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Bioactive compounds from fresh water green macro algae of Ganga water

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Abstract
Algae and their extracts are abundant sources of chemicals that are physiologically active. They have long been recognized in the development of novel biotechnology products because of their benefits for people, animals, and plants. The current investigation's goal was to determine whether any bioactive substances were present. To identify and characterize the lipids Gas chromatography-mass spectrometry (GC-MS) was used. The National Institute of Standards and Technology library was used to match the mass spectra of the chemicals discovered in the extract. Maximum % area is found for n-Hexadecanoic acid (26.15%), Hexadecanoic acid, methyl ester (14.96%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z Z) -(15.39%), Tetradecanoic acid (5.30%), Phenol, 2, 4-BIS (1,1-Dimethylethyl) -(7.81%), Benzenepropanoic acid, 3,5-bis (1, dimethyl ethynyl)-4-hydroxy-, methyl ester (4.23%), Phytol(4.84%), 1-Heptadecene(3.95%), and Pentadecane (3.73%). The biochemical functions of specific bioactive substances have been found in the extracts of the *Rhizoclonium* species, *Hydrodictyon reticulatum* species, and *Spirogyra* species prepared in methanol, chloroform, n-hexane, and MTBE (tert-Butyl Methyl Ether). The outcome of this experiment demonstrates the benefits of using green algae, which have a variety of therapeutic capabilities and are highly recommended as a biological choice with significant pharmaceutical value.

Keywords: Algae, biooil, solvent extraction, bioactive compounds, biological applications, gas chromatography mass spectrometry

Introduction
According to natural products are a distinct group of bioactive molecules with a wide range of molecular and functional characteristics [1]. Traditional treatment methods make use of a variety of natural substances. Microalgae, also known as photosynthetic microbial cell factories, can create a variety of significant biomolecules, such as carbohydrates, lipids, accessory pigments (carotenoids), polyunsaturated fatty acids, and phycobiliproteins. They are therefore seen to have the greatest potential to manufacture biofuels as well as being prospective sources of bioactive compounds that are important from a commercial standpoint, such as those utilized in medicine, nutritional supplements, and skincare [2, 3].
One of the major sources of biomass in the marine environment is marine algae. In their environment, they create a wide range of chemically active compounds, maybe as a defense mechanism against the other settling species. Numerous marine algae species offer these biogenic substances, also known as active metabolites. In addition to other uses, their antimicrobial and anti-algal characteristics are helpful in the treatment of disease.
Algae and their extracts are rich sources of biologically active substances. Their advantages for people, animals, and plants have long been understood, and they continue to be valued in the generation of new biotechnology products today. Researchers are exploring bioactive substances from natural sources that may substitute these synthetic chemicals due to the growth in antibiotic-resistant bacteria caused by the excessive use of synthetic antibacterial drugs in present-day society and the undesirable side effects of regularly used processed antioxidants [4, 5]. Due to their immense richness, microalgae constitute an underutilized source of bioactive chemicals. They generate several lipophilic products, such as eicosapentaenoic acid, fatty acid methyl esters, micro Colin A, and phthalate ester, which were shown to possess antioxidant and antibacterial characteristics [6, 7]. As a result, they offer chances for the discovery of novel bioactive substances that can be used to produce organic antioxidants and antibacterial agents. Many Asian nations utilise *Spirogyra* spp., a kind of filamentous freshwater green alga (family Zygnemataceae), as they are an important source of naturally occurring bioactive chemicals, which are frequently used for biologically beneficial functions such as antibacterial, antiviral, antioxidant, anti-inflammatory, cytotoxic, and anti-inflammatory.

Researchers working on the development of natural products and medicinal chemistry have an important challenge at hand: evaluating the chemical nature and biological activity of natural bio-resources. There are a variety of chemo metric techniques available to identify bioactive phytochemotypes and to assess biological activities using in vitro and in vivo assays that yield results that are both significant and useful in determining the pharmaceutical, therapeutic, and health-promoting properties of natural phytoproducts. These techniques have given rise to an effective and potent instrument for bio-efficacy, safety, and quality control. Additionally, *S. porticalis* methanol extract protected rats against hypoxia-induced oxidative stress and drove up the start of adaptative alterations when they were exposed to hypobaric hypoxia [8]. The bioactivity of macroalgae from the coastal region of Aceh's south-west has been investigated. This includes green algae (*Chaetomorpha crassa*, *Chaetomorpha antennina*, *Udotea* sp.), brown algae (*Sargassum* sp., *Pseudo-nitzschia* australis) [9]. It was strongly recommended to use the green alga *Halimeda macroloba's* bioactive component as a source of antioxidants [9]. It was once believed that algae's antioxidant content, which includes alkaloids, flavonoids, phenols, tannins, phlorotannin, terpenoids, pigments, glycosides, and steroids, served as a defence mechanism to keep algae from being damaged by reactive oxygen species (ROS) brought on by unfavorable conditions in the environment [10, 11]. Antioxidants in macro-algae shielded the structural elements of the species from oxidative damage caused by the environment [12]. Unbranched thick filaments define the green algal genus *Chaetomorpha* (Chlorophyte, Cladophorales) has over 70 species [13], the majority of which are rich in bioactive chemicals, making them perfect for use as nutritional supplements and alternative therapies for the treatment of illnesses [14]. These green macroalgae have shown cytotoxicity against many cancer cell lines in certain cases [15]. The active components of the green algae *Chaetomorpha* sp. were characterized and identified for the antioxidant and anticancer effects [16]. The ethanolic extract of *Rhizoclonium hieroglyphicum* had the strongest antibacterial effects and uncovered 30 different bioactive constituents, mainly including long-chain polyunsaturated and saturated fatty acids such as myristic (C14:0), palmitic (C16:0), stearic (C18:0), α-linolenic (C18:3; ω-3), and oleic (C18:1, ω-9) acids, which synergistically make this potent antibacterial action [17].

As a consequence of this, researchers are increasingly looking into new functional uses for the lipids that microalgae store but are unrelated to the creation of bioenergy. Microalgal lipid extracts have demonstrated good antioxidant and antibacterial properties in several studies, mostly as a result of the presence of diverse bioactive chemicals. However, only a small number of researchers have gone further and isolated the compounds that are responsible for the properties. The objective of the current study was to use GC-MS spectrum analysis to investigate the bioactive components of the various algal samples. The most recent research in the field of algal extracts is presented in this paper. Particular focus was placed on substances that are valuable as medicinal ingredients since they are biologically active.

Materials and Methods

Description of the study area

Algal biomass was collected in March 2022 from Ganga River Phaphamau Prayagraj, Uttar Pradesh (25.505° N, 81.868° E). Systematic authentication of the algae was done at the Botany Department CMP Degree College Prayagraj. The status assigned to the samples is *Rhizoclonium* species, *Hydrodictyon reticulatum* (L.) *Lagerheim*, and *Spirogyra* species i.e., sample-1, sample- 2, and sample-3 respectively.

Chemicals

Chemicals with names like MTBE (tert-butyl Methyl Ether), hexane, and chloroform have been purchased from LOBA CHEMIE Pvt. Ltd Mumbai, India, and methanol from Merck Life Sciences Pvt. Ltd. Mumbai, India.

Preliminary treatment of Algal biomass

To get rid of contaminating particles like sand and soil, etc., the wet algal biomass was washed with water repeatedly. After being washed, the biomass was dried in the sun for a minimum of two days for moisture content reduction up to 80% followed by oven drying at 45 °C for 24 hours. Following sample grinding, the fraction with µm particle sizes was placed in sealed plastic containers. Figure [A-K] displays images of the wet as well as dried algal biomass and the cell structure.

Analysis of Physical Parameters

HANNA Combo pH and EC Multi Meter Hi 98194 were used to analyze the physical characteristics of the water sample in situ including pH 7.4, conductivity 352, temperatures of 25 °C, and salinity 0.17.

Laboratory processes

Chloroform and Methanol solvent method

Using a mortar and pestle, 1 g of dry biomass was finely ground up. Chloroform, methanol, and water were added to the powder in a measured volume of 10 ml, 20 ml, and 10 ml respectively in a 250 ml conical flask with a stir bar. The flask with above mentioned samples and chemicals along with water set on a magnetic stirrer for 24 hours. The sample was subsequently filtered using Whatman filter paper (grade 1). 10 ml of each chloroform and DDW water were used to rinse the original flask and the filter, aiming at getting out any leftover oil. A comprehensive separation of the water/methanol (top layer) and chloroform/algal oil (bottom layer) was achieved by centrifuging the collected solvent combined with algal oil for 10 min. at 1000 rpm. A 200 µl micropipette was used to collect the bottom layer into a 2-ml Eppendorf tube. After the extraction of total oil from the solvent the oil has been again centrifuged in micro centrifuge tarsons spinwin mc-00 for 5 minutes at 1000 rpm for the removal of the remaining moisture content from the oil. The oil from algal biomass has been stored in a cold place for further analysis.

