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GC-MS Analysis of biofuel extracted from marine algae

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ABSTRACT

Depletion of fossil fuel leads the research to be focused on alternate source for biofuel production. Though various sources have been identified for the production of biofuel, their availability and cost of manufacturing was found to be high. Macroalgae are most promising feed stock to their wide spread availability and higher lipid content. Extraction of biofuel from algae was found to be the best method of biofuel production. The main aim of the project is to make use of the marine algae such as Gracilaria corticata collected from Covelong, Tamil Nadu and Ulva sp., from Kanyakumari, Tamil Nadu to extract the useful algal oil meant for biofuel production and study the GC-MS characteristics of biofuel extracted from it. Results showed that the presence of dodecamethyl (34.19% area) in Gracilaria corticata and 36.6% area decanoic acid in Ulva sp which confirmed the biodiesel.

Keywords*: Chaetomorpha antennina, Gracilaria corticata, Ulva sp.,* Extraction, biofuel

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INTRODUCTION

Climatic change and its association with emission of CO_2 has become a major concern in recent years [1]. Although numerous efforts have been made to bring this change under control, the problem still exists. According to the World Energy Outlook of the International Energy Agency, the energy demand is anticipated to increase until 2035 and this emission would rise by 20% [2]. In fact, fossil fuels will continue to be the primary source of energy generation in the short to medium term. However, in the same report this organization states that government policies can influence the pace of fossil fuel consumption. Governments have already taken solid decisions to decrease the risks associated with fossil fuel emissions. Searching of an alternate feed stock for fuel is a major research in this era. Currently, the dominant feedstock for biodiesel in Europe is rapeseed oil whereas *Glycine max* is predominantly used in the United States of America [3]. Problem with the production of greener fuels is that it leads to a raise of feedstock production and therefore leading to an increase in water consumption. There is great interest in developing algal lipids as biodiesel feedstocks among which seaweed plays a vital role.

Biofuel comprised of monoalkyl esters of long-chain fatty acids derived from vegetable oils or animal fats. It can be produced by transesterification process. Spent cooking oil plays a significant role in the production of biofuel. The other feed stocks of biodiesel include *Camelina sativa*, *Brassica napus*, *Cocos nucifera*, *Zea mays*, *Jatropha curcas* and *Helianthus annuus*. Now a day's seaweed has also been used as a feed stock for biodiesel.

India has a longer coast line when compared to many other countries. Therefore the availability of seaweed is more. Though more seaweeds are available in the Tamil Nadu coast line, only some species are being used in food and pharmaceutical industry. The other are collected in the beaches and they are treated as a waste. The usage of seaweeds in India is minimal when compared to other countries. Hence there is a vast potential to utilize this raw material for the production of biodiesel. In this study, *Gracilaria corticata* collected from Covelong, Tamil Nadu and *Ulva* sp., from Kanyakumari, Tamil Nadu to extract biofuel and its compounds were analyzed using Gas chromatography and Mass spectrometry.

MATERIALS AND METHODS

Collection of Sample:
The Marine algae sample such as *Gracilaria corticata* collected from Covelong and *Ulva sp.*, from Kanyakumari, Tamil Nadu and were identified at Madras Christian College in the department of plant biotechnology.

Preservation of sample:
The sample was washed thoroughly with distilled water to remove sand particles and epiphytes and dried under sun light. The dried samples were ground in mixer for making it as powder form. Then the powdered samples were stored.

Extraction of algal oil:
Pre weighed amount of powder algae was mixed with hexane in the ratio of 1:3 for extracting algal oil. The extraction was carried out in a soxhlet apparatus for 4hours according to UNE-EN. Then the oil was separated using separating funnel.

Transesterification Process:
Methanol and NaOH was mixed thoroughly to from sodium methoxide solution. This sodium methoxide solution was added in extracted oil. Then the mixture kept overnight. Then the bottom layer of glycerin was separated out from biofuel and then biofuel was washed with water for several time with vigorous shaking to remove the excess solvent and the traces of catalyst .After shaking the solution was kept for 16 hours to settle the biofuel and sediment layer clearly.

