl ester
20	14.320	14.275	14.420	34469	0.65	8348	0.57	4.13	3,8,11-Trioxatetracyclo[4.4.1.0(2,4).0(7,9)]undecane, (1.alpha.,2.alp
21	14.498	14.420	14.550	401002	7.51	74486	5.10	5.38	n-Hexadecanoic acid
22	14.565	14.550	14.580	53841	1.01	33618	2.30	1.60	2-Furanacetic acid, tetrahydro-5-(2-hydroxypropyl)-.alpha.-methyl-,
23	14.590	14.580	14.630	47656	0.89	23067	1.58	2.07	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester
24	15.449	15.400	15.485	49174	0.92	15793	1.08	3.11	6-Octadecenoic acid, methyl ester, (Z)-
25	15.537	15.485	15.590	142634	2.67	54865	3.76	2.60	Phytol
26	15.753	15.715	15.780	22206	0.42	8938	0.61	2.48	Heptanoic acid, 6-hydroxy-, ethyl ester
27	15.875	15.860	15.900	18078	0.34	8756	0.60	2.06	Silane, dimethyl(2,2,2-trichloroethoxy)nonyloxy-
28	15.910	15.900	15.930	14244	0.27	9432	0.65	1.51	Adamantane-1-(3,3-dichloropropyn-1-yl)
29	15.942	15.930	15.955	14433	0.27	12511	0.86	1.15	Diglycolic acid, heptyl neopentyl ester
30	15.965	15.955	15.990	13776	0.26	10645	0.73	1.29	2,6-Nonadienoic acid, 7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl
31	16.065	15.990	16.090	128851	2.41	33803	2.32	3.81	Octadecanamide
32	16.110	16.090	16.145	102433	1.92	35999	2.47	2.85	Cyclooctasiloxane, hexadecamethyl-
33	16.175	16.145	16.245	152875	2.86	30491	2.09	5.01	Diglycolic acid, pentadecyl 2,4,4-trimethylpentyl ester
34	16.305	16.245	16.340	78821	1.48	15663	1.07	5.03	18-Methyl-nonadecane-1,2-dio, trimethylsilyl ether
35	16.353	16.340	16.370	14610	0.27	11157	0.76	1.31	n-Hexadecanoic acid
36	16.419	16.400	16.435	15995	0.30	10828	0.74	1.48	Propionic acid, 3-iodo-, octadecyl ester
37	16.517	16.495	16.575	37293	0.70	13963	0.96	2.67	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
38	16.672	16.660	16.690	8455	0.16	7912	0.54	1.07	d-Mannitol, 1-decylsulfonyl-
39	16.931	16.920	16.940	7072	0.13	8071	0.55	0.88	Sarreroside
40	17.130	17.110	17.145	16623	0.31	9410	0.64	1.77	Pregn-4-ene-20-carboxylic acid, 7,12-dihydroxy-3-oxo-, methyl ester
41	17.317	17.145	17.425	1717528	32.15	285630	19.56	6.01	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)
42	17.485	17.425	17.505	94261	1.76	21412	1.47	4.40	6-(2,2,5-Trimethyl-[1,3]dioxan-4-yl)-hepta-2,4-dienoic acid, methyl
43	17.550	17.505	17.585	63670	1.19	14885	1.02	4.28	Heptasiloxane, hexadecamethyl-
44	18.483	18.420	18.550	87953	1.65	25290	1.73	3.48	Di-n-octyl phthalate
45	18.745	18.725	18.860	41391	0.77	7345	0.50	5.64	Di-n-decylsulfone
46	18.885	18.860	19.030	47999	0.90	8103	0.55	5.92	1,1,3,3-Tetraallyl-1,3-disilacyclobutane
47	19.109	19.030	19.165	52861	0.99	12254	0.84	4.31	trans-1,2-Diethoxycyclohexane
48	19.251	19.165	19.270	26166	0.49	8510	0.58	3.07	4-Decenoic acid, ethyl ester, (Z)-
49	19.655	19.630	19.670	12761	0.24	8598	0.59	1.48	1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl)amino-
50	19.815	19.760	19.845	20280	0.38	8135	0.56	2.49	4,4-Bis(dichlorofluoromethyl)-1,2-oxathietane-2,2-dioxide
				5342973	100.00	1460151	100.00		

