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Preliminary Phytochemical and GC-MS Analysis of Marine Seaweed-Acoathophora deilei (Red alga)

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Seaweeds (Marine macro algae) are multicellular marine organism and are vital constituents of the of marine ecosystem, which are abundant in the coastal areas of the world. They are tremendous source of many bioactive metabolites and have been shown to exhibit a wide range of therapeutic properties, including anti-cancer, anti-oxidant, anti-inflammatory and anti-diabetic activities. Several Asian cultures have a strong tradition of using different varieties of seaweed extensively in cooking as well as in herbal medicines preparations. As such, seaweeds have been used to treat a wide variety of health conditions such as cancer, digestive problems, and renal disorders. These plants contain important phytochemical constituents and have various potential biological activities. References regarding the use of algae in Ayurveda and Siddha system of medicine has been reported since time immemorial, but their phytochemical properties have not been reported. To identify the phytochemical constituents present in the Acoathophora deilei (Red alage) using Preliminary phytochemical and GC-MS analysis. Methods: The shade-dried of red algae were extracted with methanol and the crude methanolic extract was subjected to GC-MS analysis to identify the various bioactive components. Phytochemical investigations suggests that the Acoathophora deilei showed the presence of phytochemicals like alkaloids, phytosterols, flavonoids and diterpenes, which may contribute to its biological activities. GC-MS analysis showed the presence of 28 different compounds. The main chemical constituents found in high percentage are Hexadecanoic acid methyl ester, 2-ethyl butyric acid octadecyl ester, hexadecanoic acid, 9-octadecanoic acid methyl ester and 1,2 – Benzene dicarboxylic acid. Thus, the present analytical study of Acoathophora deilei on phytochemical and GC-MS analysis provides an important novel information to support further ongoing studies to evaluate structure of bioactive compound and its pharmacological activities.

Keywords: Acoathophora deilei; GC-MS analysis; GC-MS fingerprinting; Marine seaweeds; Phytochemical analysis.

In traditional medicine, drugs are categorized into 3 groups, namely herbal products, mineral and animal products. The source of herbal products includes not only the higher plants but also the lower plants like seaweeds (Marine macro algae)[1]. Algae are alternatives, especially marine algae, are least explored for their medicinal uses[2]. The marine algae are new potential source and it is rich in various bioactive compounds. Seaweeds are treasured resources that belong to the plant kingdom – thallophyta, which had primitive group of non-flowering plants (Cryptogams) without true root, stem and leaves. Seaweeds occur in the intertidal, superficial and upto 180m depth of

the sea and also in the backwaters. They develop on rocky shores, corals, solid rock layer, small stones and on other plants. Based on the presence of pigments, stored food materials, morphological and anatomical characters, they are categorized into four major groups such as *Chlorophyceae (*Green seaweeds), *Phaeophyceae* (Brown seaweeds), *Rhodophyceae* (Red seaweeds) and *Cyanophyceae* (Blue green algae). Marine algae are used for the preparation of various food items such as jelly, jam, chocolate, pickle, soup, salad, vegetable and porridge. Seaweeds are also utilized as animal fodder and as fertilizer for various crops[3]. To explore various nutritional benefits of seaweed consumption, there is a need for more evidence relating to the properties of seaweeds on human health. So, the current study was performed to analyze various phytoconstituents present in the methanolic extract of *Acoathophora deilei* (Red algae). GC-MS fingerprinting was also done to recognize various phytochemicals present in red algae.

MATERIALS AND METHODS

Plant material and extraction

A. deilei seaweed was collected from Rameswaram costal area, Tamil Nadu, India and it was authenticated by Dr. S. Bragadeeswaran, Marine Biologist, Centre for advanced studies in marine biology, Annamalai University, Parangipettai, Tamil Nadu. The sample was thoroughly washed with seawater to remove epiphytes followed by tap water, to remove the salts and other extraneous materials. The seaweed was washed with water, shade dried and powdered coarsely. Maceration with 95% methanol at room temperature for 72 hrs. and crude extract was obtained and the procedure was repeated till exhaustion of the material. Thereafter, the methanolic extract was distilled and dried under reduced pressure to get methanolic extract.

Phytochemical analysis

Methanolic extract *of Acoathophora deilei (*Red algae) powder was subjected to various chemical tests (qualitative) to develop profiles of the extract for its chemical composition[4].