GC-MS Analysis
GC-MS study was carried out in Sargam laboratory, Chennai, Tamil Nadu to analyze the compounds present in biofuel.

RESULTS AND DISCUSSION

In this work, marine algae were used for extracting biofuel and its compounds were analyzed to confirm the presence biodiesel. According to Singh and Singh [4], common fatty acids found in biofuels are palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). The FAME profiles determine the chemical properties of biodiesel [5]. Standard specifications have been established for biodiesel by ASTM D6751 in U.S and EN 14214 by the European Standards Organisation (EU) [6].

The average chain length metric does not distinguish between saturated and unsaturated FAME groups [7].

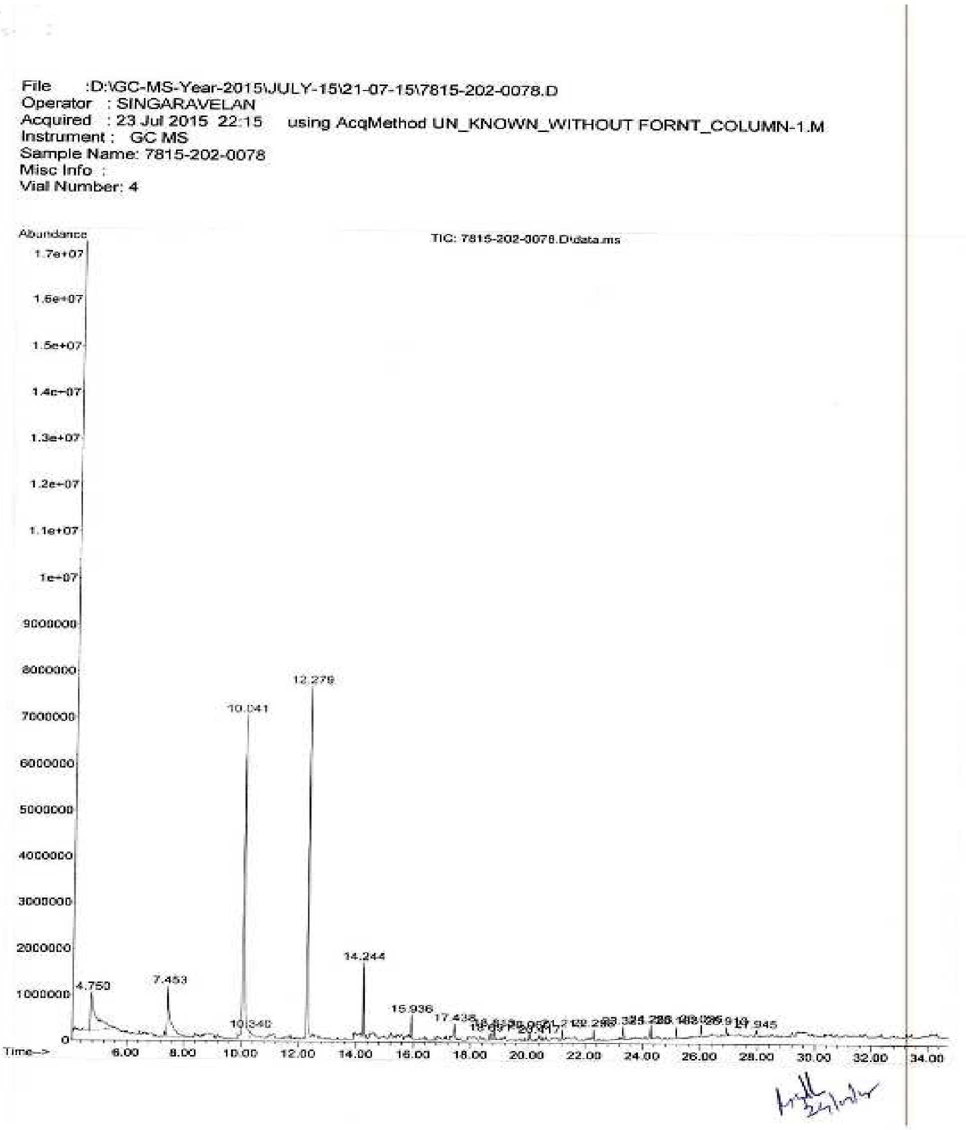

Fig.1 GC-MS analysis of *Gracilaria corticata*

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			Cyclopentasiloxane, decamethyl-	196318	000541-02-6	81
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			Cyclohexasiloxane, dodecamethyl-	225658	000540-97-6	62
			Cyclohexasiloxane, dodecamethyl-	225656	000540-97-6	46
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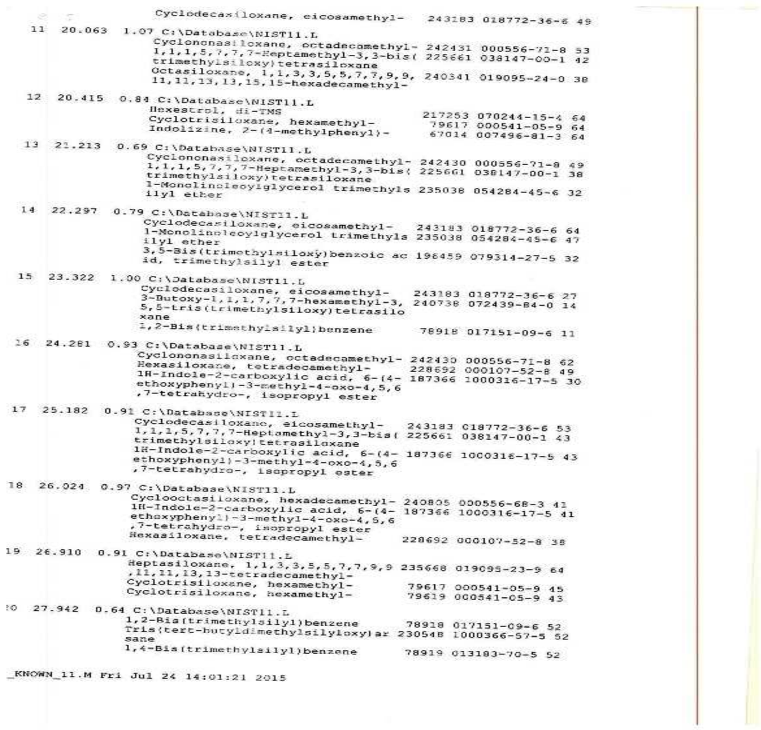
5	12.280	21.18	C:\Database\NIST11.L			
