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Research Article

Chemical Composition of *Spirulina* by Gas Chromatography Coupled with Mass Spectrophotometer (GC-MS)

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Abstract

The scope of this study was to investigate the analysis of compounds obtained while *Spirulina* was subjected to different solvent extraction. The extracts were then subjected to analysis using-by Gas chromatography coupled with mass spectrophotometer (GC-MS). The dried *Spirulina* material was purchased from industries and extracted using two polar solvents (ethanol and dichloromethane) and two non polar solvents (Hexane and Chloroform) by using soxhlet methodology. Each solvent extracts was analysed by GC-MS using DB-5 capillary column. During GC-MS analysis it was observed that mostly fatty acid compounds are present in extract. In ethanol extract totally sixteen compounds were indentified and heneicosane (45.85%), octadecane (27.04%), triacontane (10.01%), heptadecane (4.23%) and phytol as major components (2.12%). In dichloromethane extract, totally fifty one compounds were found and the most abundant components were Pentacosane (14.04%), hexacosane (12.36%), heptacosane (9.61%), heneicosane (6.88 %), heptadecane (6.66%), n-hexadecanoic acid (5.24 %) and nonacosane (4.51%). Totally twenty nine compounds were identified in hexane extract and heneicosane (24.41%), dotriacontane (14.72%), heptacosane (10.74%) and pentacosane (10.75 %) were major components. In chloroform extract, twenty five compounds were present and the major compounds were Octacosane (28.66%), heptadecane (12.28%), 1-(2-methylpropenyl) aziridine (11.28 %), n-hexadecanoic acid (3.45%).

1. INTRODUCTION

Blue green algae are phototrophic microorganism largely distributed in nature. Some of them have been used as human food for many years because of their high nutritional value. *Spirulina* is the best known genus and it was consumed by the Aztecs in Mexico Valley and the chaad lake population in Africa. At present, some countries are cultivating it on a large scale[1].

Spirulina has been found to be rich source of vitamins, minerals, essential fatty acids, and antioxidant pigments such as carotenoid[2]. *Spirulina* or its extracts shows many therapeutic properties such as ability to prevent the formation of cancer, stimulate the immunological system, reduce the blood cholesterol level, reduce the nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation[3, 4].

Spirulina has been studied because of its therapeutic properties[3] and the presence of antioxidant compound such as phenolics[5, 6]. The occurrence of phenolic compound in plant is well documented and these compounds play important role of antioxidant activity in biological system. However the antioxidant properties of algae and cyanobacteria are less well documented, although decreased cholesterol level has been reported in hypercholesterolemic patient fed *Spirulina* and the antioxidant activity of phycobiliproteins extracted from *Spirulina* has also been documented[8]. A large number of microalgae and cyanobacterial extracts and extracellular products have been found to have antibacterial or antifungal activity. The volatile compound and crude solvent extract of algae species has ability to inhibit the growth of bacteria[9, 10.]

The present study was to investigate the *Spirulina* was subjected to different solvent extraction and identify the individual components in their extract by gas chromatography coupled with mass spectrometer (GC-MS).

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2. MATERIALS AND METHODS

2.1 Sample Collection and Reagents

Dried *Spirulina* material was obtained from Sanet products limited, kodai Raod, Tamilnadu. Ethanol, Chloroform, Hexane and dichloromethane were purchased from Coimbatore Scientific Suppliers, Coimbatore, Tamil Nadu.

2.2 Preparation of various extracts of *Spirulina*

10 g of *Spirulina* was extracted separately with ethanol, dichloromethane, Hexane and chloroform by using a soxhlet apparatus for 24 hours. The resulting extracts of *Spirulina* from these solvents were kept at fridge for further use[15, 16].

2.3 GC-MS analysis

The following GC-MS conditioned maintained throughout the analysis

Column:- DB-5 Length 30m, Diameter 0.250mm, Film thickness-0.25µm or equivalent.