Phytochemical screening

Qualitative phytochemical analysis of methanolic extract of *Acoathophora deilei* was performed based on the method of sofowara (1993), Trease & Evans (1975) and Harborne (1973)[5,6,7].

Detection of alkaloids

Methanolic extract of *A.deilei* was dissolved in adequate quantity of diluted HCl and filtered.

Mayer's Test

Methanolic extract was treated with Mayer's reagent (Potassium Mercuric Iodide). The presence of alkaloids was confirmed by the formation of a yellow-coloured precipitate.

Wagner's Test

Methanolic extract was treated with Wagner's reagent (Iodine in Potassium Iodide). The presence of alkaloids was confirmed by the formation of a brown/reddish precipitate.

Dragendroff's Test

Methanolic extract was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). The presence of alkaloids was confirmed by the formation of a red precipitate.

Hager's Test

Methanolic extract was treated with Hager's reagent (saturated picric acid solution). Formation of the yellow-coloured precipitate indicates the presence of alkaloids

Detection of carbohydrates

Methanolic extract was dissolved in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

Molisch's Test

Methanolic extract was treated with 2 drops of alcoholic á-naphthol solution in a test tube. The presence of carbohydrates was confirmed by the formation of a violet ring at the junction.

Benedict's test

Methanolic extract was treated with Benedict's reagent and heated gently. Formation of orange red precipitate is an indication of the presence of reducing sugars.

Fehling's Test

Methanolic extract was hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solutions. The presence of reducing sugars was confirmed by the formation of red precipitate.

Detection of glycosides

Methanolic extract was hydrolysed with dilute HCl, and then subjected for glycosides test.

Modified Borntrager's Test

Methanolic extract was treated with ferric chloride solution and immersed in boiling water for about 5 minutes. Obtained mixture was then cooled and extraction was done with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. The presence of anthranol glycosides was confirmed by the formation of rose-pink colour.

Legal's Test

Methanolic extract was treated with sodium nitropruside in pyridine and sodium hydroxide. The presence of cardiac glycosides was confirmed by the appearance of pink to blood red colour.

Detection of saponins

Froth Test

Methanolic extract was diluted with 20ml of distilled water and this was mixed for 15 minutes. The presence of saponins was confirmed by the formation 1 cm layer of froth.

Foam Test

Using 2 ml of distilled water was mixed with 0.5 mg of methanolic extract and shaken. Formation and persistence of foam for ten minutes will indicate the presence of saponins.

Detection of phytosterols

Salkowski's Test

Methanolic extract was treated with chloroform and filtered. The filtrated extract was

Table 1. Qualitative phytochemical analysis of Acoathophora deilei

Phytochemicals	Extracts Observations	Results
Alkaloids:		
Wagner's test	Reddish Brown Solution precipitate	Present
Mayer's Test	Formation of yellow coloured precipitate	Present
Dragendroff's Test	Formation of red coloured precipitate	Present
Hager's Test	Formation of yellow coloured precipitate	Present
Flavonoids:		
Lead acetate test	Formation of yellow colour	Present
Alkaline Reagent	Formation of intense yellow colour, which becomes colourless on addition of dilute acid	Present
Phytosterols:		
Salkowski's Test :	Appearance of golden yellow colour precipitate	Present
Libermam Burchard's Test	Formation of brown ring at the junction indicates	Present
Diterpenes:		Present
Copper acetate Test	Formation of emerald green colour	
Carbohydrates:		
Molisch's test	Formation of violet ring at the junction indicates	Present
Benedict's Test	No Orange red precipitate	Absent
Fehling's Test	No formation of red coloured precipitate	Absent
Glycosides:		
Modified Borntrager's Test	No formation of rose-pink colour precipitate	Absent
Legal's Test	No formation of pink to blood red colour precipitate	Absent
Saponins:		
Froth Test	No formation of thin layer precipitate	Absent
FoamTest	Don't produced persists for 10 mintues	Absent
Phenols:		
Ferric chloride test	No formation of bluish black colour precipitate	Absent
Tannins:		
Gelatin Test	No formation of white colour precipitate	Present
Proteins and Amino acids:		
Xanthoproteic test	No formation yellow colour precipitate	Absent
Ninhydrin Test	No formation of blue colour precipitate	Absent

treated with few drops of conc. H_2SO_4, shaken and allowed to stand. Appearance of golden yellow colour is the indication of the presence of phytosterols.