Fig.1a GC-MS chromatogram of methanolic extract of *G.cervicornis* showing the presence of Phenol

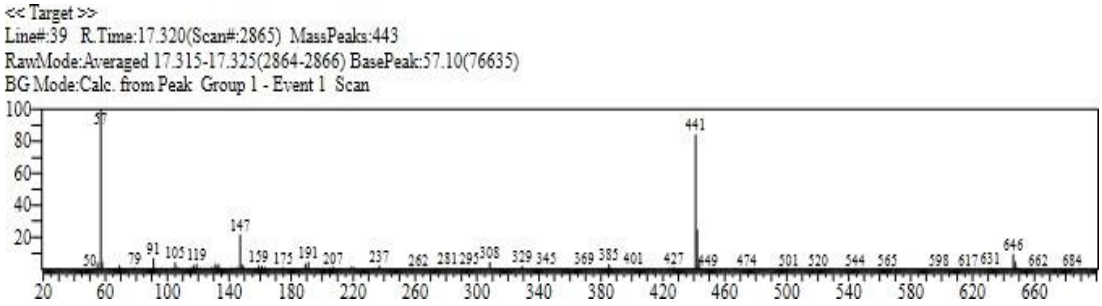


Fig 1 b. GC-MS chromatogram of methanolic extract of *G. gracilis* showing the presence of Phenol

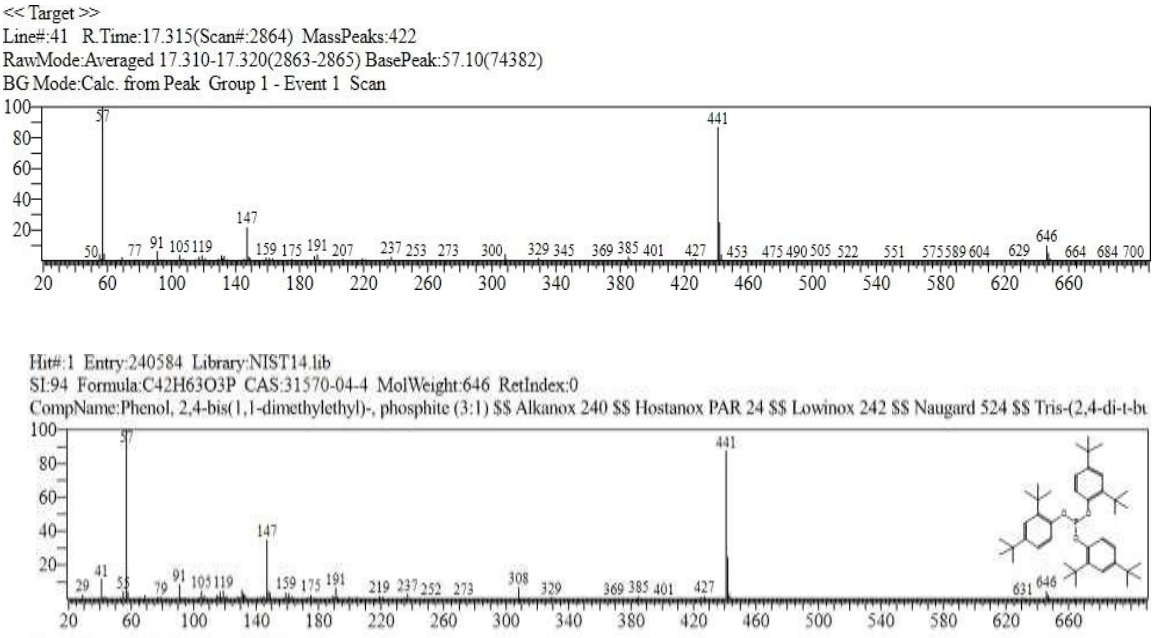


Fig 3 a. GC-MS chromatogram showing the retention time, molecular formula, molecular weight of 3,7,11,17-Tetramethyl-2-hexadecen-1-ol present in methanolic extract of *G. gracilis*