Column Flow rate – 1.0 ml/min, Detection – Mass /Abundance (AMU)

Temp. Programme:-70°C - 2min - 25°C/min - 150°C - 3°C/min - 200°C - 8°C/min - 260°C - 1.133min

Injector Temp.-250°C

Inj. Mode:- Pulsed Split

Split ratio:- 1:2

Septum Purge flow:- 3ml/min. Gas saver Mode:-20ml/min After 2min.

Aux. Temp.:- 260°C

MS Conditions

MS Source Temp.:- 230°C, MS Quad Temp:-150°C, Solvent delay:-12min. EMV mode:-Gain factor,

Gain factor: - 5.00 = 1224V, Acq Mode:- SCAN, LIBRARY - NIST

3. RESULTS AND DISCUSSION

The present study reveals that the use of organic solvents in the preparation of *Spirulina* extraction and identified more compounds by GC-MS. During GC-MS analysis of solvent extract of *Spirulina*, it was observed that ethanol extract showed sixteen compounds (Figure 1) and chloroform extracts showed twenty five compounds (Figure 2). Twenty nine compounds were identified in hexane (Figure 3) extract and dichloromethane extract showed fifty one compounds (Figure 4). Some of the compounds were present in all the extracts. Heneicosane (45.85%), Octadecene (27.04 %), Triacontane (10.01 %), Heptadecane (4.23 %) and phytol (2.12 %) were present as major components in ethanol extract (Table-1). Heneicosane (24.41 %), dotriacontane (14.72%), Heptacosane (10.74%) and Pentacosane (10.75 %) were identified as major compounds in hexane extract (Table 2). Octacosane (28.66%), Heptadecane (12.28 %), 1-(2-methylpropenyl) aziridine (11.28 %), n-Hexadecanoic acid (3.45 %) constituted in majority in chloroform extract (Table-3). In dichloromethane extract, Pentacosane (14.04%), Hexacosane (12.36 %), Heptacosane (9.61%), Heneicosane (6.88 %), Heptadecane (6.66 %), n-hexadecanoic acid (5.24 %) and Nonacosane (4.51 %) were identified as major compounds (Table -4).

Previous reported stated that the compounds such as Octadecane and Heptadecane were found in both algae and plant species shows potent antioxidant, anticancer and antimicrobial activity (Lee et al., 2007; Mishra and shree 2007). In present study, Octadecane and heptadecane were identified in ethanol, hexane, chloroform and dichloromethane extract of *Spirulina*. The methanol and acetone extract of *Spirulina platensis* and identified the hexadecane, heptadecane, Eicosane, octadecane, phytol and pentadecane by GC-MS and these compounds shows antibacterial activity against *Staphylococcus aureus* and *Salmonella typhimurium* (Vinaykumar et al., 2011). The ethyl acetate extract of *anabaena variabilis, oscillatoriaanustissima* and *anabaena flosaquae* shows potent antimicrobial activity against gram positive, gram negative, yeast and fungi and GCMS analysis of ethyl extract reveals that heptadecane, octadecane, hexadeconic acid, docosane (Hanan M et al., 2010). In present study, these similar compounds were identified. Costantino et al., (1993) reported that Phenol, 2,5-bis(1,1-dimethylethyl) has good anti-inflammatory activity in rats. In present study, Curlone was present in dichloromethane extract of *Spirulina* and Curlone is a sesquiterpenoid of *Curcuma longa rhizomes* reported by YoshinobuKiso et al., 1983. The hexane extract of *Turkish, Achillea, satureja* which contains mainly hexacosane and heneicosane and extract has exhibit the potent antibacterial activity over a board spectrum against 25 phytopathogenic bacterial strains (Kotan et al., 2010). The chemical composition of *Ceratonia siliqua* which contains nonadecane, heneicosane, hepatadecane, hexadecanoic acid, octadecanoic acid and it shows antimicrobial activity against 13 bacteria and 8 fungal strains and it also has cytotoxic effect against two tumoral human cell lines HeLa and MCF-7 (Hsouna et al., 2011). Aedes aegypti mosquito is one of the most notorious vectors of dangerous diseases like dengue hemorrhagic fever and chikangunya. The pheromone n-heneicosane (C21) has been proved to be effective in attracting the female Aedes aegypti to lay eggs in the treated water and the growth of the larva is controlled by insect growth regulator diflubenzuron (Bhutia et al., 2010). The benzene extract of *Trichodesma amplexicaule Roth*, it contains terpenoids (beta-sitosterol, alpha-amyrin, lupeol, hexacosanoic acid, ceryl alcohol and hexacosane) and bioactivities against selected pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Aspergillus flavus and Penicillium chrysogenum*. The isolated compound hexacosane was more active against *E. coli* and hexacosanoic acid had greater activity against *A. flavus* (Singh et al 2003).

