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GC-MS AND FT-IR ANALYSIS OF SOME BIOACTIVE CONSTITUENTS FROM *OSCILLATORIA TEREBRIFORMIS*

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ABSTRACT

The strain of cyanobacterial metabolites were extracted from effluent derived *Oscillatoria terebriformis* (Ag. VIAT010)and cultivated in the laboratory condition at VIAT (Vivekananda Institute of Algal Technology) through improvised CFTRI medium. Then the lipid were collected through Transesterification process and taken analysis for GC-MS and FTIR. In this result showed that the presence of 5 metabolites with retention time ranging from 16.704 to 22.081. The maximum peak was shown by 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (79.42%) followed by Phytol (11.37%), Methyl (7E)-7-hexadecenoate (3.14%) and 9, 12-Octadecadienoic acid (2.95%).

Keywords: *Oscillatoria terebriformis,* Biomass, Lipid, GC – MS, FTIR, Fatty Acids.

INTRODUCTION

Cyanobacteria are an ancient group of prokaryotic microorganisms and found in all ecological habitats. Cyanobacteria are gram-negative photoautotrophic prokaryotes having ´higher plant-type‘oxygenic photosynthesis [13,15].Certain cyanobacteria differentiate a small fraction of their cells into heterocysts, the site of aerobic nitrogen fixation. Cyanobacteria are one of the most promising groups of organisms for isolation of novel and biochemically active natural products [11,3]. Antimicrobial compounds found in cyanobacterial exudates include polyphenols, fatty acids, glycolipids, terpenoids, alkaloids, and a variety of yet to be described bacteriocins. Although this species of *Oscillatoria* is non-toxic, it did produce an unattractive surface 'scum'. It is a very 'efficient' species and outcompetes the beneficial algae which are an essential part of the fish's food chain. Lipids are the most effective source of storage energy, function as insulators of delicate internal organs and hormones and play an important role as the structural constituents of most of the cellular membranes. Bioenergy is the most important components to mitigate greenhouse gas emission and substitute of fossil fuels. The need of energy is increasing continuously because of increase in industrialization and population. Unfortunately, biodiesel from animal and plant sources cannot realistically satisfy even a small fraction of the existing demand of transport

fuels. Biodiesel are fatty acid methyl esters that are derived from triglycerides by the transesterification process [5]. The fatty acids of cyanobacteria are either saturated or unsaturated. They can also tolerate environmental stresses such as heat, cold, desiccation, salinity etc [1, 2, 12, 14]. The present work is a continuation of this study which describes the Fatty acid analysis of *Oscillatoria terebriformis,* isolated from the effluent. Lipid qualification and quantification can be carried out by several means including Fourier transform infrared micro-spectroscopy (FTIR) and Gas Chromatography (GC) with Mass Spectrometry (MS) [8].

MATERIALS AND METHODS

The strain of cyanobacterial metabolites were extracted from effluent derived *Oscillatoria terebriformis* (Ag. VIAT010)cultivated in improvised CFTRI medium and the composition is as follows [16]. The algal biomass were centrifuged and collected. Then it was dried in oven and powdered form using mortar and pestle. The algal powder was used for Lipid extraction in the method of Bligh and Dyer (1959) respectively. Then the lipid were

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collected through Transesterification process and taken analysis for GC-MS and FTIR. Lipid qualification and quantification can be carried out by several means including Fourier transform infrared micro-spectroscopy (FTIR) and Gas Chromatography (GC) with Mass Spectrometry (MS) [8]..

Gas chromatography-Mass spectroscopy Condition;

The total of *Oscillatoria terebriformis*, was analyzed by GC/MS. Preparation of extract: 1 µl of the methanolic extract of *Oscillatoria terebriformis* was employed for GC/MS analysis. Instruments and chromatographic conditions: GC-MS analysis was carried out on a GC QP 2010 [SHIMADZU] comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 240°C; ion-source temperature 200°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Identification of components: Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULT AND DISCUSSION:

