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Compounds Identification from Hypersaline *Oscillatoria Salina* Using GC-MS Analysis

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Abstract: *Marine Cyanobacteria were found to be a rich source of various active metabolites. Samples of saltpan cyanobacteria were collected from the saltpans of kanyakumari District. Oscillatoria salina was identified and isolated by serial dilution method. Biomass was produced by culturing the isolated strains in one litre of Walne's medium. After getting good biomass it was harvested, centrifuged, dried and used for GC-MS analysis to determine bioactive compounds. GC-MS analysis reveals the presence of fifteen bioactive compounds. The identification is performed by the index of NIST library.*

Keywords: *Oscillatoria salina, GC-MS, Phytochemical compounds, NIST*

1. INTRODUCTION

Marine cyanobacteria are rich sources of structurally new and biologically active metabolites with potential benefits against human disease[1,13]. Recent studies indicate the presence of some bioactive compounds from blue green algae which are shown to exhibit anticancer, antimicrobial, antifungal or anti-inflammatory and other pharmacological activities[16,8,9]. In order to isolate such active metabolites GC-MS is a technique to separates all of the components in a sample. GC-MS fingerprint has better resolution and estimation of active constituents is done with reasonable accuracy in a shorter time. GC-MS is one of the technique to identify the bioactive constituents of long chain branched chain hydrocarbons, alcohols, acids, esters, etc. Emergence concerns have been raised to establish structural and functional properties of the bioactive compounds described in algal crude extracts, up to date, over 2,400 bioactive metabolites have been isolated and identified from a diverse group of algal communities[5].The aim of the present work was to quantify the phytochemical constituents found in the methanol extracts of cyanobacteria *Oscillatoria salina.*

2. MATERIALS AND METHODS

2.1. Isolation, Culturing and Growth of Algal organisms

Samples of cyanobacteria were collected from the solar saltpans of Kanyakumari district. The sample was collected by using a mesh size of 2µ of plankton net which made of bolting silk cloth. Cyanobacteria *Oscillatoria salina* were identified by using Desikachary and Geitler, observed under microscope CX31and isolated by serial dilution method. Biomass was produced by culturing the isolated strains in one litre of Walne's medium under fluorescent lightand a facility to mix the culture with an aeration pump under laboratory condition. The algae were grown for 1 month and harvested.

2.2. Harvesting, Centrifugation and Drying

After a good biomass was developed, the algal cells were harvested. Algal cells were centrifuged at 3000 rpm for 20 minutes, removed the supernatant and collected the pellet. Wet biomass were kept in hot air oven overnight for drying. After it get dried it is transferred to a air tight bottles for further use.

3. GC-MS ANALYSIS

3.1. Preparation of Methanolic Extract (*O.salina*) for GC-MS Analysis

Oscillatoria salina was dried in hot air oven and 2 g of the powdered biomass was soaked in 95% methanol for 12 hr. Then the extract was filtered through what man No 41 filter paper along with 0.2 g of sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the

filter paper was moistened with 95% ethanol for 12 hr. The filtrate was then concentrated by bubbling nitrogen gas into the solution. An aliquot of 2 µl of this solution was employed for GC-MS analysis [10].

3.2. Gas Chromatography –Mass Spectroscopy Analysis

GC-MS analysis of the extract was performed using a Scion 436-GC Bruker system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a BR-5MS (5% Diphenyl / 95% Dimethyl poly siloxane) column, 30m x 0.25mm ID x 0.25µm df. For GCMS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/ min and an injection volume of 2 µl was employed (Split ratio of 50:1); injector temperature 280° C; Ion – source temperature 250°C. The oven temperature was programmed from 80° C (isothermal for 2 min.), with an increase of 20 ° C/min to 160° C, then 5° C / minto 280 ° C, ending with a 10 min isothermal at 300° C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds with scan range of 50-500 m/z and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area, to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass [14].

4. IDENTIFICATION OF COMPONENTS

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The 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 [4].