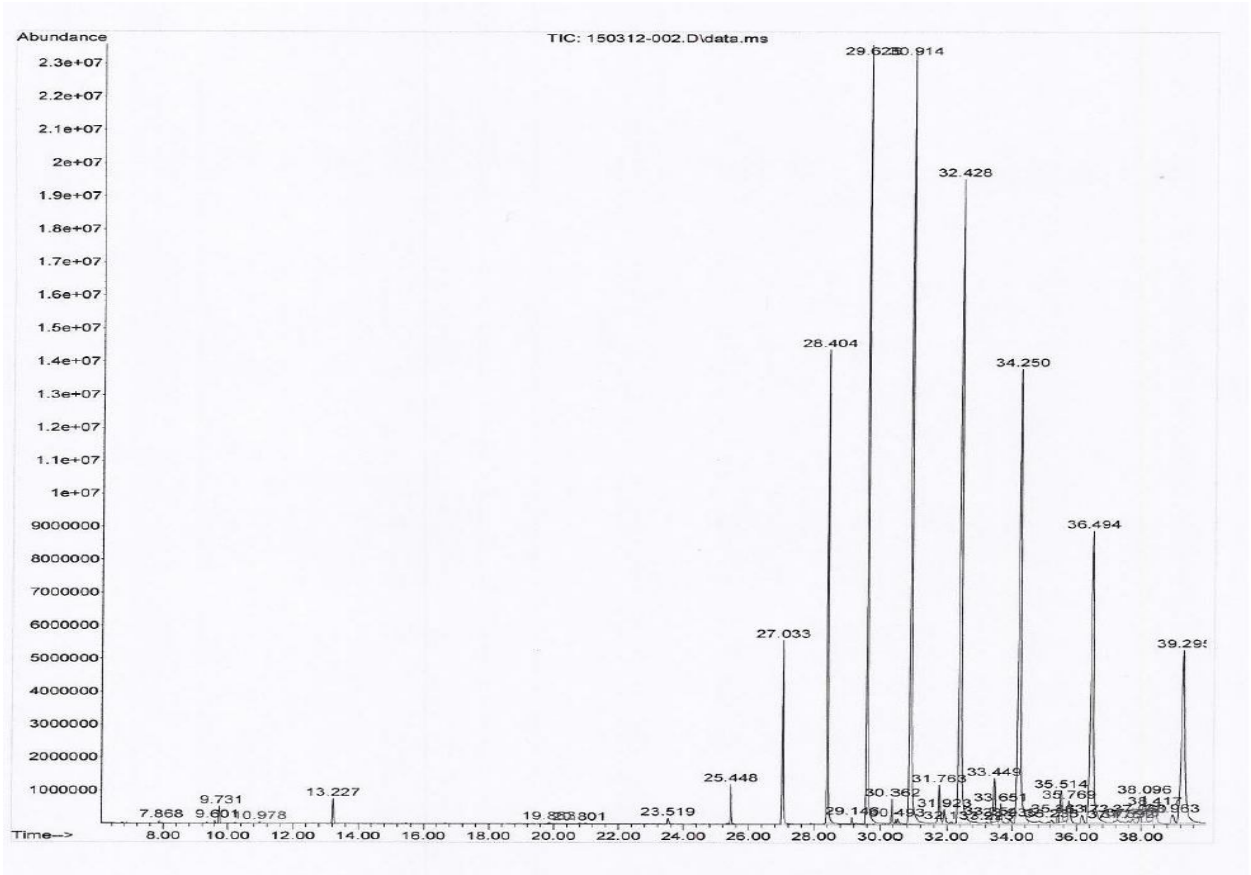

Figure 1: GC-MS chromatogram of ethanol extract of *Spirulina*.