Libermann Burchard's test

Methanolic extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. conc. H_2SO_4 was added. The presence of phytosterols was confirmed by the formation of brown ring at the junction.

Detection of phenols

Ferric Chloride Test

Methanolic extract was treated with 3-4 drops of ferric chloride solution. Appearance

Table 2. Retention time (rt), Molecular formula, Molecular weight (mw) and Percentage composition in the crude extract of the drug

SI. No	Retention time	Compound Name	Molecular formula	Molecular weight	Percentage area peak
1.	7.112	Acetamide,NN-diethyl-	$C_6H_{13}NO$	115	0.77
2	7.379	Acetamide, N- Ethyl-	C_4H_9NO	87	0.40
3	13.560	Caryophyllene	$C_{15}H_{24}$	204	0.40
4	14.542	Phenol,3,5-bis(1,1-dimethyethyl)	$C_{14}H_{22}O$	206	0.36
5	15.531	Diethyl phthalate	$C_{12}H_{14}O_4$	222	1.79
6	17.052	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	1.16
7	17.817	1-Heptadecene	$C_{17}H_{34}$	238	1.27
8	18.137	Pentadecanoic acid, Methyl ester	$C_{16}H_{32}O_2$	256	0.24
9	18.276	Neophytadiene	$C_{20}H_{38}$	278	0.42
10	18.331	2-Pentadecanone,6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.84
11	18.664	8-Octadecanone	$C_{18}H_{36}O$	268	0.64
12	19.043	Octadecane,1-Chloro-	$C_{18}H_{37}Cl$	288	0.39
13	19.176	Hexadecanoic acid, Methyl ester	$C_{17}H_{34}O_2$	270	28.58
14	19.513	Dibutyl Phthalate	$C_{18}H_{37}CL$	288	26.93
15	19.863	1-Heneicosanol	$C_{21}H_{44}O$	312	1.65
16	20.379	Hexadecanoic acid,2-hydroxy-,Methyl ester	$C_{17}H_{34}O_3$	286	1.36
17	20.643	10-Nonadecanone	$C_{19}H_{38}O$	282	0.27
18	20.872	9-Octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	296	7.20
19	21.105	Methyl stearate	$C_{19}H_{38}O_2$	298	1.59
20	21.217	Cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	0.92
21	21.432	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.30
22	21.730	n-tetracosanol-1	$C_{24}H_{50}O$	354	1.32
23	21.855	2-Ethylbutyric acid,octadecyl ester	$C_{24}H_{48}O_2$	368	12.74
24	22.296	5, 8, 11, 14- Eicosatetraenoic acid. Ethyl ester	$C_{22}H_{36}O_2$	332	0.33
25	23.137	Silane,methylvinyl(4-methylpent-2-yloxy)ethoxy	$C_{11}H_{42}O_2Si$	216	0.57
26	23.444	n-tetracosanol	$C_{24}H_{50}O$	354	1.11
27	24.558	1,2 Benzendicarboxylic acid	$C_{24}H_{38}O_4$	390	4.92
28	25.027	Octacosanol	$C_{24}H_{38}O_4$	390	0.55

Table 3. Important major compounds with their pharmacological activity of Acoathophora Deilei

No.	Chemical compounds	Molecular formula	Pharmacological activity
1.	Hexadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	Anti-bacterial and antifungal activity [18].
2.	9-octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	Anti-inflammatory, anti-androgenic, Anti- cancer activity, dermatitigenic, 5-alpha reductase inhibitor, anemiagenic and insectifuge activity [15].
3.	1,2 – Benzene dicarboxylic acid	$C_{24}H_{38}O_4$	Neurodegenerative disorders, Anti-cancer activity.

of bluish black colour is the indication for the presence of phenols.

Detection of tannins

Gelatin Test

Methanolic extract was added to 1% gelatin solution containing sodium chloride. The presence of tannins was confirmed by the formation of white precipitate.

Detection of flavonoids

Alkaline Reagent Test

Methanolic extract was treated with few drops of NaOH solution. Formation of intense yellow colour, which becomes colourless on addition of diluted acid, indicates the presence of flavonoids.

Lead acetate Test

Methanolic Extract was treated with few drops of lead acetate solution. Appearance of precipitates of yellow colour indicates the presence of flavonoids.