			Cycloheptasiloxane, tetradecamethyl-	236968	000107-50-6	60
			Cycloheptasiloxane, tetradecamethyl-	236969	000107-50-6	55
			Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	204095	003555-47-3	27
6	14.243	4.09	C:\Database\NIST11.L			
			Cyclooctasiloxane, hexadecamethyl-	240804	000556-68-3	90
			Cyclooctasiloxane, hexadecamethyl-	240805	000556-68-3	81
			Silane, [[4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)]bis[trimethyl-	228733	056114-62-6	45
7	15.934	1.01	C:\Database\NIST11.L			
			Cyclononasiloxane, octadecamethyl-	242430	000556-71-8	83
			Cyclononasiloxane, octadecamethyl-	242431	000556-71-8	78
			Cholestane, 3-thiocyanato-, (3.alpha.,5.alpha.)-	221997	020997-49-3	43
8	17.435	2.23	C:\Database\NIST11.L			
			Cyclodecasiloxane, eicosamethyl-	243183	018772-36-6	42
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	222021	000995-82-4	30
			Cycloheptasiloxane, tetradecamethyl-	236968	000107-50-6	27
9	18.694	0.60	C:\Database\NIST11.L			
			Dibenzo[b,N]-30-crown-10, 2,22-dibromo-	242793	317854-62-9	6
10	18.811	0.77	C:\Database\NIST11.L			
			Cyclooctasiloxane, hexadecamethyl-	240805	000556-68-3	83
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	225661	038147-00-1	56