Hexane solvent method

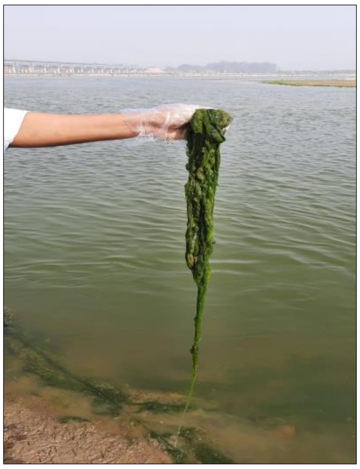
A 250 ml conical flask with 10 ml of hexane was filled with 1 g of dry biomass powder, which was then stirred with a magnetic stirrer for 6 hours. The samples were maintained in a water bath at a temperature of 65 to 75 °C after stirring. Following heating in a water bath, the organic solvent has been evaporated. And rest part of the algal sample was rinsed with 7 ml of hexane to take out the remaining oil from the sample. After evaporation oil has been collected in the Eppendorf tube for further analysis.

MTBE extraction method

A 250 ml conical flask containing 7.5 ml of methanol, 25 ml of MTBE, and 1 g of dry biomass powder, was placed in an incubator for one hour. After incubation 6.25 ml DD water was added to the conical flask and then kept for incubation again for 10 min. Following incubation, the samples were centrifuged for 10 minutes at 1000 rpm. After centrifugation, a 2 ml Eppendorf tube was used to collect the upper organic phase. Later, these oil samples were sent to the AIRF (Advanced Instrumentation Research Facility), JNU, Delhi, India for characterization using the Gas Chromatography-Mass Spectroscopy technique. By matching the spectra to the National Institute of Standards and Technology (NIST) database, the phytoconstituents contained in the organic solvent-extracted algal extracts have been determined.


A


B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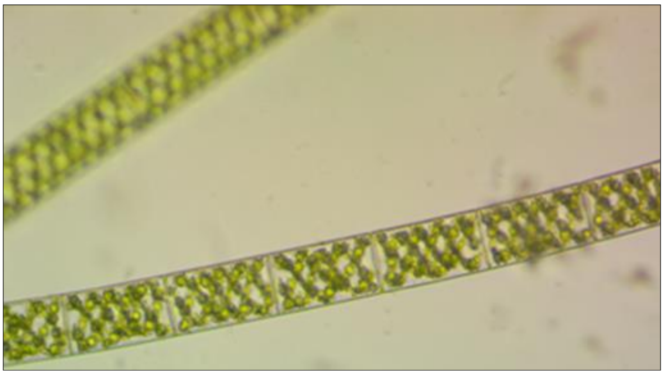
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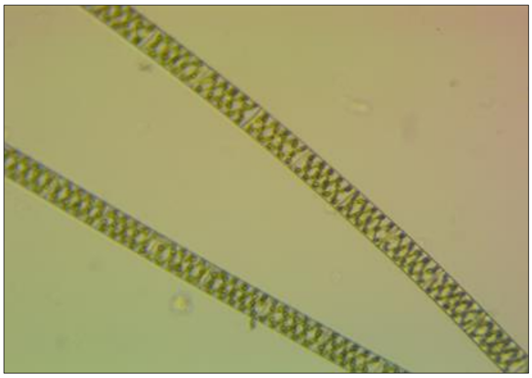
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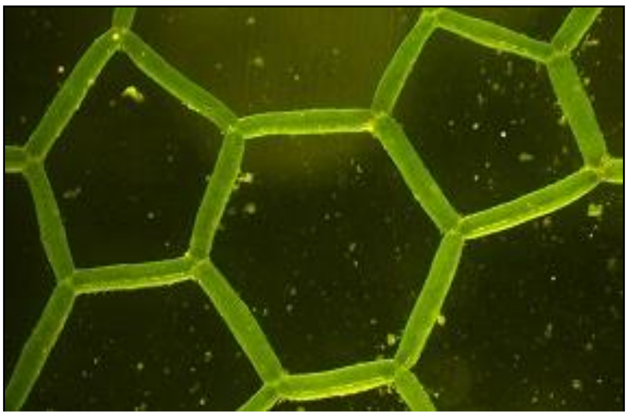
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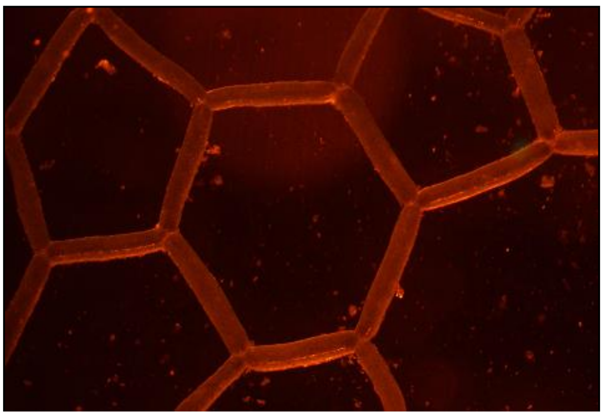
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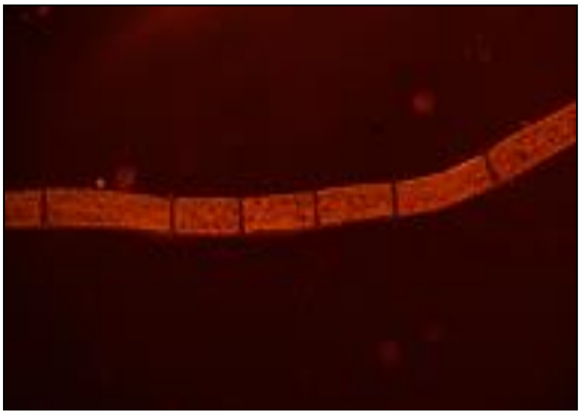

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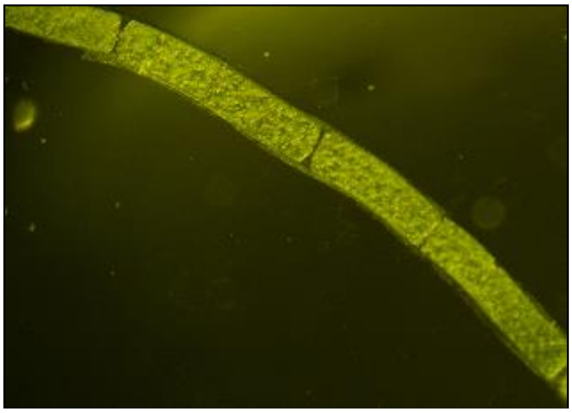

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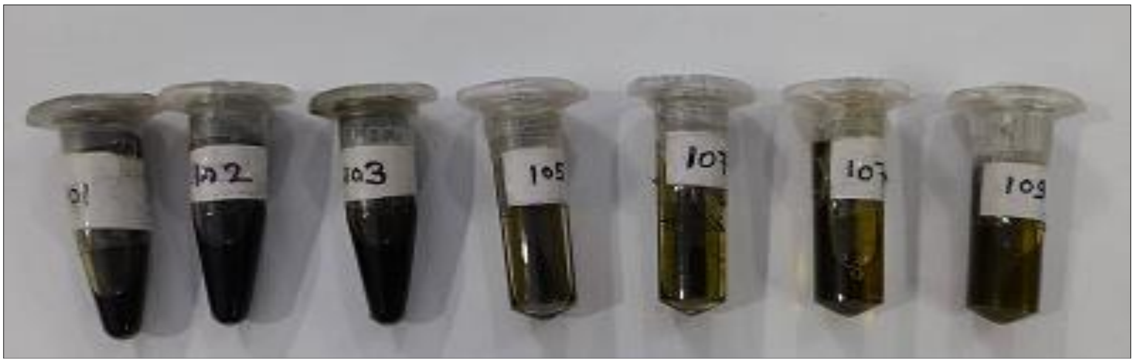


L

Fig. 1: A-C Algal biomass collected from river water; D-E: Dried Algal biomass; F-G: *Spirogyra* species; H-I: *Hydrodictyon reticulatum*; J-K: *Rhizoclonium* species; L: Algal oil with different organic solvents.

Characterization of oil

GC-MS, a highly recommended method for assessing organic substances, was used to examine various chemical contents and functional groups including fatty acids, terpenes, esters, alcohols, and aldehydes to quantify algal oil. A capillary column from the Shimadzu GC MS-QP2010 was used for the analysis. An automated split injector was used by GC-MS at 260 °C. 3.0 mL/min of bio-oil flowed through the helium gas. The temperature was maintained at 40 °C for five minutes, then raised to 300 °C and maintained there for an additional 5 minutes. All of the compounds were examined using GC-MS software, which employed an integrated library to identify each one after analysis.