Detection of proteins and amino acids

Xanthoproteic Test

Methanolic extract was treated with few drops of concentrated Nitric acid. The presence of proteins was confirmed with the appearance of yellow colour.

Ninhydrin Test

0.25% w/v ninhydrin reagent was added to the extract and boiled for few minutes. Appearance of blue colour showed the presence of amino acid.

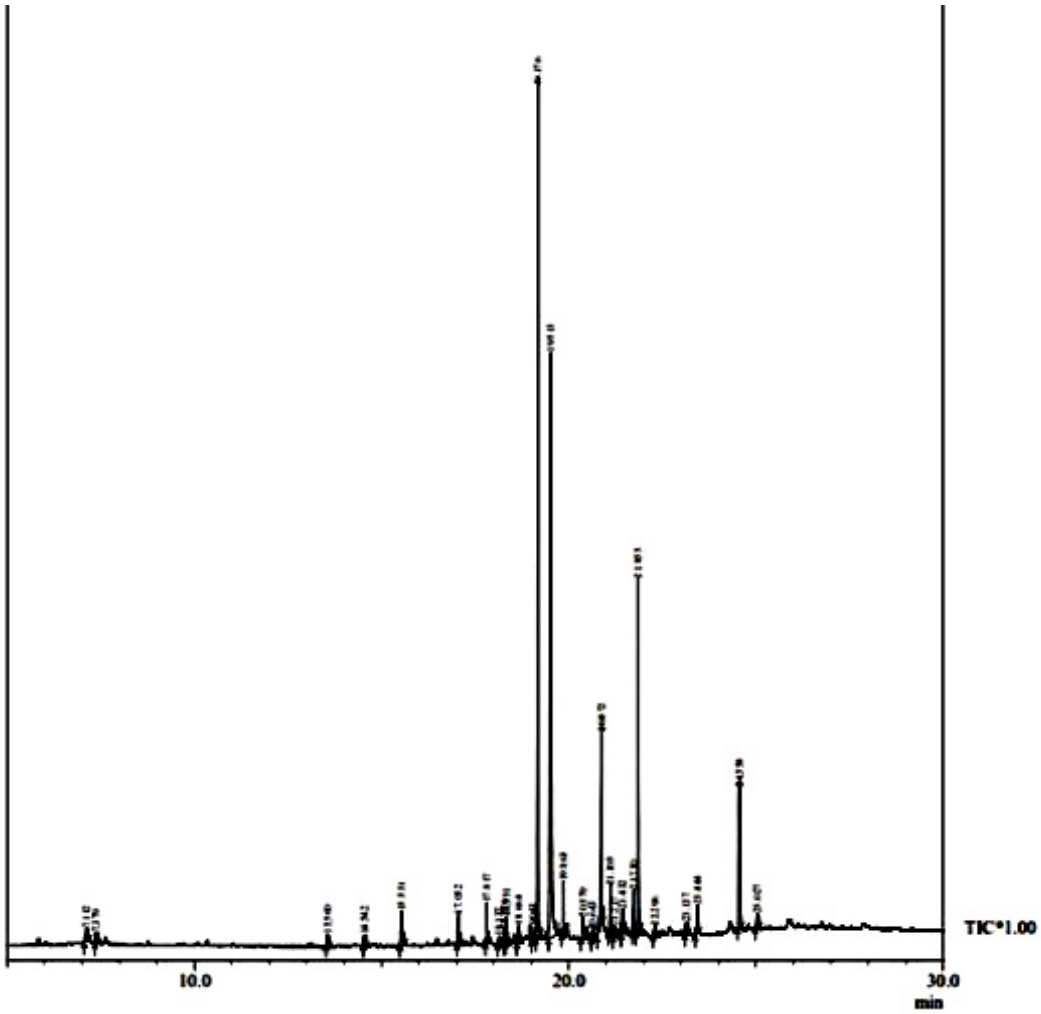