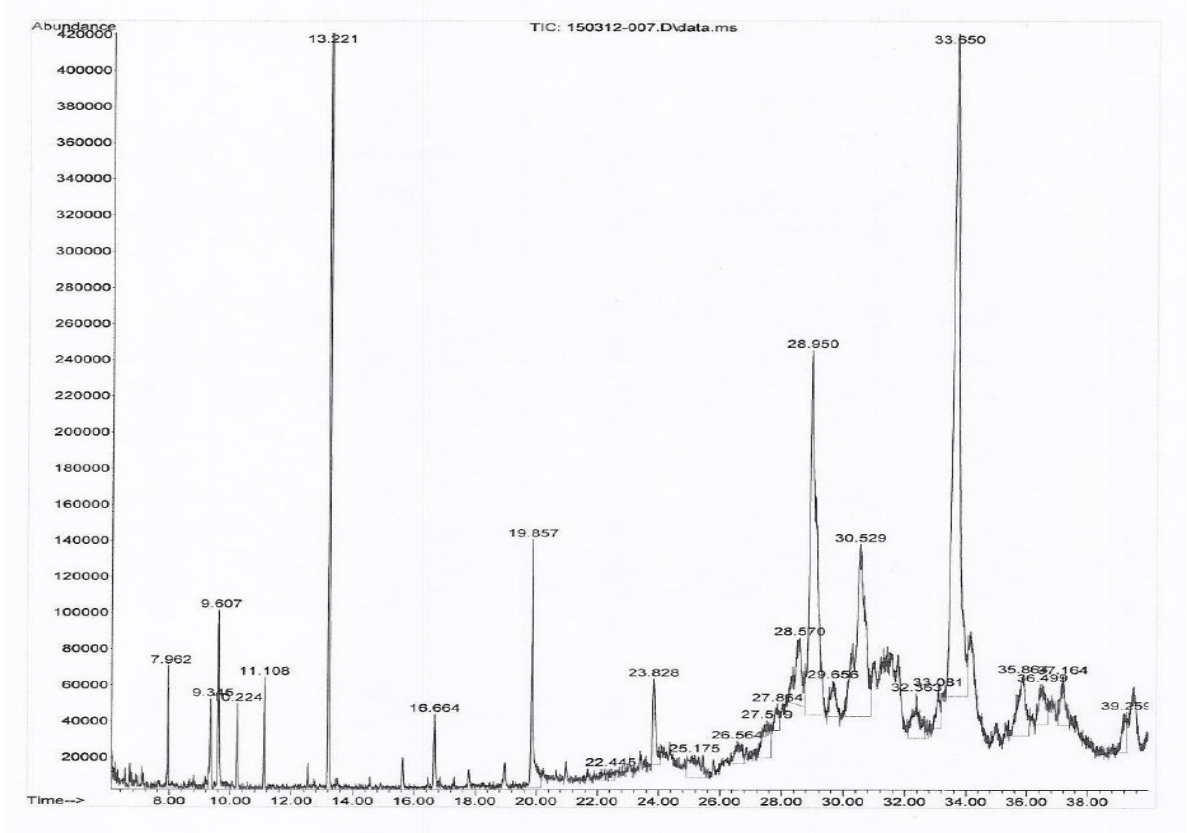

Figure 2: GC-MS chromatogram of chloroform extract of *Spirulina*

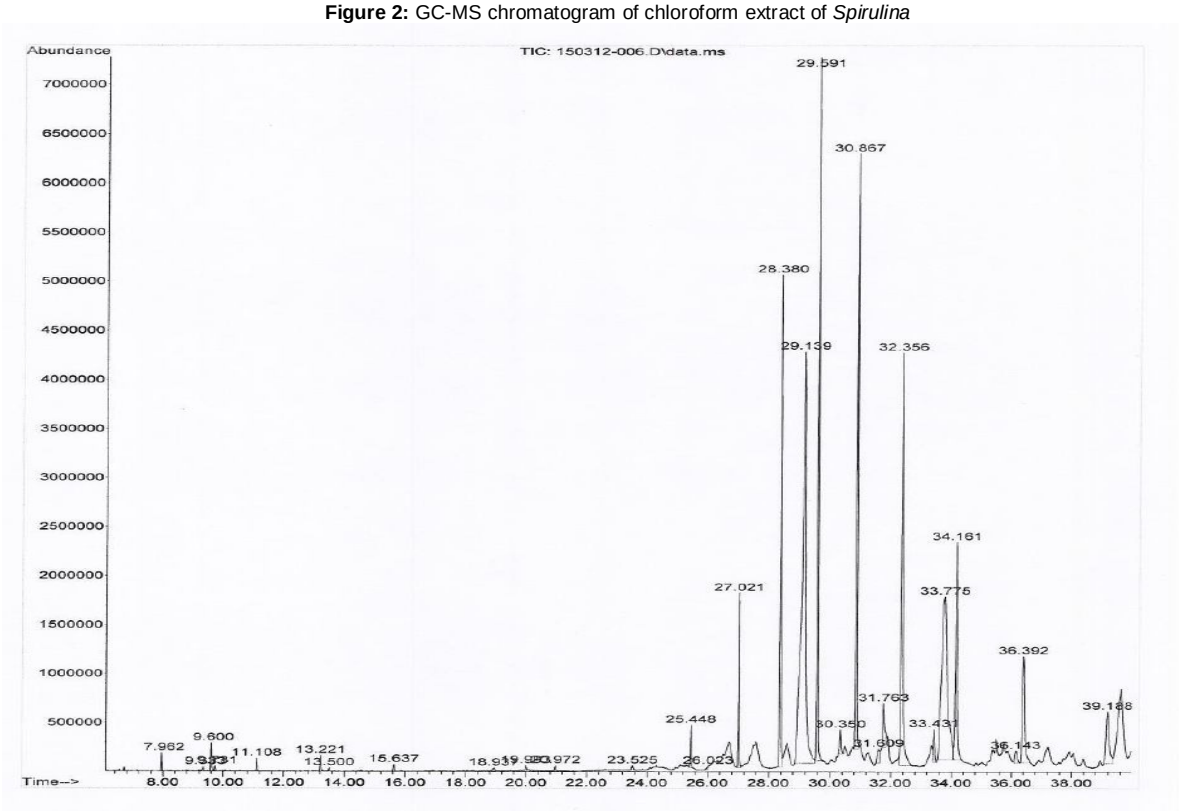

Figure 3: GC-MS chromatogram of hexane extract of *Spirulina*

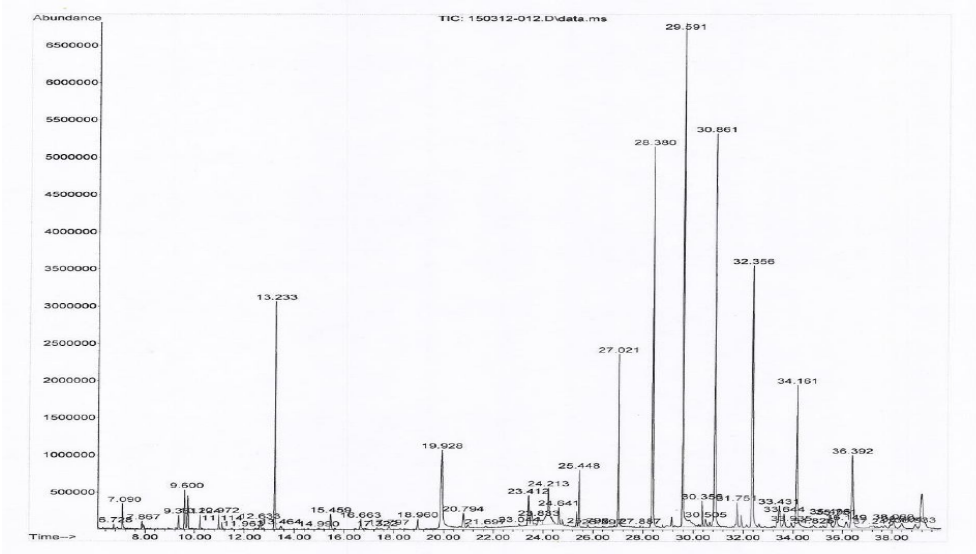

Figure 4: GC-MS chromatogram of dichloromethane extract of *Spirulina*

Table 1: GC-MS analysis of different compounds in ethanol extract of *Spirulina*

Peak	RT	Compounds	%
1	9.351	Pentadecane	0.18
2	10.22	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4 7a trimethy	0.19
3	11.1	Hexadecane	0.18
4	13.22	Hepatdecane	4.23
5	16.66	1,9-Nonanediol, methanesulfonate	0.16
6	19.88	n-hexadecanoic acid	2.5
7	23.86	Phytol	2.12
8	27.51	Docosane	1.07
9	28.57	3-Octadecane	2.43
10	29.11	Heneicosane	45.85
11	30.3	17-Pentatriacontane	0.49
12	31.26	Dodecane, 5 methyl	1.39
13	31.6	11,15- dimethylhepatatriacontane	0.83
14	33.33	Z-14-Nonacosane	1.31
15	33.74	Octadecane	27.04
16	39.61	Triacontane	10.01

Table 2: GC-MS analysis of different compounds in hexane extract of *Spirulina*

