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DETERMINATION OF BIOACTIVE COMPONENTS IN THE METHANOLIC EXTRACT OF PADINA PAVONICA USING GC-MS TECHNIQUE

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ABSTRACT

The bioactive components of *Padina pavonica* have been evaluated using GC/MS. The chemical compositions of the methanolic extract of *Padina pavonica* were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of methanolic extract of *Padina pavonica* revealed the existence of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid, bis 2-methylpro, Hexadecanoic Acid, Ethyl Ester and 2-Hexadecen-1-OL, 3,7,11,15- Tetramethyl. The results of this study offer a platform of using *Padina pavonica* as herbal alternative for various diseases.

KEYWORDS: *Padina pavonica*, GC/MS, Bioactive components, Methanolic extract.

INTRODUCTION

Marine algae are one of the largest producers of biomass in the marine environment (Bhadury et al., 2004)[2] they produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against the other settling organisms. Seaweeds are the eukaryotic organisms that live in salty water and recognized as a potential source of bioactive natural products. Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine. Today, seaweeds are the raw materials for many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries (Mishra et al., 1993).[10] Distribution of seaweed species

in India are Gulf of Kutch, Gangeya, South West Coast of India; Mandapam, Kanyakumari, Muttam and Arokiapuram Karnataka, Kerala, Lakshadweep, Tamil Nadu, Andhra Pradesh, Gopalpur coast and brackish water lake Chilika of Orissa; West Bengal and Andaman & Nicobar Islands. India presently harvests only about 20,000 tonnes of macroalgae annually (Verma, 2012).[15]

Seaweeds are reservoirs of carotenoids, pigments, polyphenols, enzymes, diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B1, B12, C, D and E (Smit, 2004)[13] and have antibacterial, antialgal, antimacrofouling and antifungal properties which are effective in the prevention of biofouling and have other likely uses, as in therapeutics (Thoudam et al., 2011). Within a decade, there was a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation identification and structure determination of phytochemicals (Roberts and Xia, 1995)[11] The aim of this study is to determine the bioactive compounds present in the *Padina pavonica* extract with the aid of GC-MS Technique, which may provide an insight in its use in convention medicine.

MATERIAL AND METHODS

Plant materials and preparation of extract

The *Padina pavonica* were collected in August 2014 from Andaman Island, India. The collected *Padina pavonica* were dried at room temperature and coarsely powdered. The powder was extracted with methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Padina pavonica* extract was stored in refrigerator until used.

GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan

interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. The relative percentage 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 Ver 5.2.0.

RESULTS AND DISCUSSION

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute 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.

GC-MS ANALYSIS

Nine compounds were identified in *Padina pavonica* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Fig 1 and Table 1). The prevailing compounds were 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid, bis(2-methylpro, Hexadecanoic Acid, Ethyl Ester and 2-Hexadecen-1-OL, 3,7,11,15-Tetramethyl. The biological activities listed (Table 2) are based on Dr. Duke's phytochemical and ethnobotanical Databases by Dr. Duke[4] of the Agricultural Research Service/USDA.

In the present study twenty chemical constituents have been identified from ethanolic extract of the plant of *Padina pavonica* by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results.

Biji Cyriac and Eswaran (2015)[3] investigated the active phytoconstituents present in the red seaweed *Gracilaria dura* using GCMS. Thirteen compounds were identified in the methanol extracts of G. duraand the major components are n-Hexadecanoic acid, Oleic Acid, Pytol, Squalene, 1,2 Benzenedicarboxylic acid, mono (2-ethylhexyl) ester, 9-Octodecenoic acid, palmitoleic acid etc and found to have antimicrobial, antioxidant, antiinflammatory, antitumor, anti-androgenic and cancer preventive properties. The findings of the present

study confirm the presence of important phytocompounds in *Padina pavonica* and helpful for further detailed study to develop the drugs from marine algae to alleviate various diseases.

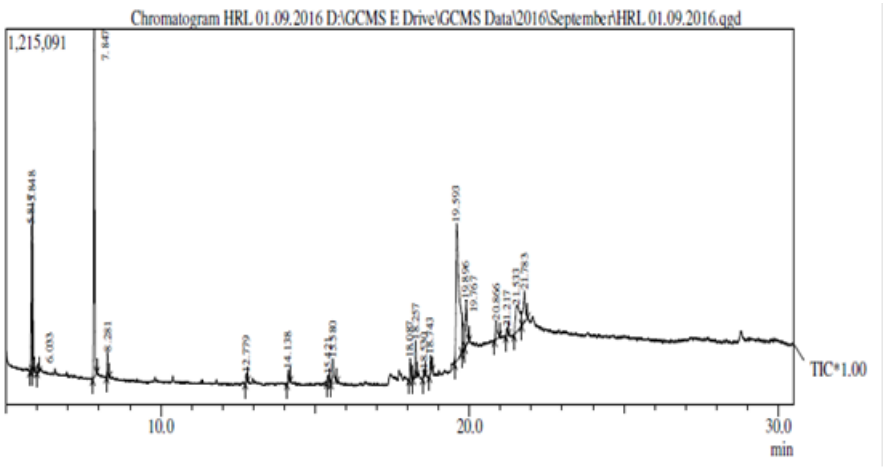

Figure 1: Bioactive components identified in *Padina pavonica* using GC-MS.

Table 1: Bioactive components identified in *Padina pavonica* using GC-MS.