Fig.2 GC-MS analysis of *Gracilaria corticata*



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                 1,1,1,5,7,7,7-Heptamethyl-3,3-bis( 225661 038147-00-1 42
                 trimethylsiloxy)tetrasiloxane
                 Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 240341 019095-24-0 38
                 11,11,13,13,15,15-hexadecamethyl-

 12  20.415  0.84 C:\Database\NIST11.L
                 Hexestrol, di-TMS                  217253 070244-15-4 64
                 Cyclotrisiloxane, hexamethyl-       79617 000541-05-9 64
                 Indolizine, 2-(4-methylphenyl)-     67014 007496-81-3 64

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                 1,1,1,5,7,7,7-Heptamethyl-3,3-bis( 225661 038147-00-1 38
                 trimethylsiloxy)tetrasiloxane
                 1-Monolinoleoylglycerol trimethyls 235038 054284-45-6 32
                 ilyl ether

 14  22.297  0.79 C:\Database\NIST11.L
                 Cyclodecasiloxane, eicosamethyl-   243183 018772-36-6 64
                 1-Monolinoleoylglycerol trimethyls 235038 054284-45-6 47
                 ilyl ether
                 3,5-Bis(trimethylsiloxy)benzoic ac 196459 079314-27-5 32
                 id, trimethylsilyl ester

 15  23.322  1.00 C:\Database\NIST11.L
                 Cyclodecasiloxane, eicosamethyl-   243183 018772-36-6 27
                 3-Butoxy-1,1,1,7,7,7-hexamethyl-3, 240738 072439-84-0 14
                 5,5-tris(trimethylsiloxy)tetrasilo
                 xane
                 1,2-Bis(trimethylsilyl)benzene      78918 017151-09-6 11

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                 Hexasiloxane, tetradecamethyl-     228692 000107-52-8 49
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                 ethoxyphenyl)-3-methyl-4-oxo-4,5,6
                 ,7-tetrahydro-, isopropyl ester

 17  25.182  0.91 C:\Database\NIST11.L
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                 1,1,1,5,7,7,7-Heptamethyl-3,3-bis( 225661 038147-00-1 43
                 trimethylsiloxy)tetrasiloxane
                 1H-Indole-2-carboxylic acid, 6-(4- 187366 1000316-17-5 43
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                 ,7-tetrahydro-, isopropyl ester

 18  26.024  0.97 C:\Database\NIST11.L
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                 ,7-tetrahydro-, isopropyl ester
                 Hexasiloxane, tetradecamethyl-     228692 000107-52-8 38

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                 ,11,11,13,13-tetradecamethyl-
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                 1,2-Bis(trimethylsilyl)benzene      78918 017151-09-6 52
                 Tris(tert-butyldimethylsilyloxy)ar 230548 1000366-57-5 52
                 sane
                 1,4-Bis(trimethylsilyl)benzene      78919 013183-70-5 52

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Table.1 Compounds present in Biofuel extracted from *Gracilaria corticata*

