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BIOACTIVE COMPOUNDS IDENTIFICATION FROM MARINE MACRO ALGAE *PADINA AUSTRALIS* USING METHANOL EXTRACT

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ABSTRACT: The investigation was carried out to determine the possible bioactive compounds marine macro algae *Padina australis* using methanol extract. The GC-MS analysis of methanol extract was performed using perkin Elmer GC clarus. Six compounds were identified from *Padina australis* showed highest peak area Pentadecanoic acid 14-methyl ester [100%] followed by 1, 2 Benzenedi carboxylic acid, butyl octyl ester [91.5%], Octadecenoic acid, methyl ester [82.3%] 10-Octadecenoic acid, methyl ester [78.6%] and Oleic acid [56.4%]. The compound 9- Octadecenoic acid [Z]; 2-hydroxy-1 [hydroxyl methyl ester] showed the lowest peak area of 34.9%.

INTRODUCTION: The seaweed gives great potential as a supplement in functional food or for the extraction of compounds. Philologically active substances in marine algae are classified into two types based on the difference in the mechanisms. They are non-absorbed high molecular materials like dietary fibers and low molecular materials. which are absorbed and which affect the maintenance of human homeostasis directly ¹. All algal fucans have complex structures but recent studies have revealed ordered repeated units in homofucans from several species. Several homofucans have large proportions of both alpha -(1-3) and alpha- (1-4) Glycoside link ages with sulphate groups at C-2, without excluding the presence of other sulphates, acetyl groups or branches at positions 2,3 or 4².



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Fast atom bombardment MS was proposed for the identification of arsenic compounds in environmental matrices ³ and successfully applied to the characterization of a partially purified *Sargassum* extract ⁴.

Positive ion electroscopy mass spectrometry (ES MS) spectra of arsenobetaine, arsenocholine, monomethylarsonic acid, DMAA, tetramethyl arsonium iodide and negative mode spectra of as (111) and As (V) were reported ⁵. Among the macromolecular constituents of the thallus of *Sargassum*, alginate was identified as the most important with respect to metal binding and selectivity ⁶.

Alginate is the common name given to a family of linear polysaccharides containing 1; 4-linked β -D-mannuronic (M) and α -L-guluronic (G) acid residues arranged in a no regular, block-wise order along the chain. The relative abundance of the M and G residues and their macromolecular conformation determine the physical properties and affinity of the alginate for divalent metals 7 .

The present study was undertaken to investigate the GC-MS analysis of methanol extract of marine macro algae *Padina australis* extract using methanol.

MATERIALS AND METHODS:

Extraction of macro algae: The marine macro algae of Padina australis was collected from Chagkumugam coast, shade dried and pulverized to powder in a mixer grinder. Required quantity of the algal powder of seaweeds were weighed, transferred to flask, treated with methanol until the powder was fully immersed, incubated overnight and filtered through a whatmann No.1 Filter paper along with sodium sulphate to remove the sediments and traces of water in the filter paper. Before filtering, the filter paper along with sodium sulphate was wet with absolute alcohol. The filtrate is then concentrated to 1ml by bubbling nitrogen gas into the solution. The extract contains both polar and non-polar components of the macro algal material. 2ml sample of the solutions was employed in GC-MS for analysis of different compounds.

Gas Chromatography - Mass Spectrometry (GC-MS analysis): GC-MS analysis of the methanol extract of *Padina australis* were performed using a Perkin Elmer GC Clarus 500 system comprising AOC-20i auto-sampler and a Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-5MS (5% Diphenyl / 95% Dimethyl Poly Siloxane) Fused silica capillary column (30 x 0.25mm 1D x 0.25mm dF). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization system was operated in electron impact mode with ionization energy of 70ev.

Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1ml/min, and an injection volume of 2ml was employed (split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed at 110°C (isothermal for 2 min), with an increase of 100 c/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C.

Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45- 450Da. The solvent delay was 0 to 2 min and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was Turbo-Mass Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a

Turbomass.

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Interpretation on mass spectrum of GC-MS was done using the database of National institute of 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.

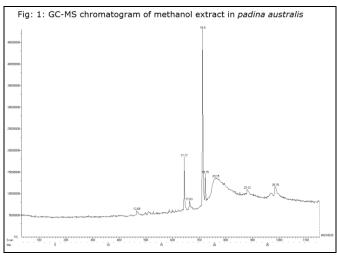
RESULTS: The results on GC-MS analysis of active fraction of methanol extract in *Padina australis* were presented in Table-1 and Fig-1. Six compounds were detected in methanol extract of *Padina australis*. The compound Pentadecanoic acid 14-methyl ester showed the peak area 100% at the RT 17.17min and MW was 104, the compound 1, 2 Benzenedi carboxylic acid, butyl octyl ester was occurred the peak area of 91.5% at RT 17.63, extraction compound of 10-Octadecenoic acid, methyl ester was showed the peak area 78.6 at RT 18.95min.