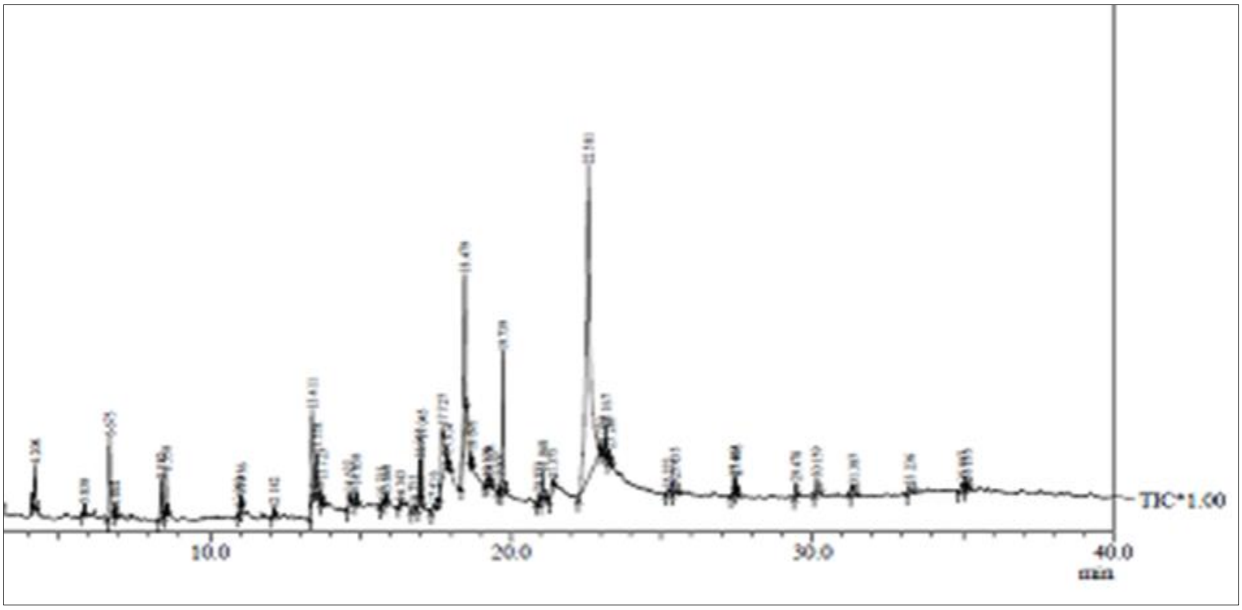


Fig 2: Chromatogram of methanol chloroform extracts of *Rhizoclonium* species

Table 1: Biochemical compounds detected in chloroform and methanol extract of *Rhizoclonium* species

S. No.	R. Time	Name of Compound	% Area	M.F.	M.W.
1.	4.208	Tetradecane	1.94	C14H30	198
2.	5.830	Nonadecane	0.48	C19H40	268
3.	6.675	2,4-Di-tert-butyl-phenol	3.55	C14H22O	206
4.	6.864	Nonadecane	0.35	C19H40	268
5.	8.382	9-Octadecene, (E)-	1.68	C18H36	252
6.	8.550	Pentadecane	1.76	C15H32	212
7.	10.980	Decane, 1-iodo-	0.14	C10H21I	268
8.	11.056	2-Propenoic acid, tridecyl ester	0.40	C16H30O2	254
9.	12.142	Hexadecane	0.27	C16H34	226
10.	13.411	Tetradecanoic acid	5.30	C14H28O2	228
11.	13.558	1-Hexadecanol	1.57	C16H34O	242
12.	13.723	Nonadecane	0.91	C19H40	268
13.	14.627	Neophytadiene	0.64	C20H38	278
14.	14.850	2-Pentadecanone, 6,10,14-trimethyl-(phytone)	0.78	C18H36O	268
15.	15.713	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.34	C20H40O	296
16.	15.860	(6Z,9Z,12Z,15Z)-Methyl octadeca-6,9,12,15-tetraenoate	0.33	C19H30O2	290
17.	16.343	Hexadecane	0.16	C16H34	226
18.	16.753	2-Methylhexacosane	0.38	C27H56	380
19.	16.961	Hexadecanoic acid, methyl ester	1.37	C17H34O2	270
20.	17.045	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	2.00	C18H28O3	292
21.	17.416	Eicosane	0.47	C20H42	282
22.	17.727	Doconexent	5.11	C22H32O2	328
23.	17.914	Cis,Cis,Cis-7,10,13-Hexadecatrienal	0.58	C16H26O	234
24.	18.479	n-Hexadecanoic acid	9.87	C16H32O2	256
25.	18.693	2-methyloctacosane	0.40	C29H60	408
26.	19.198	Propanoic acid, 3-mercapto-, dodecyl ester	0.28	C15H30O2S	274
27.	19.305	Tetradecanal	0.37	C14H28O	212
28.	19.607	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	0.33	C18H30O2	278
29.	19.739	Doconexent	5.96	C22H32O2	328
30.	20.923	9,12-Octadecadienoic acid, methyl ester	0.47	C19H34O2	294
31.	21.068	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1.54	C19H32O2	292
32.	21.375	Phytol	1.03	C20H40O	296
33.	22.581	cis,cis,cis-7,10,13-Hexadecatrienal	41.17	C16H26O	234
34.	23.019	1,E-6,Z-11-Hexadecatriene	0.27	C16H28	220
35.	23.167	1-Heneicosanol	1.20	C21H44O	312
36.	23.283	2-methyloctacosane	0.49	C29H60	408
37.	25.222	1-Tridecanol	0.28	C13H28O	200
38.	25.435	2-methyloctacosane	0.77	C29H60	408
39.	27.403	Behenic alcohol	0.67	C22H46O	326
40.	27.495	Heneicosane	0.60	C21H44	296
41.	29.478	Heneicosane	0.57	C21H44	296
42.	30.159	1,2-Benzene Dicarboxylic Acid	0.86	C24H38O4	390
43.	31.387	Heneicosane	0.86	C21H44	296
44.	33.236	Heneicosane	0.41	C21H44	296
45.	35.007	2-Methylhexacosane	0.63	C27H56	380
46.	35.155	Squalene	0.45	C30H50	410

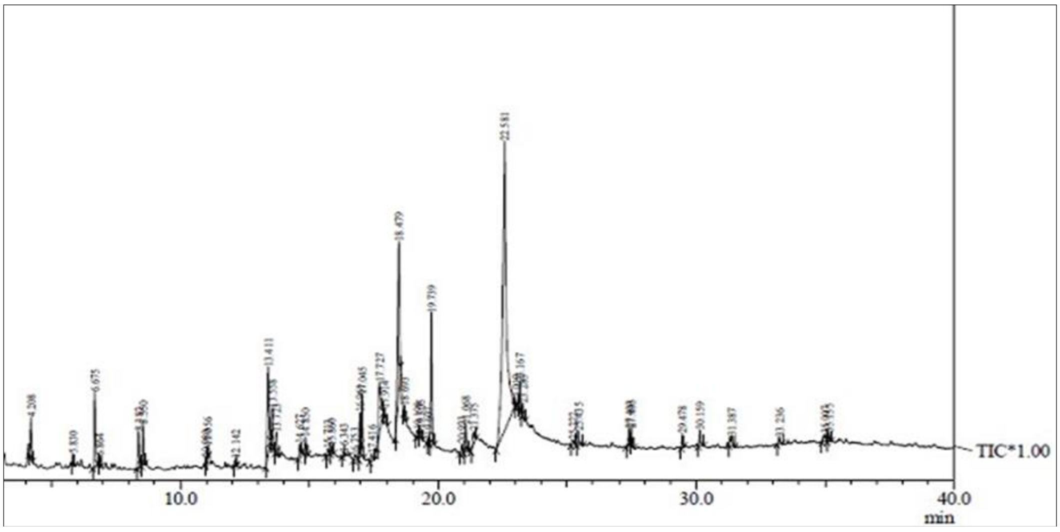


Fig 3: Chromatogram of methanol chloroform extracts of *Hydrodictyon reticulatum*

Table 2: Biochemical compounds detected in chloroform and methanol extract of *Hydrodictyon reticulatum*

