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GC-MS STUDIES ON SELECTED RED SEAWEED (ACANTHOPHORA SPECIFERA) COLLECTED FROM GULF OF MANNAR, TAMILNADU, INDIA

R. Rajeshkumar*[1] and K. Jeyaprakash[2]

[1]Research Scholar, Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613005, Tamilnadu, India.
[2]Head, PG and Research of Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613005, Tamilnadu, India.

***Corresponding Author: R. Rajeshkumar**
Research Scholar, Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613005, Tamilnadu, India.

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ABSTRACT

Acanthophora Specifera is a species of marine red seaweed and in the family Rhodomelaceae. They were freshly collected from Mandapam Coastal Area, Rameswaram Tamilnadu, India and rinsed in seawater and packed in aseptic bags for further proceedings to laboratory. Seaweeds are potential renewable resources in the marine environment. It has been used as antioxidant and antimutagen. Methanol extract was prepared for further analysis. GC-MS analysis of methanol extract of Acanthophora specifera was performed using a Shimadzu 2010 plus comprising an AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer and a RTX-5ms column. This investigation was carried out to determine the possible chemical components from Acanthophora specifera by GC-MS analysis. In the present study twenty phytocomponents have been identified from methanol extract of Acanthophora specifera by GC-MS analysis. The results of the present study revealed the presence of Phenol, 3,5-bis (1,1-dimethylethyl), Octadecanoic acid, Tetradecanoic acid, 9,12-octadecadienoic acid (z,z), 9-octadecenoic acid (z) and Hexadecanoic acid, ethyl ester were found in this sample

KEYWORDS: Red Seaweed, GC-MS, Acanthophora specifera, Chromatogram, Retention Time.

INTRODUCTION

Seaweed or benthic marine algae are the group of plants that live either in marine or brackish water environment. Like the land plants seaweed contain photosynthetic pigments and with the help of sunlight and nutrient present in the seawater, they photosynthesize and produce food. Seaweeds are found in the coastal region between high tide to low tide and in the sub-tidal region up to a depth where 0.01 % photosynthetic light is available. Plant pigments, light, exposure, depth, temperature, tides and the shore characteristics combine to create different environment that determine the distribution and variety among seaweeds. They are basically classified according to colour into three main groups i.e. green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta).

Seaweeds are potential renewable resources in the marine environment. It is generated enormous amount of bioactive compounds with immense medicinal potential. Nowadays, the uses of antibiotics have increased due to infections.[1]

The first investigation antibiotic activity carried out by Pratt et al., (1944).[2]

Since algae have been used in traditional medicine for a long time and also some algae have bacteriostatic, bactericidal, antifungal, anti viral and anti tumor activity, they have been extensively studied by several researchers. Seaweed is rich in antioxidants such as carotenoids, pigments, polyphenols, enzymes. Seaweeds are the most excellent source of Vitamin A, B1, B12, C, D and E.[3]

The mineral nutrient present in seaweeds are diverse and the main elements being magnesium, sodium, potassium and calcium. The chemical composition of seaweeds varies with species, habitat, maturity and environmental conditions.[4]

Among the different compounds with functional properties, antioxidants are the most widely studied. Antioxidants are the substances, which can defend serious human diseases including melanoma, cardiac disorders, diabetes, cancer, inflammatory that explain

their potential use in increasing shelf life of food and as medicine.[5]

Free radical induced oxidation is one of the major reasons in deterioration of nutritional quality and other physical attributes of food items under storage. Previous studies in animal models and cell culture have suggested that seaweed phytochemicals have the potential to inhibit progression of carcinoma formation.[6]

Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of micro organisms. Therefore, the present study was carried out to demonstrate the preliminary phytochemical constituents with the aid GC/MS technique of Acanthophora specifera.

MATERIALS AND METHODS

Collection of Seaweeds

Acanthophora specifera were collected from Gulf of Mannar, Rameswaram, Tamilnadu, India. The collected samples were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in sterile bags. Then the samples were washed with tap water and distilled water and spread in the dark room for drying, after which the dried samples were powdered and subsequently stored at 4°C.