SL.NO	RT	AREA%	COMPOUND
1	4.753	15.73	Cyclotetrasiloxane ,octamethyl-benzoic acid,3methyl-2-trimethyl silyloxy,trimethylsily Ester,cyclotetrasiloxane.
2	7.455	10.80	Cyclopentasiloxane,decamethyl
3	10.040	34.19	Cyclohexasiloxane,dodecamethyl
4	10.340	0.91	cyclohexasiloxane dodecamethyl
5	12.280	21.18	Cycloheptasiloxane,tetradecamethyl,trisiloxane 1,1,1,5,5,5,-hexamethy 1-3,3-bis (trimethylsily)oxy
6	14.243	4.09	Cyclopentasiloxane,hexadecamethyl-silane,([4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)]bis[trimethyl-
7	15.934	1.01	Cyclotetrasiloxane ,octamethyl.
8	17.435	2.23	Cyclotetrasiloxane,eicosamethyl-hexasiloxane1,1,3,3,5,5,7,7,9,9,11,11,-dodecamethyl-cycloheptasiloxane,tetradecamethy
9	18.694	0.60	Dibenzo[b,N]
10	18.811	0.77	Cyclooctasiloxane,hexadecamethyl-1,1,1,5,7,7,7-heptamethyl-3,3-bis trimethylsiloxy)tetrasiloxane
11	20.063	1.07	Cyclonasiloxane,octadecamethyl-1,1,1,5,7,7,7-heptamethyl-3,3-bis trimethylsiloxy)tetrasiloxane octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl
12	20.415	0.84	Hexestrol,di-tms cyclotrisiloxane,hexamethyl-indolizini,2-(4-methylphenyl)
13	21.213	0.69	Cyclonasiloxane,octadecamethyl-1,1,1,5,7,7,7-heptamethyl-3,3-bis trimethylsiloxy)tetrasiloxane 1-monolinoleoylglycerol trimethylsilyl ether 3,5-Bis(trimethylsiloxy)benzoicacid,trimethylsilyl ether
14	22.297	0.79	Cyclonasiloxane ,eicosametyl-1-monolinoleoylglycerol trimethylsilyl ether 3,5 Bis(trimethylsiloxy)benzoicacid,trimethylsilyl ether
15	23.322	1.00	Cyclonasiloxane ,eicosametyl -3-butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane 1,2-bis (trimethylsilyl)benzene
16	24.281	0.93	Cyclotetrasiloxan ,octamethyl-Hexasiloxane,tetradecamethyl-1H-Indole -2-carboxylic acid ,6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-,isopropyl ester
17	25.182	0.91	Cyclonasiloxane ,eicosametyl-1,1,1,5,7,7,7- Heptamethyl-3,3- bis trimethylsiloxy)tetrasiloxane1H-Indole -2-carboxylic acid ,6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-,isopropyl ester
18	26.024	0.97	Cycloocpasiloxane,hexadecamethyl-1H-Indole -2-carboxylic acid ,6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-,isopropyl ester Hexasiloxane,tetradecamethyl
19	26.910	0.91	Heptasiloxane 1, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-cyclotrisiloxane,hexamethyl Cycloocpasiloxane,hexadecamethyl- Cycloocpasiloxane,hexadecamethyl

A mixture of FAMEs can balance the characteristics of a biofuel. In *Garacilaria corticta*, 34.19% area was achieved by Cyclohexasiloxane and dodecamethyl ester (ester of laauric) (Fig.1, 2 &3) which indicated that the maximum amount of Cyclohexasiloxane and dodecamethyl ester was found in the extract. Next to that maximum amount of 21.18% Cycloheptasiloxane, tetradecamethyl (ester of myristic), trisiloxane 1,1,1,5,5,5,-hexamethy 1-3,3-bis (trimethylsily)oxy were found. Since the area under the peak is proportional to the amount of the substance, dodecamethyl and tetra decamethyl (Table.1) were found to be present in maximum amount in the extracted biofuel. These compounds are the main constituents of biodiesel [8].