5. RESULTS

GC-MS Chromatogram in methanol extract of *Oscillatoria salina* along with their retention time (RT) are shown in the Fig 1. Major phyto components present in the methanol extract of *Oscillatoria salina* along with molecular formula, molecular weight and peak area were presented in Table 1& Fig 1a. &b. The GC-MS chromatogram of methanol extract of *Oscillatoria salina* showed the presence of several active principle compounds. Fifteen compounds were identified in methanolic extract of *Oscillatoria salina*. The prevailing compounds were Tetradecanoic acid (16.76%), n-Hexadecanoic acid(15.42%), Phytol-acetate(12.47), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (10.37), Pentadecanoic acid(6.8%), Hexadecanoic acid - ethyl ester (5.02), (E)-9-Octadecenoic acid ethyl ester(1.9%), Undecanoic acid(1.73%), Phytol (1.58%), Propane, 1,1,3-triethoxy-(0.92%), Erucic acid (0.87%), Tetradecanoic acid - ethyl ester (0.83%), Benzoic acid (0.78%), Hexadecane(0.77%) and 2-Mercaptopropanoic acid (0.39%).

Table1. *Activity of compounds identified in the GC-MS study of Oscillatoria salina extract*

No	RT	Name of the compound	Molecular Formulae	MW	Peak Area %	Compound nature	*Activity
1	4.3	2-Mercaptopropanoic acid	$C_3H_6O_2S$	106	0.39	Sulphur compound	Antimicrobial
2	4.7	Propane,1,1,3-triethoxy-	$C_9H_{20}O_3$	176	0.92	Ether compound	No activity reported
3	5.6	Benzoic acid	$C_7H_6O_2$	122	0.78		Anasthetic Fungicide Pesticide Antisalmonella Antiseptic Expectorant Antibacterial Antyeast Antipyretic Flavor Tyrosinase inhibitor Insectifuge
4	9.5	Undecanoic acid	$C_{11}H_{22}O_2$	186	1.73	Saturated fatty acid	No activity reported
5	11	Hexadecane	$C_{16}H_{34}$	226	0.77	Alkane	No activity reported
6	13	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	16.76	Myristic acid	Nematicide Anticancer

							Hypercholestrolemic Lubricant Cosmetic Antioxidant
7	13	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	0.83	Myristic acid ester	Nematicide Anticancer Hypercholestrolemic Lubricant Cosmetic Antioxidant
8	14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	10.37	Terpene alcohol	Antimicrobial Anti-inflammatory
9	14	Pentadecanoic acid	$C_{15}H_{30}O2$	242	6.8	Saturated fatty acid	No activity reported
10	14	Phytol, acetate	$C_{22}H_{42}O_2$	338	12.47	Phytol compound	Antimicrobial Anti-inflammatory Anticancer Diuretic
11	16	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.42	Palmitic acid	5 Alpha reductase inhibitor Antiandrogenic Antioxidant Flavour Nematicide Pesticide Antioxidant Hypercholestrolemic
12	16	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	5.02	Palmitic acid	5 Alpha reductase inhibitor Antiandrogenic Antioxidant Flavour Nematicide Pesticide Antioxidant Hypercholestrolemic
13	18	Phytol	$C_{20}H_{40}O$	296	1.58	Diterpene	Antimicrobial Anti-inflammatory Anticancer Diuretic
14	19	E-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O2$	310	1.9	Oleic acid	5 Alpha reductase inhibitor Anemiagenic Anti-inflammatory Cancer preventive Dermatitigenic Perfumery Flavor Insectifuge Hypocholesterolemic Antiandrogenic
15	25	Erucic acid	$C_{22}H_{42}O_2$	338	0.87	Fatty acid compound	Antitumor Lubricant