The strain of cyanobacterial metabolites were extracted from effluent derived *Oscillatoria terebriformis* (Ag. VIAT010) cultivated in improvised CFTRI medium and it shows well growth and high biomass. The dried biomasses were used for lipid extraction and transestrification. After transesterification the lipid content was subjected to GC-MS analysis, as shown in Figure 1. It revealed the presence of 5 metabolites with retention time ranging from 16.704 to 22.081. The maximum peak was shown by 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (79.42%) followed by Phytol (11.37%), Methyl (7E)-7-hexadecenoate (3.14%) and 9, 12-Octadecadienoic acid (2.95%). On comparison of the mass spectra of the constituents with the NIST library, fifteen peaks were obtained out of which five phytoconstituents were characterized and identified (Table 1). The retention time (RT) is in minutes. The high peak areas were obtained in *Oscillatoria terebriformis* shows in FIG. A, B, C, D and E. That is Heptadecanoic Acid, Methyl Ester (Molecular formula: $C_{18}H_{36}O_2$), 9,12-Octadecadienoic acid (Z,Z) (Molecular formula: $C_{18}H_{32}O_2$). These are stereoisomers, Methyl (7E)-7-Hexadecenoate (Molecular formula: $C_{17}H_{32}O_2$), Phytol (Molecular formula: $C_{20}H_{40}O$), and 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (Molecular formula: $C_{16}H_{22}O_4$).The above table confirms the presence of the five compounds (Heptadecanoic Acid, Methyl Ester, 9, 12-Octadecadienoic Acid (Z, Z)-, Methyl (7E)-7-, Hexadecenoate, Phytol, and 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester) in *Oscillatoria terebriformis*. The fatty acids of cyanobacteria are either saturated or unsaturated. They can also tolerate environmental stresses such as heat, cold, desiccation, salinity etc [1, 2, 12, 14]. Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K [6,9]. Inruminants, the gut fermentation of ingested plant materials liberates phytol, a constituent of chlorophyll, which is then converted to phytanic acid and stored in fats. Phytol is likely the most abundant acyclic isoprenoid compound present in the biosphere and its degradation products have been used as biogeochemical tracers in aquatic environments. 9, 12-octadecadienoic acid its common name linoleic acid. Linoleic acid is essential fatty acids and is the most common fatty acid produced in agricultural seed oil production. It is present in all lipid classes. Particularly enriched in cholesterol esters, cardiolipin, phosphatidylcholine and free fatty acids. Methyl (7E)-7-hexadecenoate commonly known as palmitic acid. Concerning the biological activity of identified FA, dietary intake of palmitic acid increases risk of developing cardiovascular diseases [10]. However, another study showed that palmitic acid has no hypercholesterolaemic effect if intake of linoleic acid is greater than 4.5% of energy. On the other hand, it was shown that, if the diet contains transfatty acids, the health effects are negative, causing an LDL cholesterol increase and HDL cholesterol decrease (7). 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester its common name phthalic acid (mono (2-ethylhexyl) phthalate, phthalic acid mono(2-ethylhexyl) ester, 1-benzenecarboxylic acid mono-octyl ester (MOP), MEHP, phthalic acid mono-octyl ester). Plasticizer for PVC and other resins. Dielectric fluid. Permitted component in food packaging material. Flexible PVC industry – flooring, wire & cables, shoes, cars, pipe, tubes, profiles. Liquid used in vacuum pumps. Di (2-ethylhexyl) phthalate is widely used as a plasticizer in flexible vinyl products. Plastics may contain from 1 to 40% di(2-ethylhexyl) phthalate by weight and are used in consumer products such as imitation leather, rainwear, footwear, upholstery, flooring, wire and cable, tablecloths, shower curtains, food packaging materials and children's toys. Poly vinyl chloride (PVC) containing di(2-ethylhexyl) phthalate is also used for tubing and containers for blood products and transfusions.

Table 1. Area of percentage and retention time of fatty acids obtain from GC – MS

Peak#	R.Time	Area	Area%	Name of Compounds
1	16.704	77448	3.12	Heptadecanoic Acid, Methyl Ester
2	18.333	73068	2.95	9,12-Octadecadienoic Acid (Z,Z)-
3	18.39	78011	3.14	Methyl (7E)-7-Hexadecenoate
4	18.518	282033	11.37	Phytol
5	22.081	1970169	79.42	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
Total		2480729	100	