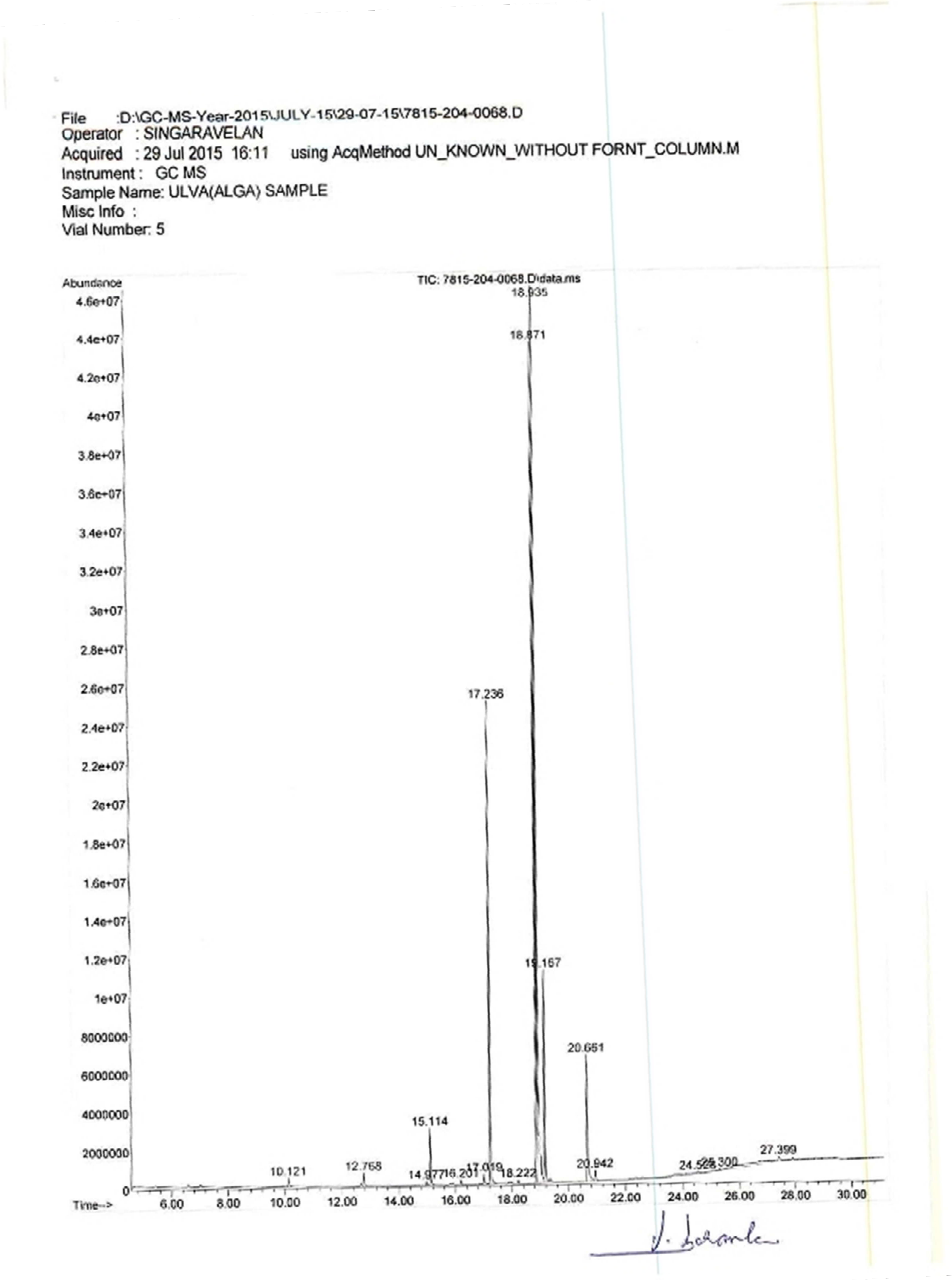

Fig.4 GC-MS analysis of *Ulva sp.*

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			Decanoic acid, methyl ester	50100	000110-42-9	95
			Tridecanoic acid, methyl ester	84483	001731-88-0	87
2	12.769	0.54	C:\Database\NIST11.L			
			Dodecanoic acid, methyl ester	72688	000111-82-0	98
			Undecanoic acid, 10-methyl-, methy l ester	72721	005129-56-6	94
			Dodecanoic acid, methyl ester	72681	000111-82-0	91
3	14.976	0.16	C:\Database\NIST11.L			
			Chloromethyl 7-chlorododecanoate	128766	080419-03-0	41
			Chloromethyl 8-chlorododecanoate	128767	080419-04-1	38
			13-Octadecenal, (Z)-	115867	058594-45-9	35
4	15.117	1.95	C:\Database\NIST11.L			
			Methyl tetradecanoate	95859	000124-10-7	99
			Methyl tetradecanoate	95861	000124-10-7	96
			Methyl tetradecanoate	95862	000124-10-7	96
5	16.201	0.21	C:\Database\NIST11.L			
			Methyl 13-methyltetradecanoate	107583	1000336-31-4	97
			Pentadecanoic acid, methyl ester	107596	007132-64-1	97
			Pentadecanoic acid, methyl ester	107593	007132-64-1	97
6	17.018	0.58	C:\Database\NIST11.L			
			9-Hexadecenoic acid, methyl ester, (Z)-	117513	001120-25-8	99
			9-Hexadecenoic acid, methyl ester, (Z)-	117507	001120-25-8	99
			Methyl hexadec-9-enoate	117464	010030-74-7	99
7	17.234	14.73	C:\Database\NIST11.L			
			Hexadecanoic acid, methyl ester	119400	000112-39-0	99
			Hexadecanoic acid, methyl ester	119407	000112-39-0	98
			Hexadecanoic acid, methyl ester	119408	000112-39-0	98
8	18.222	0.17	C:\Database\NIST11.L			
			Hexadecanoic acid, 14-methyl-, met hyl ester	131316	002490-49-5	95
			Heptadecanoic acid, methyl ester	131300	001731-92-6	94
			Heptadecanoic acid, methyl ester	131297	001731-92-6	93
9	18.868	26.52	C:\Database\NIST11.L			
			9,12-Octadecadienoic acid, methyl ester	139708	002462-85-3	99

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Fig.5 GC-MS analysis of *Ulva sp.*

BVCPS-INDIA PVT.LTD,CHENNAI Library Search Report

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ALS Vial : 5 Sample Multiplier: 1