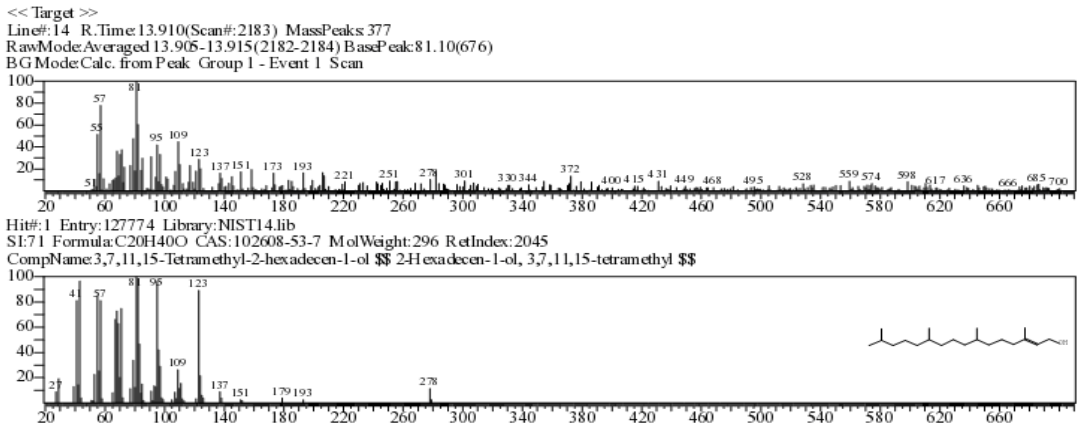


Fig 3 b. GC-MS chromatogram showing the retention time, molecular formula, molecular weight of 17 ,5-Dimethylhexyl)-10,13-dimethyl-2,3,4 present in methanolic extract of *G. gracilis*

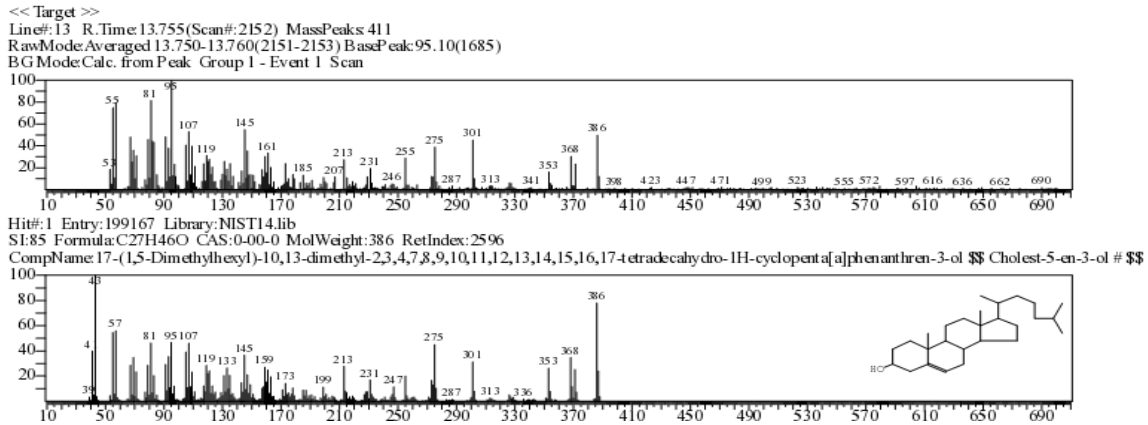


Fig 3 c. GC-MS chromatogram showing the retention time, molecular formula, molecular weight of Di-n-decylsulfone present in methanolic extract of *G. gracilis*