S. No.	R. Time	Name of Compound	% Area	M.F.	M.W.
1.	3.166	Dodecyl nonyl ether	0.33	C21H44O	312
2.	3.544	2,6-Octadiene, 2,6-dimethyl-	0.35	C10H18	138
3.	4.099	Cyclododecane	0.67	C12H24	168
4.	4.202	Tetradecane	1.90	C14H28	196
5.	5.822	Octadecane	0.48	C18H38	254
6.	6.152	Pentadecane	0.50	C15H32	212
7.	6.668	Phenol, 2,4-BIS(1,1-Dimethylethyl)-	4.85	C14H22O	206
8.	6.856	OCTADECANE	0.50	C18H38	254
9.	7.342	Decyl octyl ether	0.16	C18H38O	270
10.	7.422	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	0.25	C11H16O2	180
11.	8.372	1-Pentadecene	2.13	C15H30	210
12.	8.545	Pentadecane	1.96	C15H32	212
13.	8.676	Octanoic acid, 4-pentadecyl ester	0.21	C23H46O2	354
14.	10.277	2-Bromotetradecane	0.15	C14H29Br	276
15.	10.540	8-Heptadecene	0.69	C17H34	238
16.	10.900	Acetophenone, 2-chloro-	0.03	C8H7ClO	154
17.	10.977	Dodecane, 5,8-Dimethyl-	0.07	C14H30	198
18.	11.040	2-Propenoic acid, tridecyl ester	0.66	C16H30O2	254
19.	11.118	Heptadecane	1.07	C17H36	240
20.	11.656	Tetradecanal	0.33	C14H28O	212
21.	11.744	Dodecyl nonyl ether	0.17	C21H44O	312
22.	12.131	Docosane	0.44	C22H46	310
23.	12.214	Octyl tetradecyl ether	0.15	C22H46O	326
24.	13.550	1-Heptadecene	2.23	C17H34	238
25.	13.714	Nonadecane	1.07	C19H40	268
26.	14.619	Neophytadiene	0.21	C20H38	278
27.	14.843	2-Pentadecanone, 6,10,14-trimethyl-	4.46	C18H36O	268
28.	15.602	Heneicosane	0.11	C21H44	296
29.	16.252	Nonadecane	3.05	C19H40	268
30.	16.337	Hexadecane, 2,6,10,14-Tetramethyl-	0.13	C20H42	282
31.	16.550	7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	0.20	C17H24O3	276
32.	16.959	Hexadecanoic acid, methyl ester	1.24	C17H34O2	270
33.	17.044	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro	2.72	C18H28O3	292
34.	17.410	Eicosane	0.77	C20H42	282
35.	18.494	n-Hexadecanoic acid	26.15	C16H32O2	256
36.	18.690	Docosane	0.51	C22H46	310
37.	19.192	Propanoic acid, 3-mercapto-, dodecyl ester	0.43	C15H30O2S	274
38.	19.292	Tetradecanal	0.23	C14H28O	212
39.	19.623	2-Methyltetracosane	0.26	C25H52	352
40.	20.638	1-Octadecanesulphonyl chloride	0.43	C18H37ClO2S	352
41.	20.915	9,12-Octadecadienoic acid, methyl ester	0.52	C19H34O2	294
42.	21.066	(9E,12E)-9,12-Octadecadienoyl Chloride #	1.20	C18H31ClO	298
43.	21.207	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	0.34	C18H34O2	282
44.	21.356	Phytol	4.84	C20H40O	296
45.	22.015	Cyclotetradecanol, 1,7,11-Trimethyl-4-(1-ME	0.15	C20H40O	296
46.	22.090	C33 Botryococcane (9)	0.04	C33H68	464
47.	22.160	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.09	C20H40O	296
48.	22.277	Hexadecane, 2,6,10,14-TETRAMETHYL-	0.11	C20H42	282
49.	22.511	Undec-10-ynoic acid, decyl ester	3.99	C21H38O2	322
50.	22.997	9,12-Octadecadienoic Acid (Z, Z)-, Methyl Ester	0.31	C19H34O2	294
51.	23.161	1-Nonadecene	1.31	C19H38	266
52.	23.280	Heneicosane	0.39	C21H44	296
53.	23.373	9-Octadecenamide	0.38	C18H35NO	281
54.	23.601	Phytol, acetate	0.12	C22H42O2	338
55.	25.213	1-Tridecanol	0.32	C13H28O	200
56.	25.428	2-methyloctacosane	0.55	C29H60	408
57.	25.850	5,5-Diethylheptadecane	0.13	C21H44	296
58.	26.310	(1S,3aS,4S,7R,8aS)-1,4,9,9-Tetramethyldecahydro-4,7-(epoxymethano)azulen-3a-ol	0.17	C15H26O2	238
59.	26.514	1-Decanol, 2-hexyl-	0.17	C16H34O	242
60.	26.728	Tetracosane	0.37	C24H50	338
61.	27.394	1-Heneicosanol	0.79	C21H44O	312
62.	27.488	Heneicosane	0.41	C21H44	296
63.	29.370	5,5-Diethylheptadecane	0.04	C21H44	296
64.	29.471	Eicosane	0.50	C20H42	282
65.	30.022	Hexatriacontane	0.21	C36H74	506
66.	30.147	1,2-Benzenedicarboxylic Acid	1.47	C24H38O4	390

67.	30.838	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	8.04	C22H42O2	338
68.	31.307	Octacosanol	0.89	C28H58O	410
69.	33.229	Tetratetracontane	0.49	C44H90	618
70.	33.837	Cyclohexan-4,4-D2-OL, 5-(Methyl-D3)-2-(1-MET Hylethyl)	0.17	C10H15D5O	161
71.	34.049	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphate (3:1)	0.97	C42H63O3P	646
72.	34.589	2-Methylhexacosane	0.42	C27H56	380
73.	34.941	Heptafluorobutyric acid, n-octadecyl ester	0.69	C22H37F7O2	466
74.	36.199	L-Histidine methyl ester dihydrochloride	0.36	C7H13Cl2N3O2	241
75.	39.193	Hexacosyl pentafluoropropionate	0.28	C29H53F5O2	528
76.	43.041	dl-.alpha.-Tocopherol	1.13	C29H50O2	430
77.	44.027	1-Heptatriacotanol	5.36	C37H76O	536

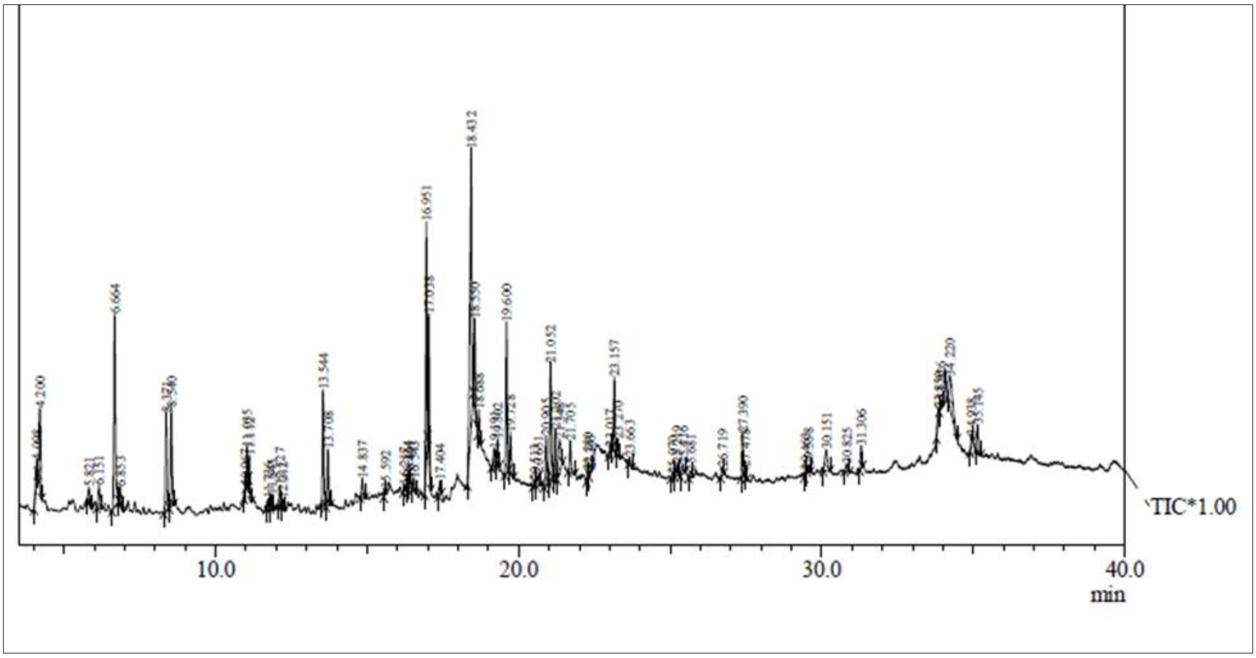


Fig 3: Chromatogram of methanol chloroform extract of *Spirogyra* species

Table 3: Biochemical compounds detected in chloroform and methanol extract of *Spirogyra* species

S. No.	R. Time	Name of Compound	% area	M.F.	M.W.
1.	4.098	Formic acid, undecyl ester	1.07	C12H24O2	200
2.	4.200	Pentadecane	3.45	C15H32	212
3.	5.821	Octadecane	0.59	C18H38	254
4.	6.151	Pentadecane	1.11	C15H32	212
5.	6.664	Phenol, 2,4-BIS(1,1-Dimethylethyl)-	7.81	C14H22O	206
6.	6.853	Octadecane	0.68	C18H38	254
7.	8.371	1-Pentadecene	3.79	C15H30	210
8.	8.540	Pentadecane	3.73	C15H32	212
9.	10.967	Borane, diethyl(decyloxy)-	0.15	C14H31BO	226
10.	11.035	2-Propenoic acid, tridecyl ester	0.96	C16H30O2	254
11.	11.112	Heptadecane, 2,6,10,15-tetramethyl-	0.87	C21H44	296
12.	11.736	Pentadecafluorooctanoic acid, tetradecyl ester	0.29	C22H29F15O2	610
13.	11.843	Octadecanoic Acid, Methyl Ester	0.44	C19H38O2	298
14.	12.127	Octadecane	0.67	C18H38	254
15.	12.212	Dodecyl nonyl ether	0.25	C21H44O	312
16.	13.544	1-Heptadecene	3.95	C17H34	238
17.	13.708	Nonadecane	2.02	C19H40	268
18.	14.837	2-Pentadecanone, 6,10,14-trimethyl-	0.79	C18H36O	268
19.	15.592	Docosane, 1-iodo-	0.22	C22H45I	436
20.	16.217	Tricyclo[4.3.1.0 2,5]Decane	0.25	C10H16	136
21.	16.334	Hexadecane	0.16	C16H34	226
22.	16.401	7-Hexadecenoic acid, methyl ester, (Z)-	0.17	C17H32O2	268
23.	16.543	Silane, Trichlorooctadecyl-	0.38	C18H37Cl3Si	386
24.	16.951	Hexadecanoic acid, methyl ester	7.29	C17H34O2	270
25.	17.038	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro methyl ester	4.23	C18H28O3	292
26.	17.404	Hexadecane, 2,6,10,14-Tetramethyl-	0.43	C20H42	282
27.	18.432	n-Hexadecanoic acid	14.36	C16H32O2	256
28.	18.550	1-Heneicosanol	2.81	C21H44O	312
29.	18.688	2-methyloctacosane	0.90	C29H60	408