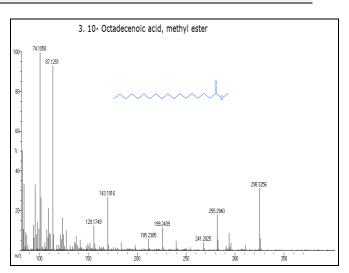
The extraction compound Octadecenoic acid, methyl ester was showed the peak area of 82.3 at RT 19.15min, the compound Oleic acid occurred the peak area of 56.4 at RT 20.15min and extraction compound of 9- Octadecenoic acid [Z]; 2-hydroxy-1 [hydroxyl methyl ester] was showed the peak area 34.9 at RT 21.56min and MW was 211.

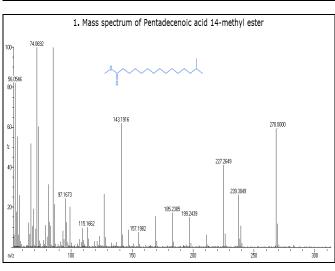
The extraction compound Pentadecanoic acid 14-methyl ester showed highest peak area [100%] at the RT 17.17min with MW was 104 and the retention time 21.56min the compound 9-Octadecenoic acid [Z]; 2-hydroxy-1 [hydroxyl methyl ester] showed the lowest peak area of [34.9%] with 211MW.

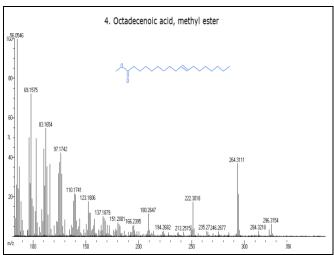
TABLE: 1: GC-MS ANALYSIS OF METHANOL EXTRACT IN PADINA AUSTRALIS

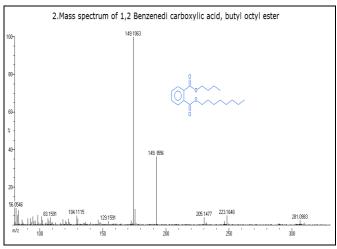
S. No.	RT	Compounds	Peak area (%)	MW
1.	17.17	Pentadecanoic acid 14-methyl ester	100	104
2.	17.63	1,2 Benzenedi carboxylic acid, butyl octyl ester	91.5	71
3.	18.95	10-Octadecenoic acid, methyl ester	78.6	249
4.	19.15	Octadecenoic acid, methyl ester	82.3	107
5	20.15	Oleic acid	56.4	247
6	21.56	9- Octadecenoic acid [Z]; 2-hydroxy-1 [hydroxyl methyl ester]	34.9	211

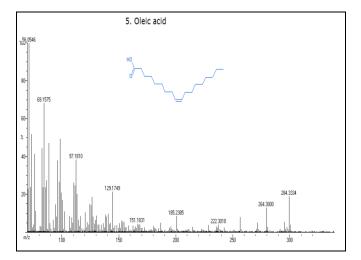


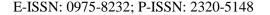


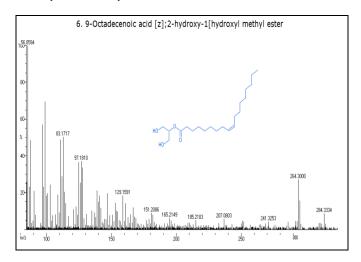












DISCUSSION: Marine chemicals have novel structures with pronounced biological activity and pharmacology. The study of such chemicals therefore is promising. High through put screening of marine metabolites for a given drug target can be achieved only if natural compounds are available as a database. Creating a database of natural products and sharing it with huge scientific community facilitates the understanding of basic mechanism of compounds and can reduce the timeline in drug discovery ⁸.

The yields of volatile compounds are better in dried seaweed rather than fresh seaweeds ⁹. The of dried by compounds Laminaria SD. Simultaneous distillation extraction and the compounds they found; those of identical to Sargassum polystum are dodecane 1-iodo, heptanal, 1- octen- 3-ol and hydrocarbons such as heptadecane and others 10. GC- MS analysis of major compounds of Cladophora glomerata second active fraction, Pentadecane, 8-hexyl- was found to be a major compound followed by the hydrocarbon compounds heptadecane, 9-hexyl and octacosane in third fraction ¹

The crude methanolic extract of *A. orientalis* was separated using gas chromatography analysis revealed the presence of seven fatty acids are Palmatic acid, Lauric acid, Myristic acid, Hexadecatrieonic acid, Stearic acid, Oleic acid, and alpha linolenic acid ¹². Mass spectrum of the peak at 18.16 min was due to phthalide and the peak at 18.34 min was due to n-tetradecane, which had been added in a known amount as an internal standard for purposes of quantitating the products ¹³

The GC –MS analysis of essential oil components of *D. dichotoma* found two compounds namely n-Tridecane (4.11min) and n-Eicosane (12.65min) ¹⁴.

In the present study, six compounds were detected in methanol extract of *Padina australis*. The extraction compound Pentadecanoic acid 14-methyl ester showed highest peak area [100%] .The same work was reported by Ronald A. Hites, 1992.

CONCLUSION: In the present investigation, six bioactive compounds were identified using methanol extract of potential macro algae *Padina australis*. The presence of various bioactive compounds justifies the use of the macro algae for various ailments by traditional practitioners.

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