Peak	RT	Compounds	%
1	7.96	Tetradecane	0.19
2	9.33	Pentadecane	0.11
3	9.6	Phenol, 2,5-bis(1,1-dimethyethyl)	1.01
4	9.73	Butylatedhydroxytoluene	0.07
5	11.1	Hexadecane	0.19
6	13.21	Hepatadecane	0.31
7	13.5	Hepacosane	0.07
8	15.63	Octadecane	0.14
9	18.93	Dodecane, 2,6,11 trimethy	0.08
10	19.99	1,2-Benzenedicarboxylic acid, butyl 2- methyl propyl ester	0.11
11	20.97	Eicosane	0.11
12	23.53	Hepatcosane	0.09
13	25.44	Decosane	0.72
14	26.02	Octacosane	0.07
15	27.02	Tricosane	1.63
15	27.02	Tricosane	2.63
16	28.38	Tetracosane	7.22
17	29.13	Heneicosane	24.41
18	29.59	Pentacosane	10.75
19	30.35	Pentacosane	0.23
20	30.86	Hexacosane	10.63
21	31.6	Tetracosane, 1- bromo	0.38
22	31.76	Tetracosane, 9-octyl	1.13
23	32.35	Hepatacosane	10.74
24	33.43	Octacosane	0.82
25	33.77	Dotriacontane	14.72
26	34.16	Octacosane	6.3
27	36.143	Oxalic acid, isobutyl octadecyl ester	0.46
28	36.392	Heptacosane	4.03
29	39.188	Triacontane	1.49

Table 3: GC-MS analysis of different compounds in chloroform extract of *Spirulina*