30.	19.191	Propanoic acid, 3-mercapto-, dodecyl ester	0.73	C15H30O2S	274
31.	19.302	Heptadecanal	0.86	C17H34O	254
32.	19.600	Sulfuric acid, 5,8,11-heptadecatrienyl methyl ester	5.23	C18H32O3S	328
33.	19.728	cis-5,8,11,14,17-Eicosapentaenoic acid	1.40	C20H30O2	304
34.	20.513	4A-Hydroxy-4-Nitro-Octahydro-Naphthalen-1-One	0.12	C10H15NO4	213
35.	20.651	1-Octadecanesulphonyl chloride	0.66	C18H37ClO2S	352
36.	20.905	9,12-Octadecadienoic acid, methyl ester	1.89	C19H34O2	294
37.	21.052	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	4.54	C19H32O2	292
38.	21.202	11-Octadecenoic acid, methyl ester	1.65	C19H36O2	296
39.	21.348	2-Hexadecen-1-OL, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]-	2.14	C20H40O	296
40.	21.705	Methyl stearate	1.25	C19H38O2	298
41.	22.280	Tetracosane	0.24	C24H50	338
42.	22.363	Hexadecane	0.09	C16H33I	352
43.	23.017	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl	0.26	C19H34	262
44.	23.157	1-Heneicosanol	2.58	C21H44O	312
45.	23.270	Heneicosane	0.55	C21H44	296
46.	23.663	Pentafluoropropionic acid, heptadecyl ester	0.23	C20H35F5O2	402
47.	25.070	Androstan-3-One, Oxime, (5.ALPHA.)-	0.11	C19H31NO	289
48.	25.219	1-Tridecanol	0.57	C13H28O	200
49.	25.416	2-Tridecanone	0.49	C13H26O	198
50.	25.681	4, 2-Cresotic acid, 6-methoxy-, bimol. ester, methyl ester, 4,6-dimethoxy-o-toluate	0.25	C29H30O10	538
51.	26.719	Heptadecane, 8-methyl-	0.30	C18H38	254
52.	27.390	n-Tetracosanol-1	1.44	C24H50O	354
53.	27.478	Heneicosane	0.23	C21H44	296
54.	29.463	Heneicosane	0.12	C21H44	296
55.	29.558	2,2,3,3,4,4 Hexadeutero Octadecanal	0.27	C18H30D6O	274
56.	30.151	1,2-Benzenedicarboxylic Acid	1.38	C24H38O4	390
57.	30.825	2-Methylhexacosane	0.43	C27H56	380
58.	31.306	n-Tetracosanol-1	0.69	C24H50O	354
59.	33.850	Eicosanoic Acid, Methyl Ester	0.42	C21H42O2	326
60.	33.926	Cyclohexyl Palmitate #	0.41	C22H42O2	338
61.	34.220	Propanoic acid, 3,3'-thiobis-, didodecyl ester	3.15	C30H58O4S	514
62.	34.938	n-Nonadecanol-1	0.43	C19H40O	284
63.	35.145	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23	1.08	C30H50	410

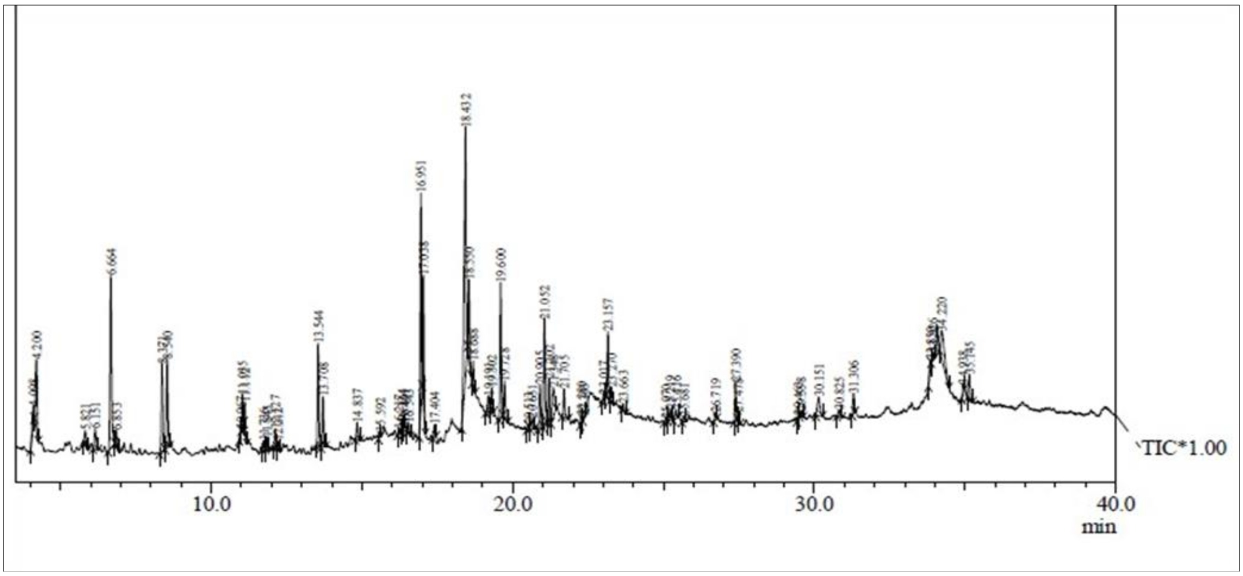


Fig 4: Chromatogram of Hexane extract of *Hydrodictyon reticulatum*

Table 4: Biochemical compounds detected in Hexane extract of *Hydrodictyon reticulatum*

S. No.	R. Time	Name of Compound	% area	M.F.	M.W.
1.	4.176	Tetradecane	0.64	C14H30	198
2.	4.334	Decane, 3,8-Dimethyl-	0.21	C12H26	170
3.	4.394	Heneicosane	0.15	C21H44	296
4.	5.143	Heneicosane	0.37	C21H44	296
5.	5.266	Tetradecane	0.67	C14H30	198
6.	5.520	Dodecane, 4,6-dimethyl-	0.35	C14H30	198
7.	5.717	Hexadecane	0.24	C16H34	226
8.	5.802	Heptadecane	0.73	C17H36	240
9.	5.987	Heptadecane	0.32	C17H36	240

10.	6.128	Pentadecane	1.07	C15H32	212
11.	6.445	1-Dodecanol, 2-hexyl-	0.37	C18H38O	270
12.	6.642	Phenol, 3,5-bis(1,1-dimethylethyl)-	1.80	C14H22O	206
13.	6.836	Eicosane	1.08	C20H42	282
14.	7.084	1-Dodecanol, 2-hexyl-	0.46	C18H38O	270
15.	7.326	Dodecyl nonyl ether	0.34	C21H44O	312
16.	8.527	Tetradecane	0.45	C14H30	198
17.	9.125	Heneicosane	0.18	C21H44	296
18.	10.260	Heneicosane	0.35	C21H44	296
19.	10.965	Eicosane	0.62	C20H41I	408
20.	11.103	Heptadecane	1.51	C17H36	240
21.	11.208	Heptadecane, 2,6,10,15-tetramethyl-	0.17	C21H44	296
22.	11.725	1-Decanol, 2-hexyl-	0.50	C16H34O	242
23.	12.116	Eicosane	1.04	C20H41I	408
24.	12.198	1-Decanol, 2-hexyl-	0.26	C16H34O	242
25.	12.365	Nonyl tetradecyl ether	0.20	C23H48O	340
26.	12.492	1-TRIDECANOL	0.29	C13H28O	200
27.	12.721	1-Dodecanol, 2-hexyl-	0.39	C18H38O	270
28.	13.323	1-Tridecanol	0.29	C13H28O	200
29.	14.425	Pentadecanoic acid, methyl ester	0.48	C16H32O2	256
30.	14.826	2-Pentadecanone, 6,10,14-trimethyl-	1.18	C18H36O	268
31.	15.584	Heneicosane	0.33	C21H44	296
32.	16.213	1,8,11,14-Heptadecatetraene, (Z,Z,Z)-	0.37	C17H28	232
33.	16.317	Heptadecane, 3-Methyl-	0.11	C18H38	254
34.	16.384	9-Hexadecenoic Acid, Methyl Ester, (Z)-	0.29	C17H32O2	268
35.	16.535	Silane, Trichlorooctadecyl-	0.46	C18H37Cl3Si	386
36.	16.945	Hexadecanoic acid, methyl ester	9.89	C17H34O2	270
37.	17.400	Eicosane	0.95	C20H42	282
38.	17.775	Dibutyl phthalate	0.29	C16H22O4	278
39.	18.366	n-Hexadecanoic acid	1.55	C16H32O2	256
40.	18.683	Heneicosane	0.49	C21H44	296
41.	19.287	Octadecanal	0.65	C18H36O	268
42.	19.596	2-Methylhexacosane	0.24	C27H56	380
43.	20.577	Eicosane	0.10	C20H42	282
44.	20.644	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	0.43	C18H30O2	278
45.	20.897	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	3.78	C19H34O2	294
46.	21.050	8,11,14-Eicosatrienoic acid, methyl ester	9.07	C21H36O2	320
47.	21.196	11-Octadecenoic acid, methyl ester	0.98	C19H36O2	296
48.	21.308	Heneicosane, 10-methyl-	0.58	C22H46	310
49.	21.698	Methyl stearate	0.74	C19H38O2	298
50.	22.272	Hexadecane, 2,6,10,14-Tetramethyl-	0.40	C20H42	282
51.	23.268	Heneicosane	3.77	C21H44	296
52.	23.661	1-Decanol, 2-hexyl-	0.35	C16H34O	242
53.	25.167	C33 Botryococcane (9)	0.09	C33H68	464
54.	25.207	1-Nitrododecane	0.12	C12H25NO2	215
55.	25.425	Heneicosane	7.28	C21H44	296
56.	26.501	Octadecyl octyl ether	0.24	C26H54O	382
57.	26.720	Eicosane	0.65	C20H42	282
58.	27.491	Heneicosane	9.45	C21H44	296
59.	28.728	2-methyloctacosane	0.22	C29H60	408
60.	29.474	Heneicosane	7.78	C21H44	296
61.	30.000	5,5-Diethyltridecane	0.27	C17H36	240
62.	30.131	Di-n-octyl phthalate	1.36	C17H36	240
63.	30.663	Tetratetracontane	0.74	C44H90	618
64.	30.835	Hexatriacontane	0.88	C36H74	506
65.	31.377	Heneicosane	6.04	C21H44	296
66.	32.528	Docosane	0.37	C22H46	310
67.	32.707	Pentacosane	0.52	C25H52	352
68.	33.226	Docosane	3.91	C22H46	310
69.	33.830	Tetracosanoic Acid, Methyl Ester	0.57	C25H50O2	382
70.	34.226	2-Methylhexacosane	0.19	C27H56	380
71.	34.328	Tricosane	0.35	C23H48	324
72.	34.510	3-Methylheptacosane	0.34	C28H58	394
73.	34.995	Glyceryl TRI-2,2-Dideuterio Dodecanoate	2.79	C39H68D6O6	644
74.	35.135	1-Coprosten-3-one semicarbazone	0.47	C28H47N3O	441
75.	36.145	Celidoniol, Deoxy-	0.29	C29H60	408
76.	36.340	2-methyloctacosane	0.33	C29H60	408
77.	36.907	Docosane	1.43	C22H46	310
78.	39.258	Tetracosane	0.80	C24H50	338