Search Libraries: C:\Database\NIST11.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - 7815-204-0068.E

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			10,13-Octadecadienoic acid, methyl ester	139716	056554-62-2	99
10	18.935	36.60	C:\Database\NIST11.L			
			cis-13-Octadecenoic acid, methyl e ster	141299	1000333-58-3	99
			9-Octadecenoic acid (Z)-, methyl e ster	141302	000112-62-9	99
			11-Octadecenoic acid, methyl ester	141291	052380-33-3	99
11	19.165	6.91	C:\Database\NIST11.L			
			Methyl stearate	143126	000112-61-8	99
			Methyl stearate	143127	000112-61-8	99
			Methyl stearate	143130	000112-61-8	98
12	20.659	5.68	C:\Database\NIST11.L			
			9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	154825	000141-24-2	93
			Methyl 12-hydroxy-9-octadecenoate	154792	1000336-28-8	90
			9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	154823	000141-24-2	74
13	20.941	0.51	C:\Database\NIST11.L			
			Methyl 18-methylnonadecanoate	166215	1000352-20-6	97
			Eicosanoic acid, methyl ester	166219	001120-28-1	97
			Eicosanoic acid, methyl ester	166218	001120-28-1	96
14	24.529	0.21	C:\Database\NIST11.L			
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			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9 ,11,11,13,13-tetradecamethyl-	235668	019095-23-9	68
			2,4-Cyclohexadien-1-one, 3,5-bis(1 ,1-dimethylethyl)-4-hydroxy-	79250	054965-43-4	46
15	25.301	0.25	C:\Database\NIST11.L			
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	240341	019095-24-0	90
			Indole-2-one, 2,3-dihydro-N-hydrox y-4-methoxy-3,3-dimethyl-	66750	1000129-52-1	38
			Benzo[h]quinoline, 2,4-dimethyl-	67018	000605-67-4	38
16	27.396	4.62	C:\Database\NIST11.L			
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9 ,11,11,13,13-tetradecamethyl-	235668	019095-23-9	62

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Fig.6 GC-MS analysis of *Ulva sp.*

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Operator : SINGARAVELAN
Sample : ULVA(ALGA) SAMPLE
Misc :
ALS Vial : 5 Sample Multiplier: 1

Search Libraries: C:\Database\NIST11.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - 7815-204-0068.E