Table 2. Shows the FTIR Chart

S.No	Wave number (cm-1)	Molecular Motion	Functional Group
1	3582.84	O-H Free	Alcohols and Phenols
2	3471.93	N-H Stretch	Hydro cyclic Amine
3	3332.09	N-H Stretch	Aromatic Primary Amine
4	3286.76	N-H Stretch	Aromatic Primary Amine
5	3265.54	N-H Stretch	Aromatic Primary Amine
6	3227.93	N-H Stretch	Aromatic Primary Amine
7	2959.82	C-H (Asymmetric/ Symmetric Stretch)	Methylene
8	2927.03	C-H (Asymmetric/ Symmetric Stretch)	Methylene
9	2855.66	C-H (Asymmetric/ Symmetric Stretch)	Methylene
10	1657.85	N-H Bend	Secondary Amine
11	1641.45	N-H Bend	Secondary Amine
12	1612.52	N-H Bend	Secondary Amine
13	1379.13	C-H (Asymmetric/ Symmetric Bend)	Methyl
14	1267.25	C-N Stretch	Aromatic Primary Amine
15	1239.29	O-H stretch	Aromatic Ether
16	1128.38	C-H in Plane Bend	Aromatic
17	1032.90	C-H in Plane Bend	Aromatic
18	946.10	C-H_2 Medium	Alkanes
19	809.15	C-H out of Plane Bend	Aromatic
20	750.32	O-H Bend	Phenols