Preparation of extract

A dried sample of Acanthophora specifera was pulverized to powder in a mechanical grinder. 50g of dried seaweed powder was extracted with methanol for 72h by maceration until the powder was fully immersed, incubated over night and filtered through whatmann no.41 filter paper. The filtrate is then concentrated by bubbling nitrogen gas into the solution. The extract employed in GC-MS for analysis of different compounds.

GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150ºC, then 8ºC/min to 250ºC, ending with a 20min 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 51.25min. 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 Turbo Mass Ver 5.2.0 (Srinivasan et al., 2013).[7]

Identification of components

Interpretation on GCMS 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 (Dr. Dukes, 2013).[8]

RESULTS AND DISCUSSION

GCMS analysis was carried out in crude extract of seaweed Acanthophora Specifera. In the present study twenty chemical constituents have been identified from methanolic extract of the Acanthophora Specifera by GCMS analysis. The total ion chromatogram of methanolic extract showing the GCMS profile of the compounds identified is given in the figure 1 & Table 1.

The present of twenty compounds has been observed which belonging to broad groups of compounds such as long chain aliphatic compounds, acids, esters, ethers, amine, alcohols and aromatic compounds with hetero atoms such as oxygen, nitrogen also prominently seen.

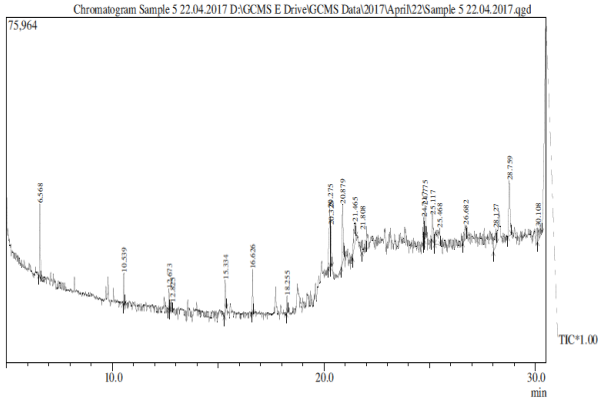

Figure 1: Chromatogram of Acanthophora specifera sample.

This analysis revealed the presence of major constituents like tetradecane, 14-butanediol, Bis-(3,5,5-Trimethylhexyl)ether, 1,2-benzene dicarboxylic acid, Diisooctyl ester, 35-octanedione etc,. most of the identified major compounds were generally reported as having various biological activities (Table 2). 1,2-benzene dicarboxylic acid Diisooctyl ester has been reported to elicit potent inhibition of human platelet phospholipase A2 (labow et al.,1988)[9] and rapidly increases protein phosphorylation in HeLa cells via protein kinase C and casein kinase I(lahouse et al.,2006).[10]

Table 1: Identification of active compounds in Sample using GCMS.