Fig.1. GC-MS Analysis of Methanolic Extract of *Acoathophora Deilei*

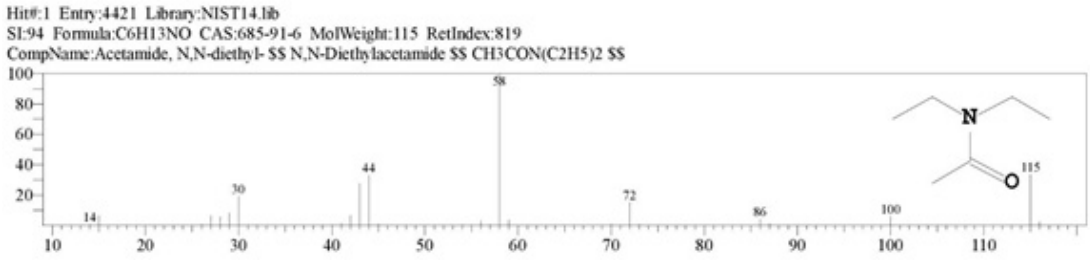

Figure. 2.1. Acetamide N,N diethyl-

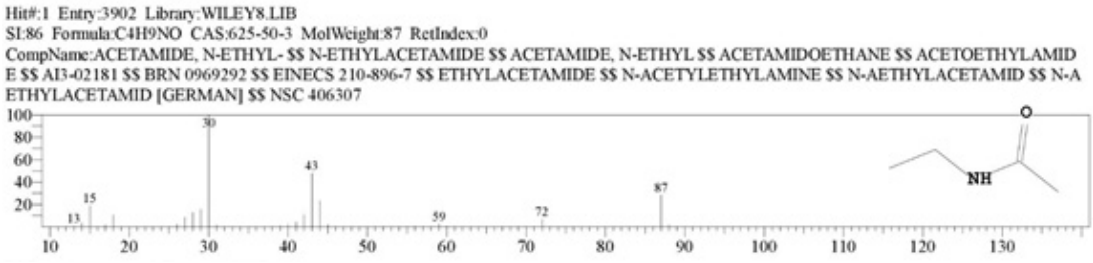

Figure. 2.2. Acetamide, N -Ethyl

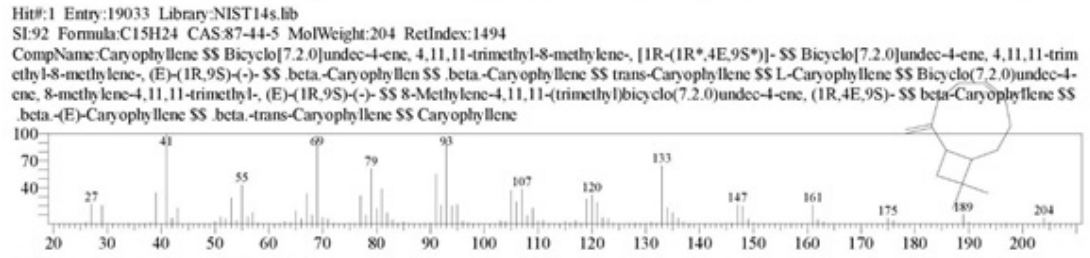

Figure. 2.3. Caryophyllene

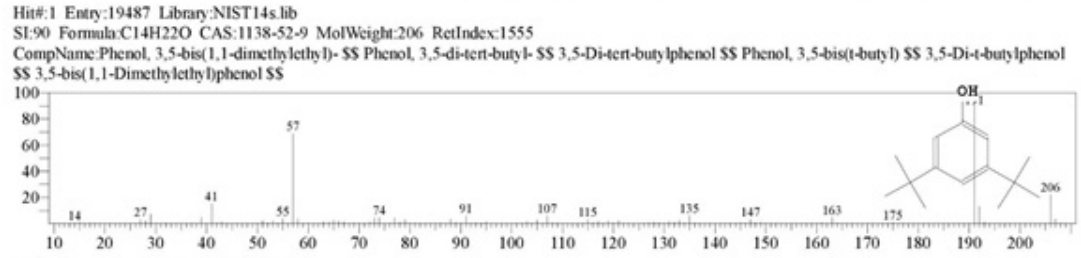

Figure. 2.4. Phenol, 3,5-bis (1,1-dimethylethyl)