Fig.1. The GC shows the different fatty acid compounds

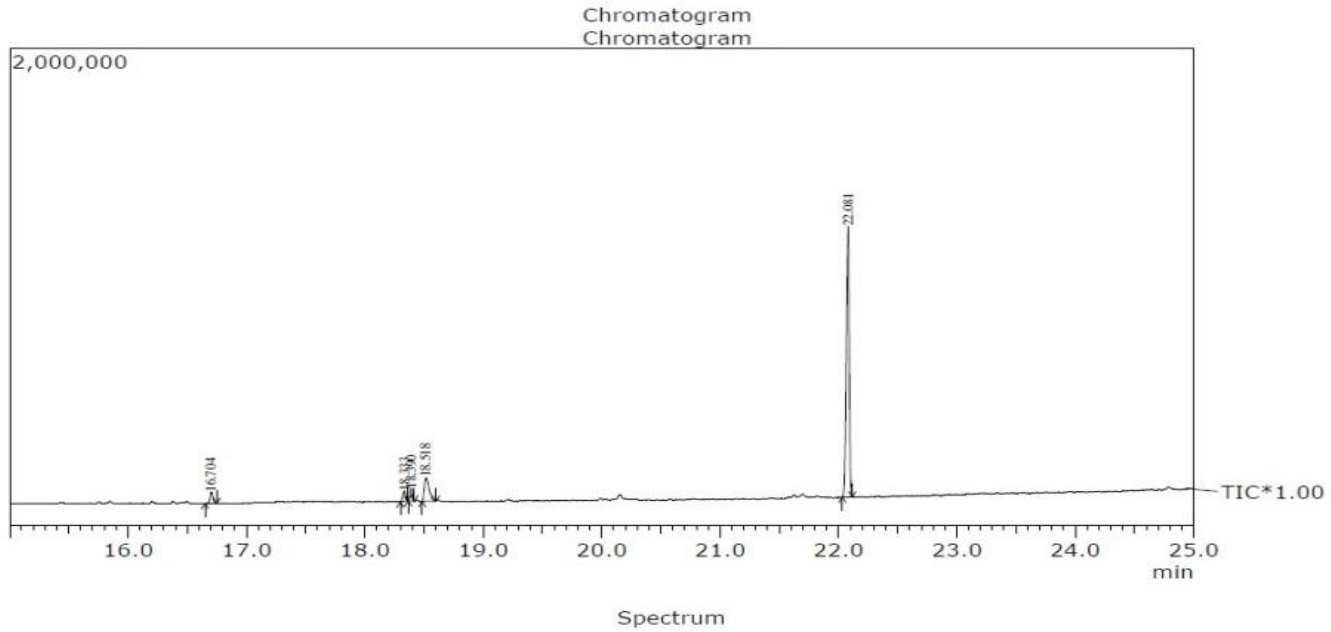
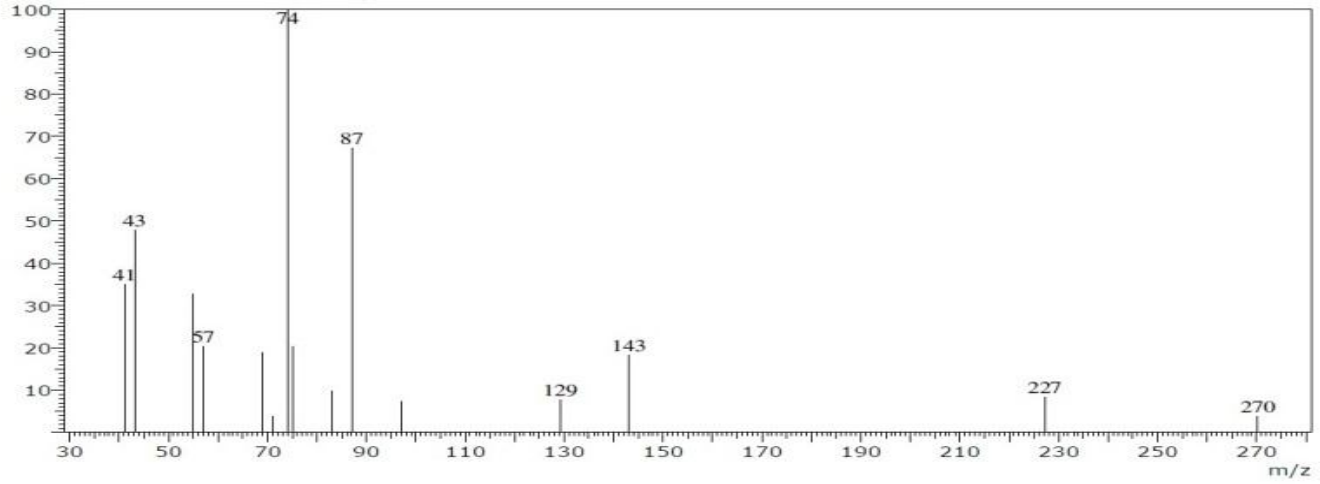
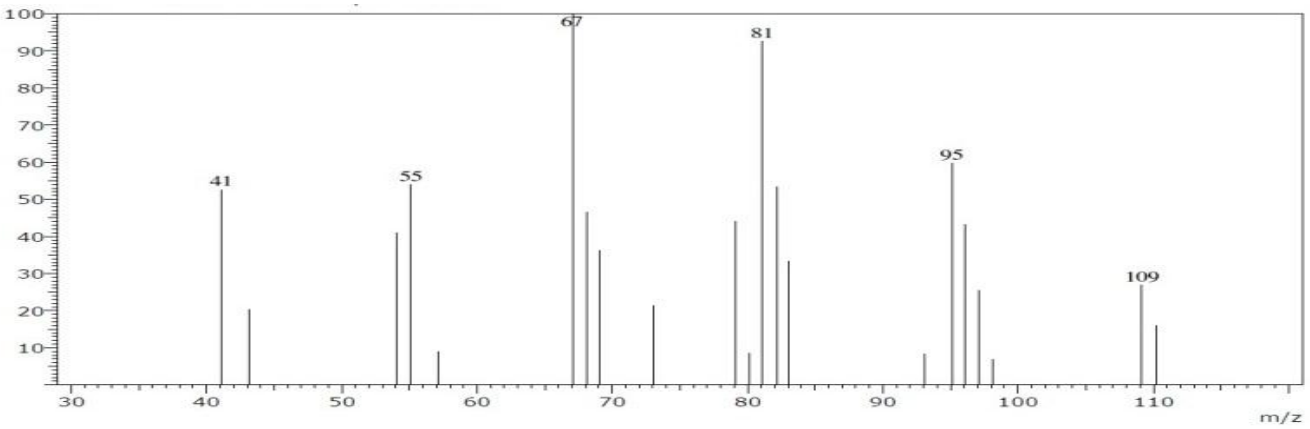


Fig. 2. MS Spectrum of Heptadecanoic Acid, Methyl Ester (A), 9,12-Octadecadienoic Acid (Z,Z)- (B), Methyl (7E)-7-Hexadecenoate (C), Phytol (D), 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (E)