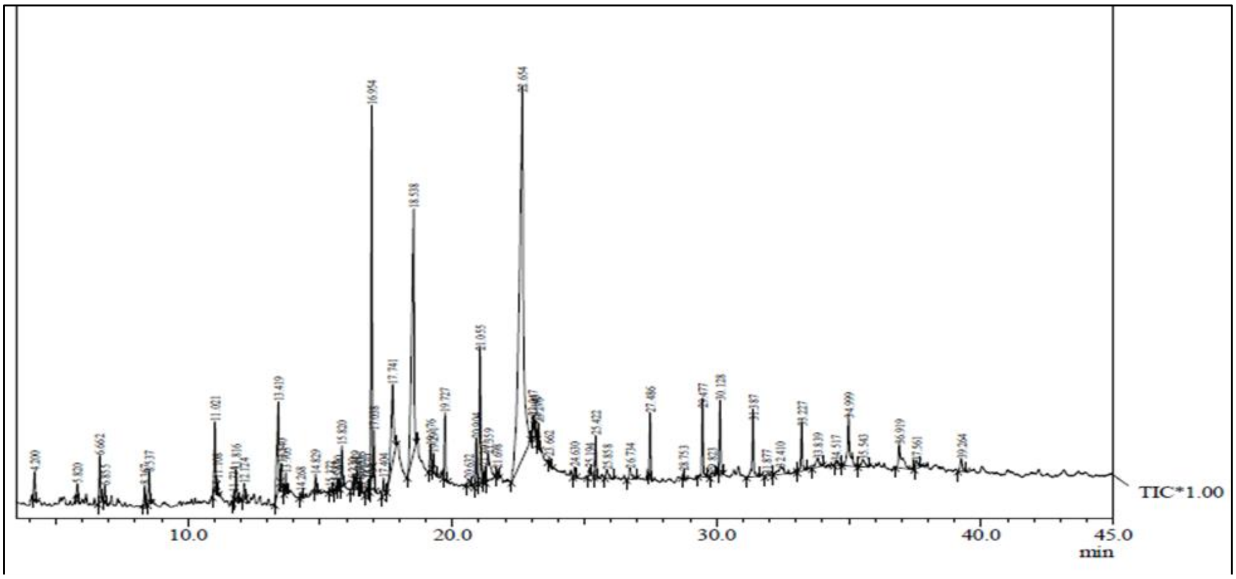


Fig 5: Chromatogram of MTBE extract of *Rhizoclonium* species

Table 5: Biochemical compounds detected in MTBE extract of *Rhizoclonium* species

S. No.	R. Time	Name of Compound	% area	M.F.	M.W.
1.	4.200	Tetradecane	0.77	C14H30	198
2.	5.820	Eicosane	0.34	C20H42	282
3.	6.662	Phenol, 2,4-BIS(1,1-Dimethylethyl)-	1.14	C14H22O	206
4.	6.855	Hexadecane, 2,6,11,15-tetramethyl-	0.43	C20H42	282
5.	8.367	1-Pentadecene	0.45	C15H30	210
6.	8.537	Pentadecane	0.75	C15H32	212
7.	11.021	Dodecyl acrylate	1.64	C15H28O2	240
8.	11.108	Nonadecane	0.18	C19H40	268
9.	11.734	1-Decanol, 2-hexyl-	0.16	C16H34O	242
10.	11.816	Methyl tetradecanoate	0.72	C15H30O2	242
11.	12.124	Eicosane	0.34	C20H42	282
12.	13.419	Tetradecanoic acid	3.30	C14H28O2	228
13.	13.540	1-Octadecene	0.50	C18H36	252
14.	13.705	Nonadecane	0.46	C19H40	268
15.	14.268	Tetradecanal	0.12	C14H28O	212
16.	14.829	2-Pentadecanone, 6,10,14-trimethyl-	0.44	C18H36O	268
17.	15.372	Phthalic acid, cis-hex-3-enyl isobutyl ester	0.13	C18H24O4	304
18.	15.583	Hexadecane	0.07	C16H34	226
19.	15.690	4,7,10-Hexadecatrienoic acid, methyl ester	0.18	C17H28O2	264
20.	15.820	(6Z,9Z,12Z,15Z)-Methyl octadeca-6,9,12,15-tetraenoate	1.00	C19H30O2	290
21.	16.230	Octane, 2-bromo-	0.17	C8H17Br	192
22.	16.329	Hexadecane	0.12	C16H34	226
23.	16.463	Octadecane	0.06	C18H38	254
24.	16.536	Silane, Trichlorooctadecyl-	0.25	C18H37Cl3Si	386
25.	16.737	Decane, 1-iodo-	0.07	C10H21I	268
26.	16.830	(Z)-14-Tricosenyl formate	0.21	C24H46O2	366
27.	16.954	Hexadecanoic acid, methyl ester	7.98	C17H34O2	270
28.	17.038	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	0.71	C18H28O3	292
29.	17.404	Eicosane	0.48	C20H42	282
30.	17.741	Doconexent	4.11	C22H32O2	328
31.	18.538	n-Hexadecanoic acid	11.81	C16H32O2	256
32.	19.176	Propanoic acid, 3-mercapto-, dodecyl ester	0.64	C15H30O2S	274
33.	19.291	Heptadecanal	0.40	C17H34O	254
34.	19.727	Arachidonic acid	1.56	C20H32O2	304
35.	20.632	Pyridinium, 1-Hexadecyl-, Chloride, Monohy	0.30	C21H38N	304
36.	20.904	9,12-Octadecadienoic acid, methyl ester	1.10	C19H34O2	294
37.	21.055	3,6-Octadecadienoic Acid, Methyl Ester	3.65	C19H34O2	294
38.	21.201	11-Octadecenoic acid, methyl ester	0.33	C19H36O2	296
39.	21.359	Phytol	1.84	C20H40O	296
40.	21.698	Methyl stearate	0.21	C19H38O2	298
41.	22.654	cis, cis, cis-7,10,13-Hexadecatrienal	30.81	C16H26O	234
42.	23.047	Octadecanoic acid	0.37	C18H36O2	284
43.	23.143	1-Tricosene	0.25	C23H46	322
44.	23.270	Heneicosane	0.47	C21H44	296

45.	23.662	17-Pentatriacontene	0.16	C35H70	490
46.	24.630	Doconexent	0.24	C22H32O2	328
47.	25.194	Ethylcyclodocosane	0.20	C24H48	336
48.	25.422	Heneicosane	0.95	C21H44	296
49.	25.858	Eicosane	0.40	C20H42	282
50.	26.734	Eicosane	0.51	C20H42	282
51.	27.486	Heneicosane	1.34	C21H44	296
52.	28.753	(6Z,9Z,12Z,15Z)-Methyl octadeca-6,9,12,15-tetraenoate	0.19	C19H30O2	290
53.	29.477	Heneicosane	1.93	C21H44	296
54.	29.823	Acetamide, N-n-heptyl-	0.19	C9H19NO	157
55.	30.128	1,2-Benzenedicarboxylic Acid	1.89	C24H38O4	390
56.	31.387	Heneicosane	1.98	C21H44	296
57.	31.877	Fumaric acid, but-3-yn-2-yl dodecyl ester	0.18	C20H32O4	336
58.	32.410	1,54 Dibromotetrapentacontane	0.95	C54H108Br2	914
59.	33.227	Heneicosane	1.50	C21H44	296
60.	33.839	TETRACOSANE	0.63	C24H50	338
61.	34.517	Hexatriacontane	0.24	C36H74	506
62.	34.999	Glyceryl Tri-2,2-Dideuterio Dodecanoate	2.35	C39H68D6O6	644
63.	35.543	Pentatriacontane	0.52	C35H72	492
64.	36.919	Tetratetracontane	1.66	C44H90	618
65.	37.561	Pentatriacontane	0.41	C35H72	492
66.	39.264	Docosane	0.54	C22H46	310