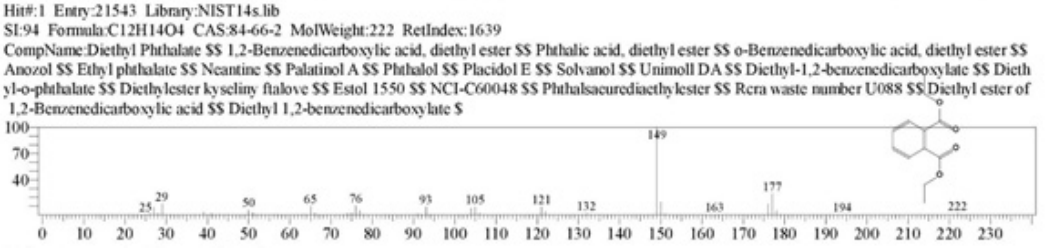

Figure. 2.5. Diethyl

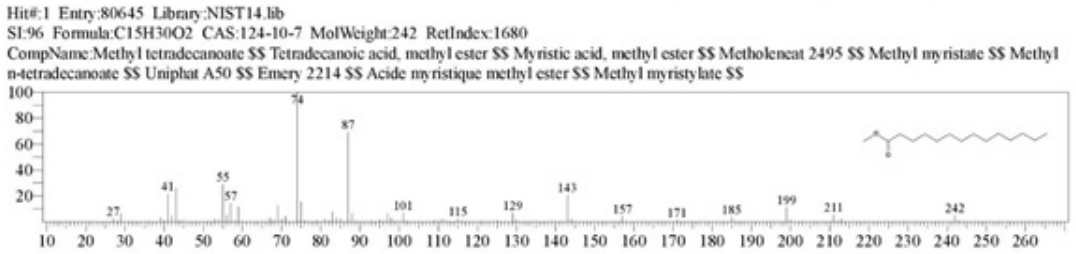

Figure. 2.6. Methyl tetradecanoate

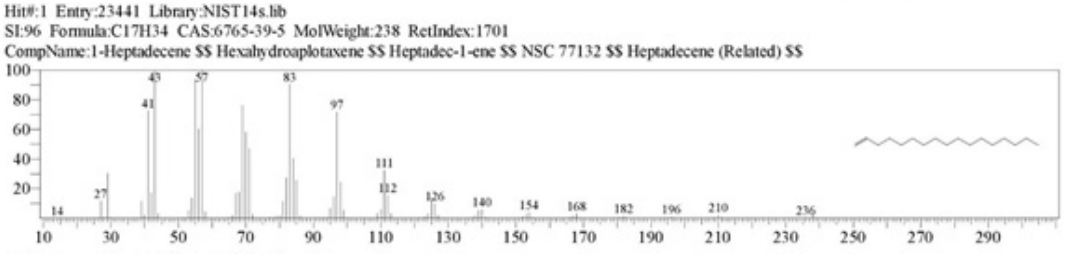

Figure. 2.7. 1-Heptadecene

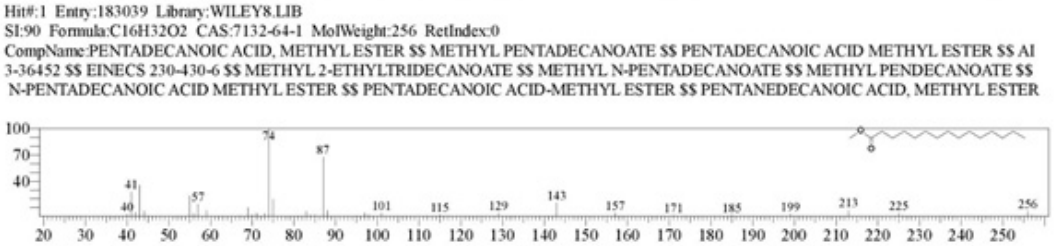

Figure. 2.8. Pentadecanoic acid, Methyl ester

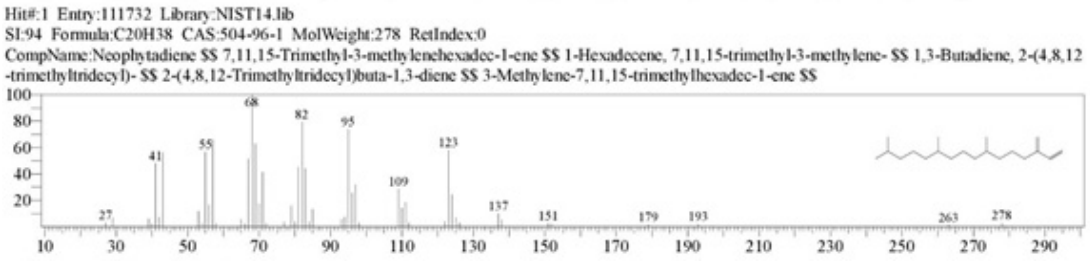