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			1,3-Benzodioxole, 5-(2-nitro-1-pro penyl)-	66648	005438-41-5	53
			Silane, trimethyl[5-methyl-2-(1-me thylethyl)phenoxy]-	79147	055012-80-1	45

N_KNOWN_11.M Wed Jul 29 16:52:36 2015 Page: 3

Fig.7 GC-MS analysis of *Ulva sp.*

Table.2 Compounds present in Biofuel extracted from *Ulva sp.*

SL.NO	RT	AREA%	COMPOUND
1	10.125	0.36	Decanoic acid,methyl ester Decanoic acid,methyl ester, Tridecanoicacid,methyl ester
2	12.769	0.54	Decanoic acid,methyl ester undecanoic acid,10methyl -,methyl ester Decanoic acid,methyl ester
3	14.976	0.16	Chloromethyl 7-chlorododecanoate Chloromethyl 8- chlorododecanoate
4	15.117	1.95	Methyltetradeconoate Methyltetradeconoate Methyltetradeconoate
5	16.201	0.21	Methyl 13- Methyltetradeconoate Pentadecanoic acid , methyl ester Pentadecanoic acid , methyl ester
6	17.018	0.58	9-Hexadecanoic acid, methyl ester(z)- 9-Hexadecanoic acid ,methyl ester(z)- methyl hexadec-9-enoate
7	17.234	14.73	Hexadecanoic acid, methyl ester Hexadecanoic acid, methyl ester Hexadecanoic acid, methyl ester
8	18.222	0.17	Heptadecanoic acid ,14-methyl ,methyl ester Heptadecanoic acid, methyl ester Heptadecanoic acid, methyl ester
9	18.868	26.52	9,12-Octadecadienoic acid ,methyl ester Octadecadienoic acid(z,z)-methy ester 10-13- Octadecadienoic acid ,methyl ester
10	18.935	36.60	Cis-13 Octadecadienoic acid ,methyl ester 9- Octadecadienoic acid (z),methyl ester 11- Octadecadienoic acid ,methyl ester
11	19.165	6.91	Methyl stearate Methyl stearate Methyl stearate
12	20.659	5.68	9- Octadecadienoic acid,12-hydroxy-,methyl ester ,[R-(z)]-methyl 12- hydroxyl-9-octadecenoate9- Octadecadienoic acid,12-hydroxy-,methyl ester ,[R-(z)]-
13	20.941	0.51	Methyl 18-methyl nonadecanoate Eicosanoic acid,methyl ester Eicosanoic acid,methyl ester
14	24.529	0.21	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-2,4-Cyclohexadien-1-one,3,5-bis (1,1-dimethylethyl)-4-hydroxy-
15	25.301	0.25	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-Indole-2-one,2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-Benzo[h]quinoline,2,4-dimethyl-
16	27.396	4.62	Heptasiloxane1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethy 1,3-Benzodioxole,5-(2-nitro-1-propenyl)-Silane,trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-

In *Ulva sp.* 36.6% 9- Octadecadienoic acid (linolenic acid) was present (Fig.4,5,6 &7). Presence of eicosanoic acid (arachidic acid), Methyl stearate (6.91%), Hexadecanoic acid (14.73%) (Palmitic acid) also confirmed the biodiesel (Table.2).

CONCLUSION

Low temperature operability is an important characteristic of biofuel [5]; a poor cold flow temperature is an attribute of long-chain, saturated fatty acid esters dominating the biodiesel [5]. The longer the carbon chain the poorer the low temperature operability. Maintaining proper engine temperature is important for efficient engine operation. Since the presence of long chain saturated fatty acid such as plamitic acid, myristic acid, arachidic acid were considerably less, poor cold flow temperature could have been reduced.

Oxidative stability is another important property with respect to performance of biodiesel [9]. Oxidative stability is related to the degree of [7]. The higher the unsaturation of biodiesel, the lower the stability of the biodiesel [7]. Since the presence of unsaturated fatty acids such as, linolenic acid may reduce oxidative stability of biodiesel, this may be considered as major disadvantage and will take necessary steps to reduce this poor oxidative stability in future work.

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