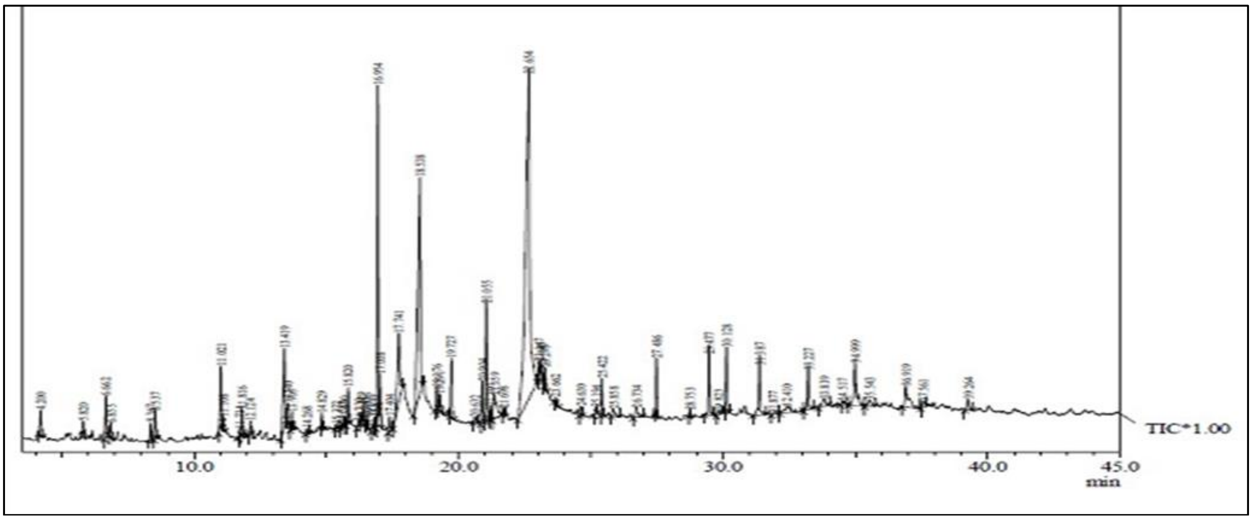


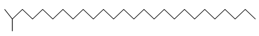
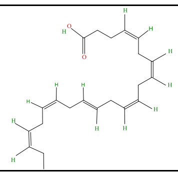
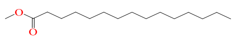
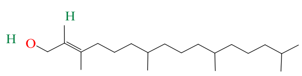
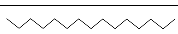
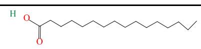
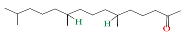
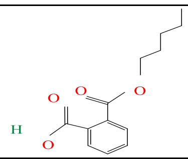
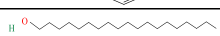
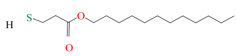
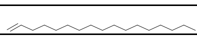
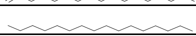
Fig 6: Chromatogram of MTBE extract of *Spirogyra* species

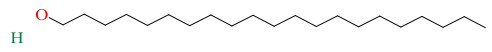
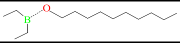
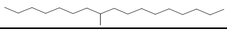
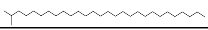
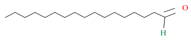
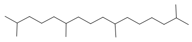
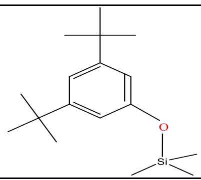
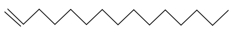
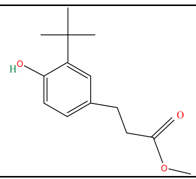
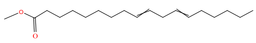
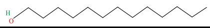
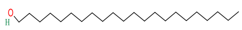
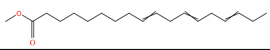
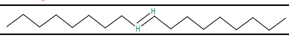
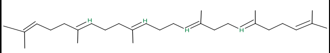
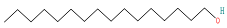
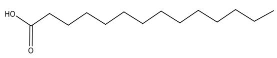
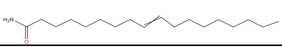
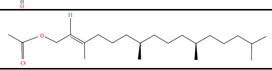
Table 6: Biochemical compounds detected in MTBE extract of *Spirogyra* species

S. No.	R. Time	Name of Compound	% area	M.F.	M.W.
1.	4.060	1H-Pyrazole, 4,5-Dihydro-5,5-Dimethyl-	0.23	C5H10N2	98
2.	4.157	2-(Diethylamino)acetonitrile	0.98	C6H12N2	112
3.	4.232	Heptadecane	0.35	C21H44	296
4.	6.706	Phenol, 3,5-bis(1,1-dimethylethyl)-	3.84	C14H22O	206
5.	8.400	1-Tridecene	1.89	C13H26	182
6.	8.568	Tridecane	2.58	C13H27I	310
7.	10.987	Sulfurous acid, 2-ethylhexyl isohexyl ester	0.63	C14H30O3S	278
8.	11.149	Decane, 2,3,5,8-tetramethyl-	0.96	C14H30	198
9.	11.751	2-Tridecene, 2-Chloro-1,1,1-Trifluoro-, (Z)-	0.64	C13H22ClF3	270
10.	12.140	Sulfurous acid, hexyl octyl ester	0.68	C14H30O3S	278
11.	12.516	1-Heptanol, 6-methyl-	0.69	C8H18O	130
12.	13.349	2-Tridecene, 2-Chloro-1,1,1-Trifluoro-, (Z)-	0.64	C13H22ClF3	270
13.	13.564	1-HEXADECENE	2.75	C16H32	224
14.	13.721	2-Bromo dodecane	2.42	C12H25Br	248
15.	16.267	Bicyclo [5.1.0] Octan-4-OL, Stereoisomer	2.17	C8H14O	126
16.	16.433	9-Octadecenoic acid (Z)-, methyl ester	2.12	C19H36O2	296
17.	16.544	2-Decen-1-OL, (Z)-	0.57	C10H20O	156
18.	16.978	Hexadecanoic acid, methyl ester	14.96	C17H34O2	270
19.	17.046	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro	1.54	C18H28O3	292
20.	18.554	1-Nonadecene	4.49	C19H38	266
21.	18.691	2-Bromo dodecane	2.16	C12H25Br	248
22.	19.622	8,11,14-Eicosatrienoic acid, (Z, Z, Z)-	2.43	C20H34O2	306
23.	20.932	9,12-Octadecadienoic acid, methyl ester	7.25	C19H34O2	294

24.	21.079	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	15.39	$C_{19}H_{32}O_2$	292
25.	21.220	9-Octadecenoic acid (Z)-, methyl ester	4.51	$C_{19}H_{36}O_2$	296
26.	21.380	Oxirane, [(hexadecyloxy)methyl]-	0.59	$C_{19}H_{38}O_2$	298
27.	21.725	Methyl stearate	2.12	$C_{19}H_{38}O_2$	298
28.	230159	n-Nonadecanol-1	2.42	$C_{29}H_{60}$	408
29.	23.273	2-methyloctacosane	1.14	$C_{29}H_{60}$	408
30.	23.662	Oxirane, [(Dodecyloxy)Methyl]-	0.81	$C_{15}H_{30}O_2$	242
31.	24.558	cis-7,10,13,16-Docosatetraenoic acid, methyl ester	1.20	$C_{23}H_{38}O_2$	346
32.	24.710	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	2.24	$C_{21}H_{32}O_2$	316
33.	25.426	2-Methyltetracosane	0.83	$C_{25}H_{52}$	352
34.	26.726	Allyl n-octyl ether	0.69	$C_{11}H_{22}O$	170
35.	27.399	n-Pentadecanol	1.43	$C_{15}H_{32}O$	228
36.	27.487	2-methyloctacosane	0.72	$C_{29}H_{60}$	408
37.	29.471	Heptadecane, 7-methyl-	1.00	$C_{18}H_{38}$	254
38.	30.152	Octadecanoic Acid, 10-Methyl-, Methyl Ester	2.72	$C_{20}H_{40}O_2$	312
39.	31.300	Cyclohexanone, 2-Isopropyl-2,5-Dimethyl-	0.46	$C_{11}H_{20}O$	168
40.	31.377	2-Methyltetracosane	0.53	$C_{25}H_{52}$	352
41.	33.763	1H-Pyrazole-5-carboxylic acid, 4-amino-, hydrazide	0.05	$C_4H_7N_5O$	141
42.	33.830	Docosanoic Acid, Methyl Ester	0.79	$C_{23}H_{46}O_2$	354
43.	34.910	3,3-Dimethyl-2-Azabicyclo [2.2.2] Oct-5-Ene-Hy	0.20	$C_9H_{16}ClN$	173
44.	34.993	2-Methyltetracosane	0.95	$C_{25}H_{52}$	352
45.	35.137	Squalene	1.45	$C_{30}H_{50}$	410
46.	35.534	5-Tert-Butyl-2-(Hydroxymethyl) Cyclohexa	0.81	$C_{11}H_{22}O_2$	186

Table 7: Bioactive compounds identified from the algal samples with different solvents and their applications

