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Hypersaline Microalgae – A Prolific Source of Bioactive Compounds

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ABSTRACT

Microalgae are an important untapped aquatic resource offering novel secondary and discovery of new drugs. Microalgae produce a wide variety of toxins and other biomedically interesting bioactive compounds. The present study was intended to investigate the phytochemical composition of the hypersaline microalgae Amphora sp using gas chromatography-mass spectrometry. GC-MS analysis reveals the presence of eleven bioactive compounds. The results provided insights into the presence of several pharmacologically active constituents.

KEYWORDS Amphora sp, GC-MS, Phytochemical compounds, NIST

Introduction

Microalgae are cosmopolitan and possess high potential for adoption to diverse environmental factors. Microalgae have a vast range of applications in different aspects. The potential of microalgae as a source of a variety of compounds such as polysaccharides, lipids, proteins, vitamins, sterols, enzymes, pharmaceuticals and other fine chemicals is well recognized, and their demand is now on an increasing trend. One of the most prompt areas among researchers is currently the production of bioactive compounds from hypersaline microalgae. The algae secrets some bioactive metabolites to acclimatize to salt stress and also to balance as per the surroundings osmotic pressure [6]. There are over 50,000 different species of microalgae of which only a few have been characterized [1].

Screening of bioactive metabolites of algal crude extracts is enforced in clinical practice, where antibacterial, anti-plasmodia and cytotoxicity [8], antifungal [10] and antiviral [9] activity have been accessed to these metabolites. Although many studies regarding the microalgae are available, less attention has been paid for diatoms because of difficulties in the isolation and cultivation. Thus the presence of the complex network of products in marine diatoms opens intriguing questions about the role of active compounds. Many compounds from microalgae could be useful for welfare of mankind if proper investigation done. However, in spite of its tremendous uses, the aim of the present work was to quantify the phytochemical constituents found in the hexane extracts of diatom *Amphora* sp. GC-MS is one of the most reliable biophysical method for its specificity and repeatability, was utilized for the phytochemical profiling of *Amphora* sp.

Materials and methods

Isolation, Culturing and Growth of Algal organisms

Samples of microalgae were collected from the Puthalam solar saltpans. The sample was collected by using a mesh size of 2µ of plankton net which made of bolting silk cloth. Microalgae *Amphora sp* were identified by using monographs, observed under microscope CX31 and isolated by serial dilution method. Biomass was produced by culturing the isolated strains in one litre of Walne's medium under fluorescent light and a facility to mix the culture with an aeration pump under laboratory condition. The algae were grown for 1 month and harvested.

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.

GC-MS analysis

Preparation of hexane extract (*Amphora sp*) for GC-MS analysis

Amphora sp was dried in hot air oven and 2 g of the powdered biomass was soaked in 95% solvent for 12 hr. Then the extract was filtered through whatman 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% solvent hexane 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 [4].

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 0.25m 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 / min to 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 [6].

Identification of compounds

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 [2].

Results

The results of the GC-MS analysis revealed the presence of several bioactive constituents in hexane extract of *Amphora sp*. The GC spectra of *Amphora sp* are presented in Figure 1&2 and the identified compounds are enlisted in Table 1. Eleven compounds were identified in hexane extract of *Amphora sp*. The prevailing compounds were 2-Mercaptopropanoic acid (0.39%), Benzoic acid (0.78%), Tetradecanoic acid (16.76%), Tetradecanoic acid - ethyl ester (0.83%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (10.37%), Phytol-acetate (12.47%), n-Hexadecanoic acid (15.42%), Hexadecanoic acid – ethyl ester (5.02%), trans-13-Octadecenoic acid (6.78%), Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester (0.54%), Bis(2-ethylhexyl) phthalate (2.00%).

Table.1. Activity of compounds identified in the GC-MS study of *Amphora sp* extract

No.	RT	Name of the compound	Molecular Formulae	MW	Peak Area %	Compound nature	*Activity
1	4.26	2-Mercaptopropanoic acid	$C_3H_6O_2S$	106	0.39	Sulphur compound	Antimicrobial
2	5.63	Benzoic acid	$C_7H_6O_2$	122	0.78		Anasthetic, Fungicide, Pesticide, Antisalmonella, Antiseptic, Expectorant, Antibacterial, Antyeast, Antipyretic, Flavor, Tyrosinase inhibitor, Insectifuge
3	12.6	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	16.76	Myristic acid	Nematicide, Anticancer, Hypercholestrolemic, Lubricant, Cosmetic, Antioxidant
4	13	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	0.83	Myristic acid ester	Nematicide, Anticancer, Hypercholestrolemic, Lubricant, Cosmetic, Antioxidant
5	13.7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	10.37	Terpene alcohol	Antimicrobial, Anti-inflammatory
6	14.45	Phytol, acetate	$C_{22}H_{42}O_2$	338	12.47	Phytol compound	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic
7	16.2	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
8	16.4	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
9	18.9	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	6.78	Unsaturated fatty acid compound	No activity reported
10	20.7	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	0.54	Palmitic acid ester	5 Alpha reductase inhibitor, Antiandrogenic, Antioxidant Flavour, Nematicide, Pesticide, Antioxidant, Hypercholestrolemic
11	25.2	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	2.00	Plasticizer compound	Antimicrobial, Antifouling