Peak	RT	Compounds	%
1	7.96	Tetradecane	0.81
2	9.34	Decane 2,3,5 - trimethy	1.05
3	9.6	Phenol, 2,5-bis(1,1-dimethyethyl)	1.75
4	10.22	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4 7a trimethy	0.6
5	11.1	Hexadecane	0.79
6	13.22	Heptadecane	12.3
7	16.66	8-Azabicyclo[3.2.1] octane	0.9
8	19.85	n-Hexadecanoic acid	3.45
9	22.45	Pentafluropropionic acid, undecyl ester	0.54
10	23.82	Phytol	1.53
11	25.17	Dichloroacetic acid, tri decyl ester	1.75
12	26.56	Sulfurous acid, butyl tetra decyl ester	0.99
13	27.51	Octadecane, 1 - iodo	1.93
14	27.86	1-Hentetracontanol	0.63
15	28.57	Tetrapentacontane	3.1
16	28.95	Pentacosane	15.8
17	29.65	Nonahexacontanoic acid	1.25
18	30.52	1-(2-methylpropenyl) aziridine	11.28
19	32.36	Undecane	2.1
20	33.08	Hepataflurobutanoic acid, heptadecyl ester	0.94
21	33.65	Octacosane	28.66
22	35.86	1-Decanol, 2-hexyl	3.25
23	36.49	Tetrapantacontane, 1,54 -dibromo	1.86
24	37.16	Tetrapantacontane, 1,54 -dibromo	1.65
25	39.25	1-Hentetracontanol	1.09