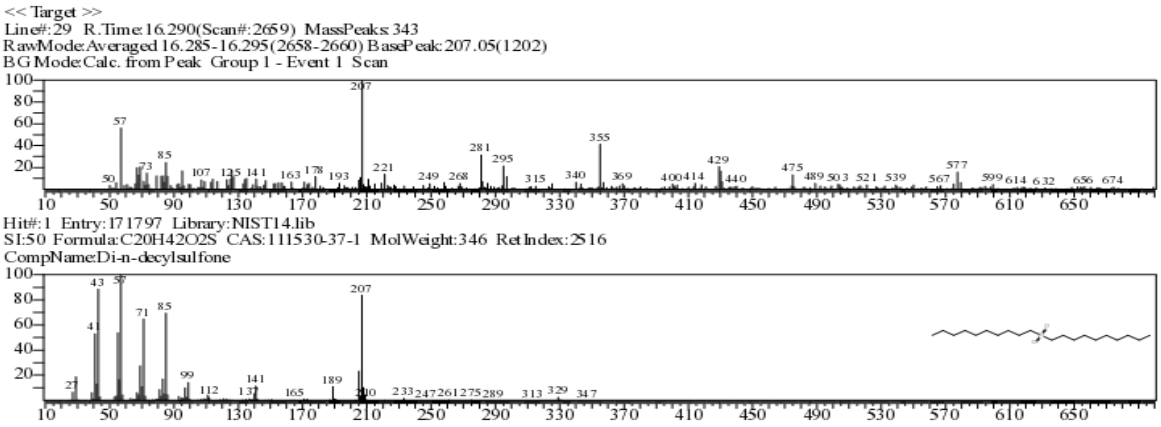
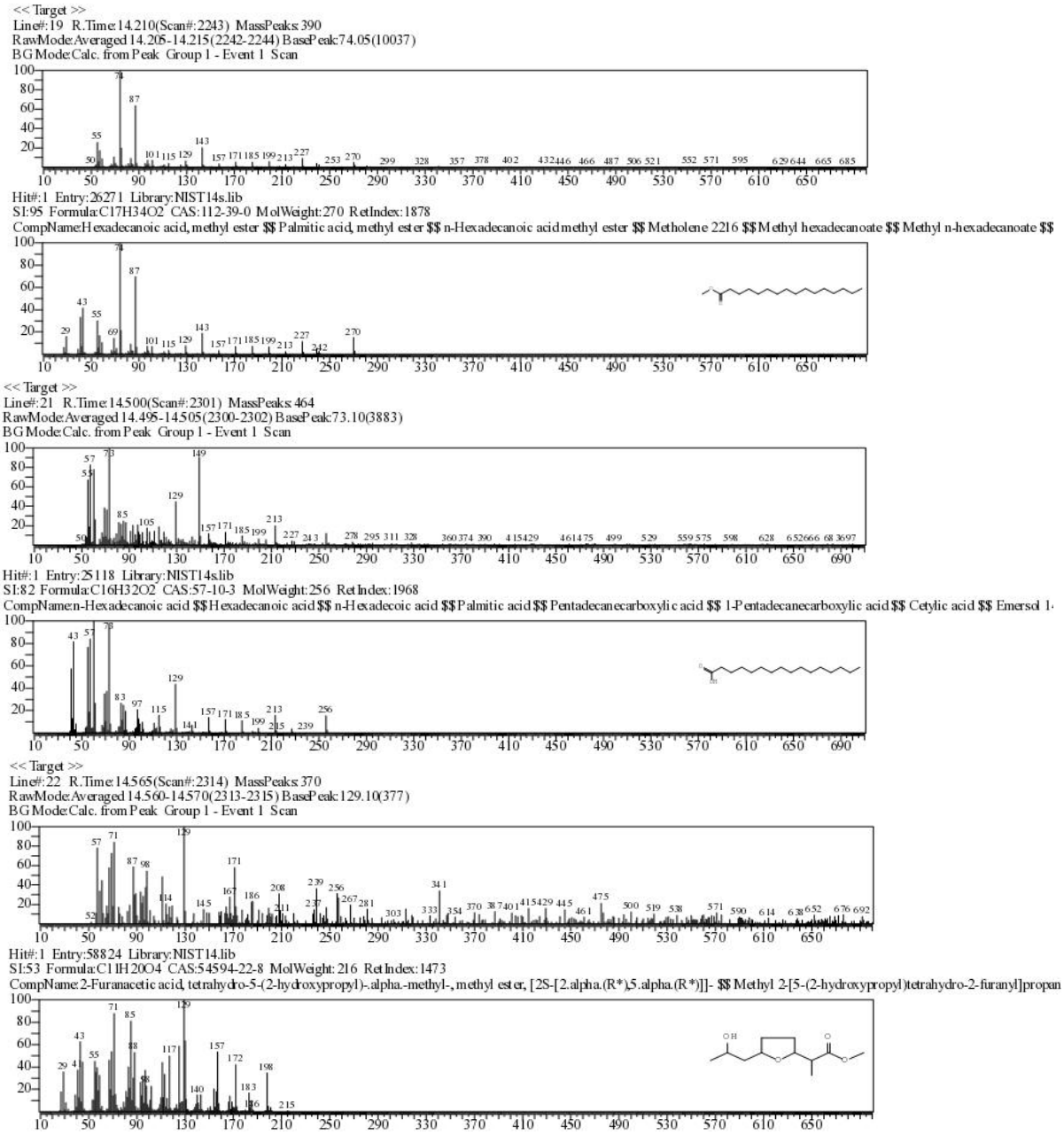