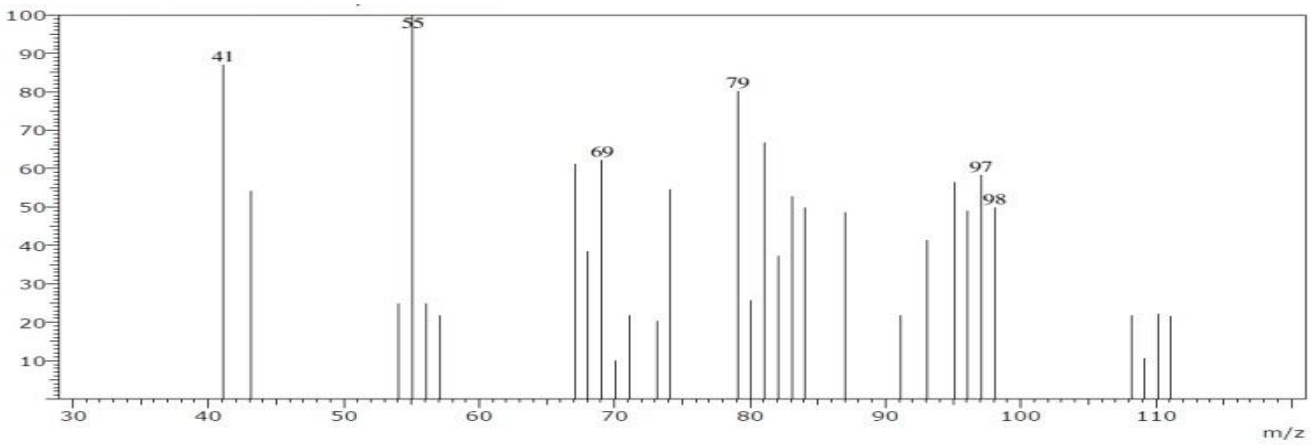
A



B



C



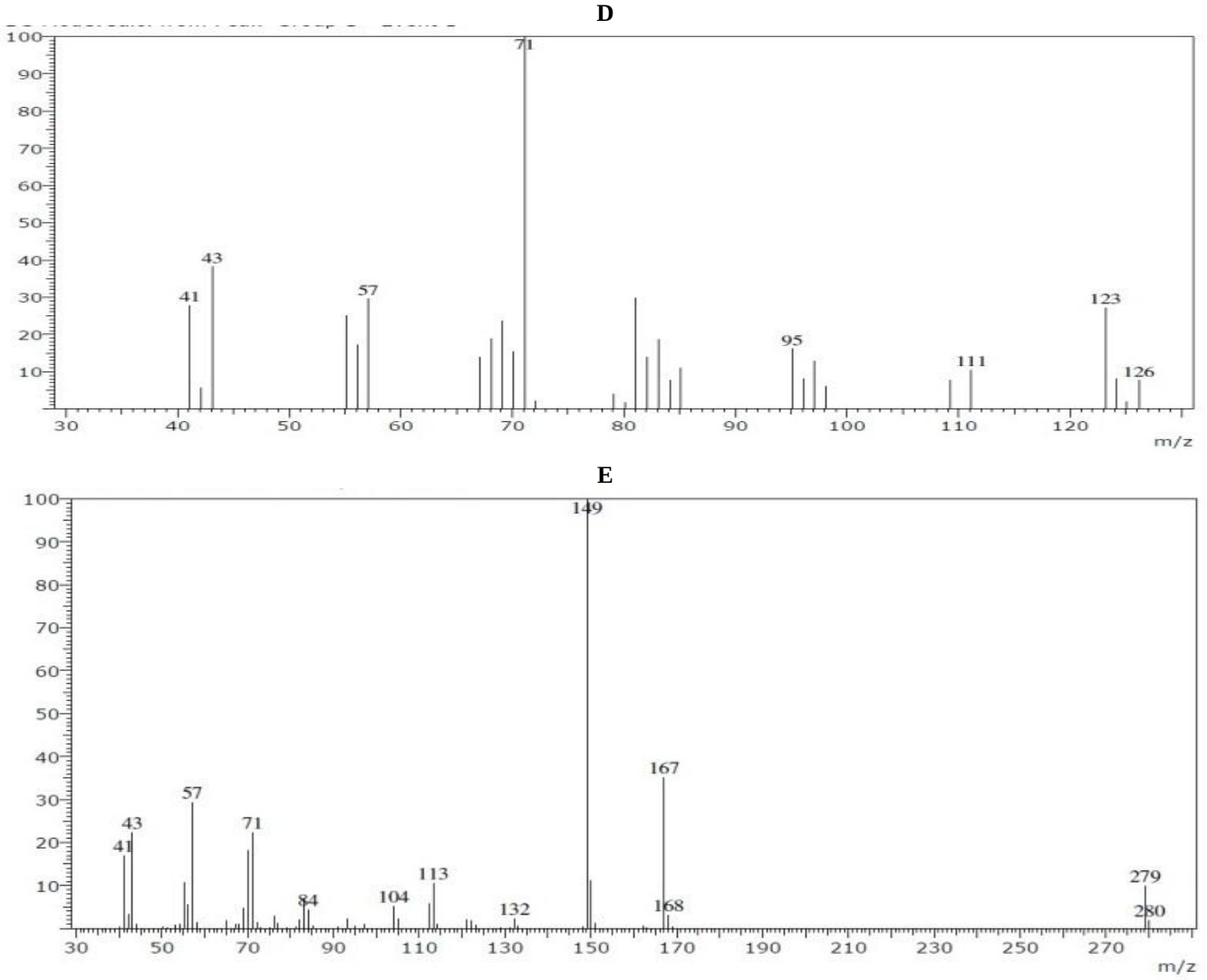
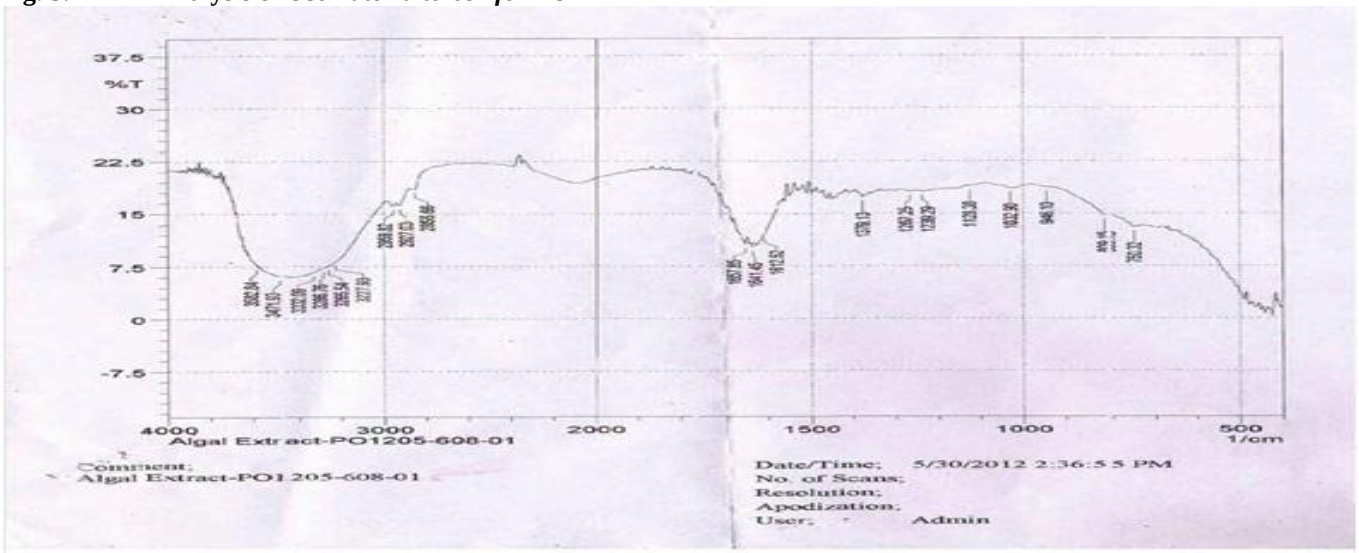


Fig. 3. FT-IR Analysis of *Oscillatoria terebriformis*



CONCLUSION

In this study the effluent derived *Oscillatoria terebriformis* confirmed that the presence of the five different types of fatty acids compounds and these fatty acid compounds are useful to pharmaceutical industry, Plastic industry and chemical industry. Also this cyanobacteria used as phytoremediation process in industrial effluent.

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