Peak	R.Time	Area %	Height %	Molecular Formula	Molecular Weight	Name of the compound
1	5.817	8.83	12.84	$C_9H_{20}O_2$	160	Butane, 1,1-diethoxy-3-methyl-
2	5.848	7.97	14.82	$C_9H_{20}O_2$	160	Pentane, 1,1-diethoxy-
3	6.033	0.50	0.78	$C_9H_{20}O_2$	160	3,3-Diethoxy-2-Butanone
4	7.847	20.23	30.65	$C_{11}H_{24}O_4$	220	Propane, 1,1,3-triethoxy-
5	8.281	1.40	2.25	$C_{10}H_{22}O_3$	190	1,1,3-Triethoxybutane
6	12.779	0.61	0.83	$C_{13}H_{28}$	184	Decane, 2,3,8-trimethyl
7	14.138	0.95	1.15	$C_{13}H_{28}$	184	Nonane, 3,7-Dimethyl-
8	15.421	0.51	0.62	$C_{14}H_{30}O_3S$	278	Sulfurous acid, 2-ethylhexyl isohexyl ester
9	15.580	2.61	2.09	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic Acid, Diethyl
10	18.087	1.45	1.79	$C_{17}H_{34}O_2$	270	Isopropyl myristate
11	18.257	2.20	3.23	$C_{20}H_{38}$	278	2,6,10-Trimethyl,14-Ethylene-14-Pent
12	18.530	0.45	0.61	$C_{20}H_{40}O$	296	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
13	18.743	1.47	1.68	$C_{17}H_{32}O$	252	8-Hexadecenal, 14-methyl- (Z)-
14	19.593	26.31	12.07	$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid
15	19.767	5.05	3.32	$C_{16}H_{22}O_4$	278	1,2-Benzenedicarboxylic acid, bis(2-methylpro
16	19.896	4.63	3.96	$C_{18}H_{36}O_2$	284	Hexadecanoic Acid, Ethyl Ester
17	20.866	2.10	1.66	$C_{19}H_{40}O$	284	n-Nonadecanol-1
18	21.217	0.62	0.67	$C_{20}H_{40}O$	296	2-Hexadecen-1-OL, 3,7,11,15-Tetramethyl
19	21.533	7.42	2.27	$C_{20}H_35F_3O_2$	364	Oleyl alcohol, trifluoroacetate
20	21.783	4.71	2.71	$C_{18}H_{34}O_2$	282	Ethyl 9-hexadecenoate

Table 2: Pharmacological activities of components identified in *Padina pavonica.*

Peak	R.Time	Name of the compound	Molecular Weight	BIOLOGICAL ACTIVITY**
1	15.580	1,2-Benzenedicarboxylic Acid, Diethyl	222	Used as a plasticizer for vinyl foams, which are often used as floor tiles. Other uses are in traffic cones, food conveyor belts, and artificial leather
2	18.530	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	Antimicrobial
3	19.593	n-Hexadecanoic acid	256	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, anti-inflammatory,Flavor,Hemolytic
4	19.767	1,2-Benzenedicarboxylic acid, bis(2-methylpro	278	Plasticizer, Antimicrobial
5	19.896	Hexadecanoic Acid, Ethyl Ester	284	Antioxidant, Flavour, Hypocholestrolemic Nematicide, Pesticide, Lubricant Antiandrogenic, Hemolytic,5- Alpha reductase inhibitor, anticancer
6	21.217	2-Hexadecen-1-OL, 3,7,11,15- Tetramethyl	296	Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents

**Source: Dr. Duke's phytochemical and ethnobotanical database (online database)

Balamurugan *et al* (2013)[1] studied for the richness of its bioactive compounds in *Hypnea musciformis* (red seaweed). The ethanolic extract was subjected to GC-MS analysis revealed 26 chemical constituents. Seaweed exhibits potentially bioactive major constituents like n-hexadecanoic acid-tetradecanoic acid, oleic acid-9-octadecenoic acid-6-octadecenoic acid, hexadecanoic acid-ethyl ester-ethyl tridecanoate and octadecanoic acid. Further biochemical and mineral analyses presented the phenolic content, total carbohydrate, total protein, fat, moisture content, ash, vitamins A, C and E and lipid soluble and water-soluble antioxidants. Chemical elements, such as Ca, Mg, Na, K and Fe were in highest proportion in the red seaweed.

Kavitha and Palani (2016)[7] reported the phytochemical screening of seaweed *Chlorococcum humicola* was performed to know the phytochemical constituents and GC-MS analysis was done to analyze and identify different constituents present in the extract of *Chlorococcum humicola*. The results of GC-MS study indicated the presence of 14 different compounds.

The compounds exhibited a wide range in their nature. Largest peak area was observed for the compound 1-Propene, 3- (2-cyclopentenyl)-2-methyl-1,1-diphenyl-. The compound with highest molecular weight was cartilagineol.

The study indicated that six compounds having significant biological functions that were showed in Table 2. Most of the compounds had antimicrobial, antioxidant, antiinflammatory, antitumour and cancer preventive properties. It was in agreement with the results of Mayer and Lehmann (2001)[9], Mayer and Hamann (2004)[8], Smit (2004)[12], Flora and Maria Victorial Rani (2013)[5], Gihan et al., (2014-2015)[6] It could be concluded that *Padina pavonica* contains various bioactive compounds. So it is recommended for the development of pharmaceutical compounds for therapeutic use.

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