Table 4: GC-MS analysis of different compound in dichloromethane extract of *Spirulina*

Peak	RT	Compounds	%
1	6.72	Cinnamaldehyde	0.2
2	7.09	2-Butenoic acid, 2-propenylidene ester	0.59
3	7.86	1-Tetradecane	0.33
4	9.35	Pentadecane	0.44
5	9.6	Phenol, 2,5-bis(1,1-dimethylethyl)	1.78
6	10.22	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4 7a trimethy	0.4
7	10.97	1-Hexadecane	0.38
8	11.11	Hexadecane	0.2
9	11.96	Iso-valeraldehydepropyleneglycol acetyl	0.12
10	12.63	Ar-tumerone	0.43
11	12.23	Heptadecane	6.66
12	13.46	Curlone	0.15
13	14.99	2-(2- xycyclohexyloxy)pyridine-n-oxide	0.1
14	15.46	1-Octadecane	0.57
15	16.66	Bicyclo[3.1.1]heptan, 2,6,6-trimethy-, (1.alpha., 2.beta.,5.alpha)	0.54
16	17.32	Dodeca-1,6-dien-12-ol, 6,10-dimethyl	0.14
17	17.8	7-Octadecyne, 2-methyl	0.11
18	18.96	Pentadecanoic acid, 14-methyl ester	0.47
19	19.93	n-hexadecanoic acid	5.24
20	20.79	Cycloeicosane	0.58
21	21.7	n-hexadecanoic acid	0.12
22	23.04	cis,cis,cis-7,10,13 - hexadecatriena	0.13
23	23.41	9,11-Octadecadienoic acid methyl ester	1.83
24	23.83	Phytol	0.69
25	24.21	1-pentadecyne	3.13
26	24.64	Acetic acid isopropylidene-hydrazine	0.51
26	24.64	Acetic acid isopropylidene-hydrazine	0.51
27	25.44	Docosane	2.14
28	25.79	1,19-Eicosadiene	0.1
29	26.39	3-methyhexyl isothiocyanate	0.11
30	27.02	Heptadecane, 3-methyl	4.83
31	27.88	Tetrapentacontane	0.08
32	28.38	Tetracosane	9.57
33	29.59	Pentacosane	14.09
34	30.35	Pentacosane	0.85
35	30.5	Tetracosane, 3-ethyl	0.36
36	30.86	Hexacosane	12.36
37	31.75	Tetracosane	1.5
38	32.35	Heptacosane	9.61
39	33.43	Octacosane	1.38
40	33.64	Hexacosane	0.74
41	33.93	1-Docosene	0.26
42	34.16	Heneicosane	6.88
43	34.82	Dichloroaceticacid, tridecyl ester	0.27
44	35.49	Hexacosane	1.21
45	35.75	Heptacosane	0.72
46	36.14	1-Hexacosane	0.34
47	36.39	Nonacosane	4.51
48	37.24	17-Pentatriacontane	0.49
49	38.06	Eicosane	0.7
50	38.4	Eicosane	0.63
51	38.93	Cyclopentane, 1,1,3-trimethyl	0.38

4. CONCLUSION

This study revealed a high level of chemical composition characteristic of fatty acids extracted from *Spirulina* and analysis by GC-MS. From GC MS data, identification of more compounds in their extract and it previously reported that these compounds has antibacterial, antifungal, antioxidant and anticancer activity but further researches should be made to isolation and purification of natural products in their extract.

5. ACKNOWLEDGEMENT

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