S. No.	Compounds	Structure	Application	References
1.	2-Methylhexacosane		role in sexual behavior as a part of a blend of contact pheromones in *Mallodonda systomus*	[18]
2.	Doconexent		Development of the sensory, perceptual, cognitive, and motor neural systems during the brain growth spurt.	[19]
2.	TETRACOSANE		most potent inhibitor of β amyloid aggregation weakly inhibited acetylcholinesterase (AChE)	[20]
3.	n-Tetracosanol-1		active antimutagenic; Anti-bacterial activity, nematicidal, anticancer, antioxidant, and antimicrobial activity	[21-23]
4.	Hexadecanoic acid, methyl ester		have antitumor, immunostimulant properties, antioxidant, anticholesteremic, and anti-inflammatory properties; antibacterial	[24-25]
5.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]-		Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1 with antimicrobial, anticancer, anti-inflammatory, anti-diuretic, immune-stimulatory, and anti-diabetic properties.	[26]
6.	HENEICOSANE		exhibited excellent antimicrobial activity against *Streptococcus pneumoniae* and *Aspergillus fumigatus*; Antineoplastic, oviposition-attractant pheromone (for trapping mosquitoes	[27-28]
7.	PENTADECANE		Antimicrobial activity	[29]
8.	OCTADECANE		antimicrobial effect especially on *Staphylococcus aureus* and *Escherichia coli*	[30]
9.	n-Hexadecanoic acid		prostaglandin-E2 9-reductase inhibitor	[28]
10.	2-Pentadecanone, 6, 10, 14-trimethyl- (phytone).		antibacterial, anti-nociceptive, and anti-inflammation activities	[31, 32]
11.	1,2-BENZENEDICARBOXYLIC ACID		Antibacterial, fungicidal, and algicidal properties	[33]
12.	n-Nonadecanol-1		antibacterial activity	[34]
13.	Propanoic acid, 3-mercapto-, dodecyl ester		Antibacterial (extract of *Moringa stenopetala*)	[35]
14.	1-Heptadecene		Show the toxicity to larvae of *Plutella xylostella*	[36]
15.	Hexadecane		Antibacterial activity	[37]
16.	1-Heneicosanol		Activity against *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) as well as against	[38]

			*Candida albicans* (NIM 982879) and *C. krusei*(ATCC 6258)	
17.	Borane, diethyl(decyloxy)-		antifungal, antibacterial, and anticancer activity	[39]
18.	NONADECANE		Good energy storage potential for building applications.	[40]
19.	Heptadecane, 8-methyl-		Effective in antimicrobial and antioxidant potential (Oils from *Daphne mucronata*)	[41]
20.	2-methyloctacosane		Antimicrobial activity	[42]
21.	Heptadecanal		Possessed anti-bacterial properties against *Staphylococcus aureus* and *Salmonella typhimurium*	[43]
22.	HEXADECANE, 2,6,10,14-TETRAMETHYL-		Study of the origin and genetic properties of entomogenous fungi	[44]
23.	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-		Natural antifungal compound	[45]
24.	1-Pentadecene		Could be used in the form of an attractive trap or a repellent dispenser for *Tribolium castaneum*, depending on its concentration.	[46]
25.	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester		has antifungal, and antioxidant activities	[47]
26.	9,12-Octadecadienoic acid, methyl ester		Hepatoprotective, antihistaminic, hypocholesterolemic, anti -eczemic	[48]
27.	1-TRIDECANOL		antibacterial activity against *S. aureus*	[34]
28.	Behenic alcohol		used as an emulsifier, emollient, and thickener in cosmetics also has antimicrobial properties	[49]
29.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-		anti-inflammatory activity	[50]
30.	9-Octadecene, (E)-		Antioxidant activity (Extract of stem bark *C. sebestena*)	[51]
31.	Squalene		LDP which protects skin and is adjunctive to cancer therapy; Antibacterial, Antioxidant, Antitumor, Anti-inflammatory, Antinociceptive, Potential antiplatelet components, Hypoglycemic, Hypolipidemic effects, Sedative action, Antihistaminic, Hepatoprotective activities, Immunostimulant	[28, 52]
32.	1-Hexadecanol		Show antioxidant activity	[53, 21]
33.	Tetradecanoic acid		Significant larvicidal and repellent activity against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes; Antioxidant, Nematicidal, Hypocholesterolemic, Anticancer	[54, 52]
34.	9-Octadecenamide		a natural sleep-inducing lipid	[55]
35.	Phytol, acetate		rheumatoid arthritis and especially for chronic inflammatory diseases (*D. villosa* extracts)	[56]

Result

Due to its potential health advantages, algae are expanding the supplementary medicine field all around the world. Algae have been utilized in traditional medicine for a long time, mostly in the treatment of various diseases. The purpose of this study is to determine the beneficial outcomes of the bioactive substances produced by the chosen green algae species.

Nearly 35 biologically indispensable bioactive compounds were found in 3 green algal samples with 3 different organic solvents by GC-MS analysis. Table 1-7 lists the biologically essential bioactive compounds along with their retention time, molecular weight, formula, and concentration (%) as well as the applications for which they are used. The dominating chemical compounds were Hexadecanoic acid, methyl ester (14.96%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z,Z)- (15.39%), Tetradecanoic acid (5.30%), phenol, 2,4-bis(1,1-dimethylethyl)- (7.81%), Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (4.23%), Phytol (4.84%), 1-Heptadecene (3.95%), PENTADECANE (3.73%), and n-Hexadecanoic acid (26.15%). The biological advantages of presented bioactive compounds were observed in the methanol chloroform, n-hexane & MTBE (tert- Butyl Methyl Ether) extracts of the *Rhizoclonium* species, *Hydrodictyon reticulatum*, and *Spirogyra* species.

Discussion

The most prevalent bioactive substances found in each of the three algal samples in all three organic solvents are n-Hexadecanoic acid shows the highest area % 26.15 in chloroform and methanol extract of *Hydrodictyon* species and phenol, 2,4-BIS(1,1-dimethylethyl)- shows highest area % 7.81 in chloroform and methanol extract of *Spirogyra* species pentadecane shows highest area % 3.73 in chloroform and methanol extract of *Spirogyra* species and tetradecanoic acid shows area % 5.30 in chloroform and methanol extract of *Rhizoclonium* species. The outcome of this experiment discloses the potential advantages of using green algae, which have a variety of therapeutic features and are strongly suggested as a biological solution with significant pharmaceutical value.

Hexadecanoic acid, methyl ester

Hexadecanoic acid, methyl ester (methyl palmitate) belongs to a class of organic compounds i.e. fatty acid methyl esters identified in chloroform and methanol extract of *Rhizoclonium* species (1.37%) *Hydrodictyon reticulatum* (1.24%) & *Spirogyra* species (7.29%), Hexane extract of *Hydrodictyon reticulatum* (9.89%), MTBE extract of *Rhizoclonium* species (7.98%) & *Spirogyra* species (14.96%) showed strong larvicidal activity, insecticidal activity, and pesticide activity, respectively, against cotton leafworm (*Spodoptera littoralis*) and cotton aphid (*Aphis gossypii*). Besides algae, this compound is also found in ethyl acetate extracts of fungus *Beauveria bassiana* and *Trichoderma herzianum* [57].

N Hexadecanoic acid

N Hexadecanoic acid is present in the higher plant (*Santalum album)* as well as lower chlorophyllous thalli. This compound exhibits hemolytic, strong mosquito larvalicide, anti-androgenic taste, anti-inflammatory, antioxidant, hypercholesterolemia nematicide, and pesticide properties. [58-62].

Doconexent (DHA)

DHA is not found in higher plants and can only be obtained exogenously from marine sources including fish oils, krill oil, and algae [63]. During the brain's growth surge, DHA is one of the nutrients that is absolutely necessary for the development of the sensory, perceptual, cognitive, and motor neural systems. The cell membranes and axons of the neurons' dendritic extensions are continually developing. The greatest fluidizing component in cell membranes is DHA, which is necessary for a growing membrane to be reasonably fluid. Even synapses, which are the main functional components of brain circuits, are constructed from membranes that are excessively rich in DHA [19]. In this paper, results show that Chloroform and methanol extract and MTBE extract of *Rhizoclonium* species have area % of DHA (5.96%), (4.11%) respectively.

2, 4-Di-tert-butyl-phenol

This biologically significant molecule is also present in green algae, where it has a good percentage area of (3.55%), and it gives to the antioxidant capacity observed in the peel of *Nephelium lappaceum* [64].

Phytol

This is aromatic diterpene alcohol that has applications as an antimicrobial, immunostimulant, antioxidant, anti-allergic, anti-inflammatory, antinociceptive and anti-allergen identified in chloroform-methanol and MTBE extracts of *Rhizoclonium* sp. (1.84%) and chloroform-methanol extract of *Hydrodictyon reticulatum* (4.84%) instead of green filamentous algae this bioactive compound also detected in ethyl acetate extract from *Santolina chamaecyparissus* [65, 66].

Conclusion

The current study concluded that GC-MS analysis of green algal extracts confirms the existence of multiple bioactive substances responsible for a variety of pharmacological actions, which supports the significance of green algae in the field of medicine. The use of GC-MS to extract chemical compounds can provide pharmaceutical companies with a wide range of molecules from algae that they can use to create future medications. Hence, green algae may be used shortly to discover new medications. However, further research is required to identify novel biologically active substances and toxicity profiles.

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