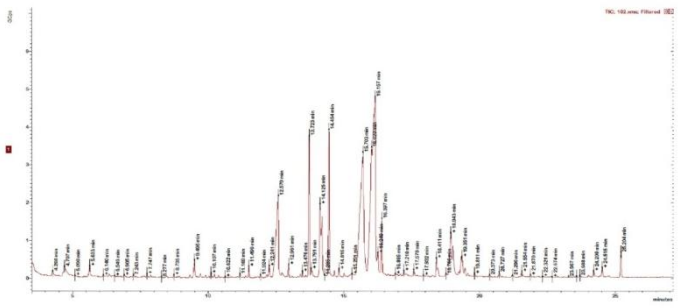

Fig1. *GC-MS Chromatogram of the methanol extract of Oscillatoria salina*

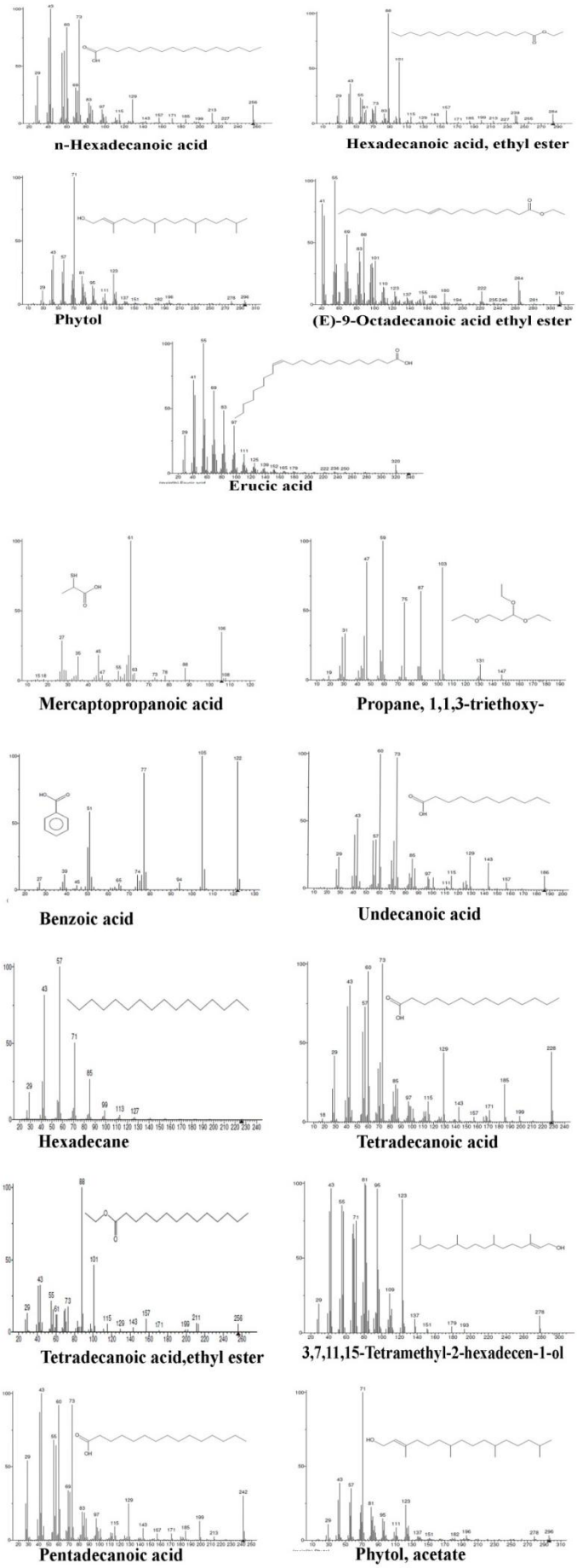

Fig1a.*GC-MS spectrum of some compounds present in the methanol extract of Oscillatoria salina*

6. DISCUSSION

The GC-MS analysis of *Oscillatoria salina* revealed the presence of fifteen compounds. The identified compounds possess many biological properties. Among the identified phytochemicals, produced by *Oscillatoria salina*Tetradecanoic acid and n-Hexadecanoic acid representing 16.76, 15.42% respectively. Tetradecanoic acid has been reported to be a major volatile component in many *Oscillatoria* sp. Earlier studies reported that *Oscillatoria angustissima* consisted of Tetradecanoic acid(3.52%) and Hexadecanoic acid(5.16%) [7] which is in good agreement with our results.(E)-9-Octadecenoic acid ethyl ester(1.9%) is one among the phytocomponents in *Oscillatoria salina* was found to have potential cancer preventive, anti-inflammatory and antiarthritic activities. Phytol was found to give good as well as preventive and therapeutic results against arthritis [12]. 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 [2,11].This study explores the adverse effect of cyanobacteria, these compounds can be used for the production of natural pharmaceutical substances. Natural substances play an important role to prevent diseases as synthetic drug causes various harmful diseases to human beings. Owing to the use of such a important bioactive substance marine cyanobacteria needs an broad studies for each and every isolated bioactive compounds.

7. CONCLUSION

The result of the present studies reveals that the methanolic extract of *Oscillatoria salina* holds anti-inflammatory, anticancer, antioxidant, anti tumour, Anemiagenic and antimicrobial properties. The GC-MS analysis of *Oscillatoria salina* reveals the presence of phyto constituents. The importance of this study is due to the biological activity of these compounds which are needed for the pharmaceutical industry.

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