Peak#	R.Time	Area%	Height%	Molecular Formula	Name of the compounds
1	6.568	7.32	11.84	$C_{15}H_{32}$	Tetradecane, 2-Methyl-
2	10.539	2.53	5.06	$C_{12}H_{20}O_3$	6-Nonenoic Acid, 2,7-Dimethyl-3-Oxo-, Methyl Ester, (E)-
3	12.673	2.07	3.28	$C_{12}H_{25}NO_2$	12-Aminododecanoic Acid
4	12.825	1.51	1.48	$C_2H_7BO_2$	Boronic acid, ethyl-
5	15.334	3.13	5.35	$C_{13}H_{22}O_4$	Oxalic acid, cyclobutyl heptyl ester
6	16.626	4.17	7.03	$C_{14}H_{29}I$	Tetradecane, 1-iodo
7	18.255	1.65	2.72	$C_{15}H_{24}O_5$	2-Naphthaleneacetic Acid, Decahydro-1,5-Dihydroxy-.Alpha.,4a-Dimethyl-8-Oxo-, Methyl Ester,
8	20.275	9.33	9.42	$C_{10}H_{22}O_2$	14-Butanediol, 2,3-Diethyl-2,3-Dimethyl
9	20.325	6.25	6.66	$C_{15}H_{32}O_2$	1-(2-Hydroxyethoxy)Tridecane
10	20.879	9.90	9.52	$C_{18}H_{38}O$	Bis-(3,5,5-Trimethylhexyl) Ether
11	21.465	6.45	3.40	$C_{10}H_{11}F_7O_2$	35-Octanedione, 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-
12	21.808	2.58	1.80	$C_{17}H_{19}N_3O$	1-(Benzotriazoyl-1-Yl)-3-(1-Cyclohexenyl)Propargyl Ethyl Ether
13	24.717	2.63	4.02	$C_{17}H_{15}NO_3$	N-Dibenzo[B,D]Furan-3-Yltetrahydro-2-Furancarboxamide
14	24.775	4.03	5.41	$C_{12}H_{22}O_2$	2-(1-Methylcyclohexyloxy)-tetrahydropyran
15	25.117	4.03	4.10	$C_{22}H_{36}OSi_4$	2-Trisilanol, 1,1,1,3,3,3-Hexamethyl-2-[9-(Trimethylsilyl)-9h-Fluoren-9-Yl]
16	25.468	8.30	2.73	$C_{12}H_{34}O_5Si_4$	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy
17	26.682	3.54	2.33	$C_{13}H_{19}NO_4Si$	Glycine, N-[4-[(trimethylsilyl)oxy]benzoyl]-, methyl ester
18	28.127	4.93	2.76	$C_{15}H_{22}O_2$	1,2-Naphthalenedicarboxaldehyde
19	28.759	12.15	8.94	$C_{24}H_{38}O_4$	1,2-Benzenedicarboxylic Acid, Diisooctyl Ester
20	30.108	3.52	2.15	$C_8H_{24}O_4Si_4$	Cyclotetrasiloxane, Octamethyl
		100.00	100.00		

The antioxidant and cytoprotective activities have also been reported for 1, 1, 3, 3, 5, 5, 7, 7- octamethyl. The various biological activities reported for these phytocomponents to be evaluate the pharmacological activity of methanolic extract of Acanthophora Specifera. The capability of oleic acids and hexadecane that exhibited antimicrobial and antioxidant capabilities have also been reported.

Identification of bioactive compounds in Sample extract by GC MS analysis

Twenty compounds were identified in Sample by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds are Phenol, 3,5-bis (1,1-dimethylethyl), Octadecanoic acid, Tetradecanoic acid, 9,12-octadecadienoic acid (z,z), 9-octadecenoic acid (z) and Hexadecanoic acid, ethyl ester were found in this Sample. The biological activities of the selected compounds were listed in table 2. The presence of various bioactive compounds justifies the use of the Sample for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting its biological activity will definitely give fruitful results.

Table 2: Bioactivity of components identified in sample by GC-MS.

Peak#	R. Time	Name of the compounds	Biological activity**
1	6.568	Tetradecane, 2-Methyl-	Antimicrobial Activity, Wound healing activity, Anti-viral and Antitumor activities
6	16.626	Tetradecane, 1-iodo	Antimicrobial Activity, Wound healing activity,
9	20.325	1-(2-Hydroxyethoxy)Tridecane	Antimicrobial Activity
15	25.117	2-Trisilanol, 1,1,1,3,3,3-Hexamethyl-2-[9-(Trimethylsilyl)-9h-Fluoren-9-Yl]	Antimicrobial activity
16	25.468	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy	Antioxidant and Antimicrobial
19	28.759	1,2-Benzenedicarboxylic Acid, Diisooctyl Ester	Inhibition of human platelet phospholipase A2 (Labow et al., 1988).[9] Rapidly increases protein phosphorylation in HeLa cells via protein kinase C and casein kinase1 (Lahousse et al., 2006).[10]

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

CONCLUSION

Isolation of individual phytochemical constituents from Acanthophora Specifera and subjecting them to meticulous biological screening can give fruitful results. From the results it could be concluded that Acanthophora Specifera contains various bioactive compounds. Therefore it is recommended as seaweed of phyto pharmaceutical importance.

Conflict of Interest

The authors declare that there are no conflicts of interest. The research received no specific grant from any funding agency in the public, community, or non-for profit sectors.

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