Fig.2 GC-MS chromatogram showing the retention time, molecular formula, molecular weight of Fatty acids



Antifungal activity against *M. phaseolina* and *L. theobromae*

Ballesteros *et al.*, (1992) reported that most dominant plants in the mediteranean phytobentic communities such as seagrasses, *Cytoseira* sp, *Halopteris* sp, *Codium* sp and *Mesophyllus lichenoides* strongly inhibit the growth of fungi. Khaled *et al.*, (2012) also reported that methanolic extracts of *P. pavonica* and *S. vulgare* showed antifungal effect against *Candida* sp strains. In one more study Saleh and Mariri (2017) proved that methanolic extract of *U. lactuca* exhibited lowest MIC value of 0.106 mg ml^{-1} against *A. niger* and *Candida albicans.* In line with the previous studies in this study also methanolic extract of *G. cervicornis* recorded inhibition

zone of 20.7 mm against *M. phaseolina* and the acetone extract of the same showed 16.6 mm. Among the two solvents used methanol seems to be effective in extraction as it could extract bioactive compounds from the seaweed efficiently. *M. phaseolina* is more sensitive to both seaweed extracts than *L. theobromae.*

Phytochemical analysis by GC-MS

The antimicrobial activity of phenolic compounds is attributed by changing the microbial cell permeability, leakage of macromolecules or cellular integrity loss which may lead to cell death (Abu-Ghannam and Rajauria, 2013). The presence of phenols as major portion in the extracts of both *G. gracilis* and *G. cervicornis* contributes for their antifungal activity against *M. phaseolina* and *L. theobromae*. The absence of phenols in chromatogram of *Endocladia muricata* strongly proves that the phenolic compounds have played main role in the antifungal activity exhibited by the other two seaweeds. The study of Ammar *et al.,* (2017) is also in accordance with the present research finding as the phenolic acid and flavonoids in the methanolic extract of *Sargassum vulgare* inhibited the mycelia growth by 51% in *Pythium aphanidermatum.*

Aliya *et al.,* (1995) have recorded highest amounts of tridecanoic acid and palmitic acid in *Bryopisis pennata* and *Valoniopsis panchynema.* In *Laurentia brandenii,* the major component of the active fraction was found to be octadecanoic acid (49.75%) followed by hexadecanoic acid (14.24%) and it was also observed that the higher % of octadecanoic acid contributed for the biological activity (Aseer Manilal *et al.,* 2010). Recently, Corato *et al.,* (2017) has demonstrated that higher fatty acid content of *Laminaria digitata*, *Undaria pinnatifida* and *Porphyra umblicalis* may have influence on fungal suppression as the extracts strongly reduced the incidence of brown rot of peaches and green mould on lemons. The presence of a variety of fatty acids in the extracts of both *Gracilaria* in this study substantiates the previous findings.

The compound ethyl isoallocholate has been shown to exhibit anti-inflammatory and antimicrobial activity (Sarada *et al.,* 2011) and the same is also present in the extract of *G. cervicornis* used in the present study. Antibacterial property of 3,7,11,17-Tetramethyl-2-hexadecen-1-ol against *A. flavus* and *A.niger* and 17 1,5-Dim-ethylhexyl)-10,13-dimethyl-2,3,4 (Santhanam *et al.,* 2019) and biological activity including antifungal activity of Di-n-decylsulfone1, 2-Bis (trimethylsilyl) benzene (Susheela Mary *et al.,* 2017) have been reported. The presence of these compounds in chromatogram of *G. cervicornis* (Fig 3 a, b, c) with height % of 4.62, 6.0 and 3.8 respectively substantiate for the antifungal activity exhibited by this seaweed against *M. phaseolina* and *L. theobromae.*

In the present study, the formation of inhibition zone by the methanolic extracts of *G. cervicornis* and *G. gracilis* against the root and leaf fungal pathogens *viz., M. phaseolina* and *L. theobromae* and their chromatographic cataloging shows that these two seaweeds can be effectively utilized for the extraction of antimicrobial compounds against fungal pathogens.

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