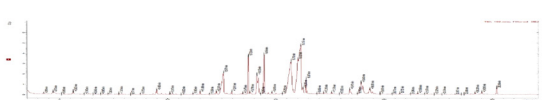
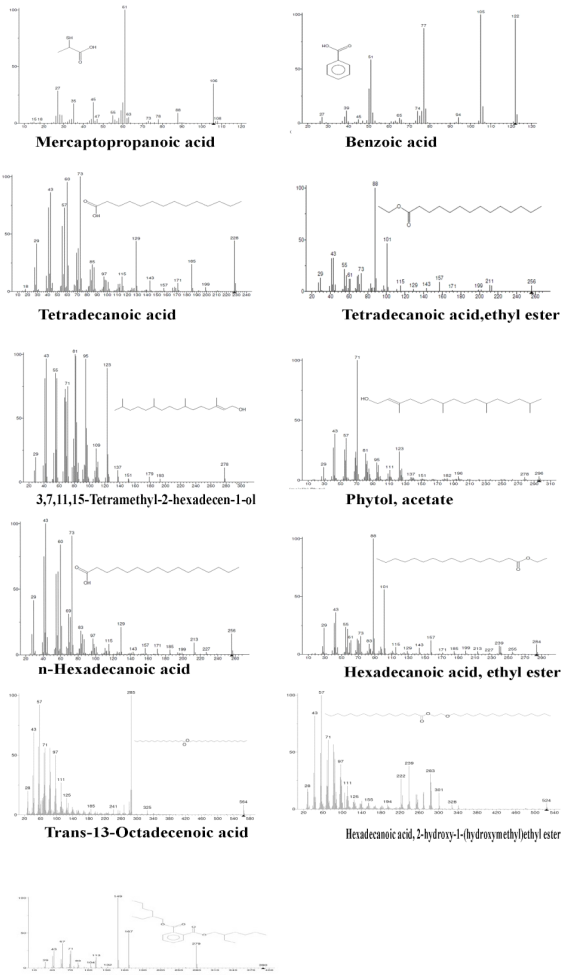


Fig.1 GC-MS Chromatogram of the hexane extract of Amphora sp

Fig.2.GC-MS spectrum of some compounds present in the hexane extract of *Amphora* sp



Discussion

Phytochemical profiles of microalgae vary heavily depending on variation in culture, strain as well as environmental parameters. In the present study, phytochemical extraction was performed using a hexane extraction method. The GC-MS analysis of *Amphora sp* revealed the presence of eleven compounds. The identified compounds possess many biological properties. Among the identified phytochemicals, produced by *Amphora sp* Tetradecanoic acid and n-Hexadecanoic acid representing 16.76, 15.42% repectively.

Integration of the principles of molecular pharmacology with contemporary high-content screening technologies is essential for the success of these discovery activities [(3)]. The diatom compounds responsible for inhibitory effects during copepod embryogenesis as 2-trans-4-cis-7-cis-decatrienal, 2-trans-4-trans-7-cis-decatrienal, and 2-trans-4-trans-decadienal [(5)]. The compounds isolated were already proved that they are having antioxidant and antimicrobial, anti-inflammatory**,** antiseptic, antineoplastic, anti-allergic, antipyretic and analgesic effects. The formation of reactive aldehydes was not a universal property of all diatoms, but is highly variable among species and strains [(11)].

Conclusion

The result of the present studies reveals that the hexane extract of *Amphora* sp holds anti-inflammatory, anticancer, antioxidant, antitumour, anemiagenic and antimicrobial properties. The GC-MS analysis of *Amphora* sp reveals the presence of phytoconstituents. Large scale cultivation strategies ensure the adequate supply of this compound. But till date, there are no reports on chromatographic analysis of hexane extract of this microalgae. The activities and properties of identified compounds should also be tested in future to reveal the clear sketch of active mechanism.

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