Figure. 2.9. Neophytadiene

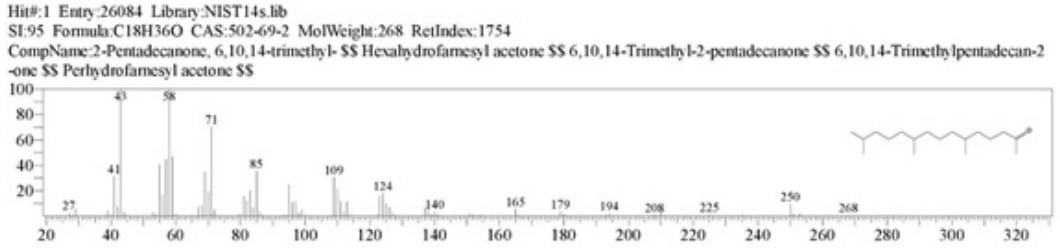

Figure. 2.10. 2-Pentadecanone,6,10,14-trimethyl-

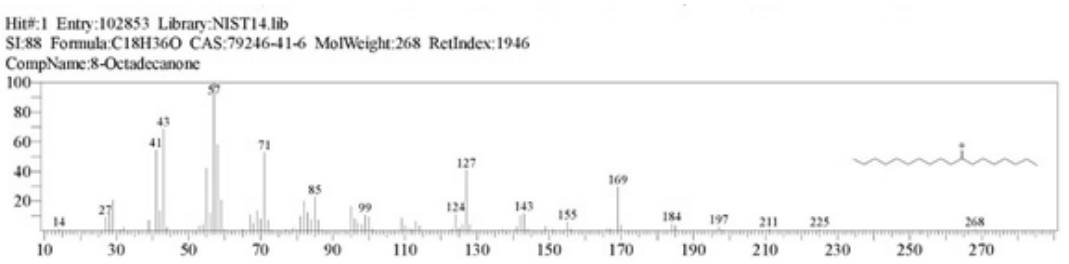

Figure. 2.11. 8-Octadecanone

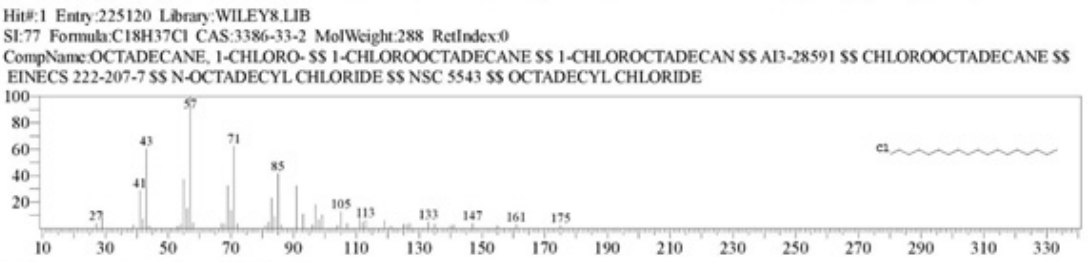

Figure. 2.12. Octadecane,1-Chloro-

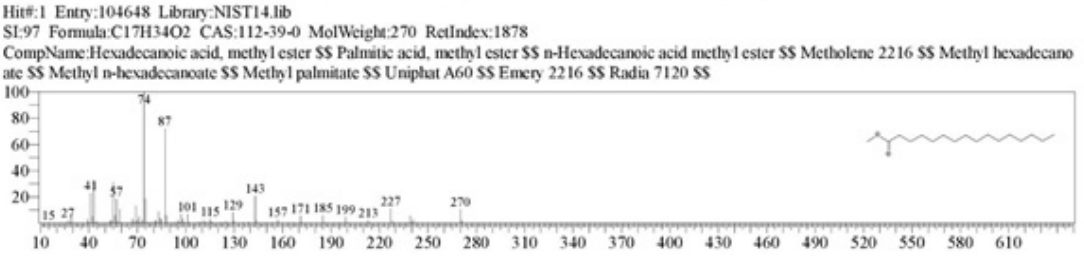

Figure. 2.13. Hexadecanoic acid, methyl ester

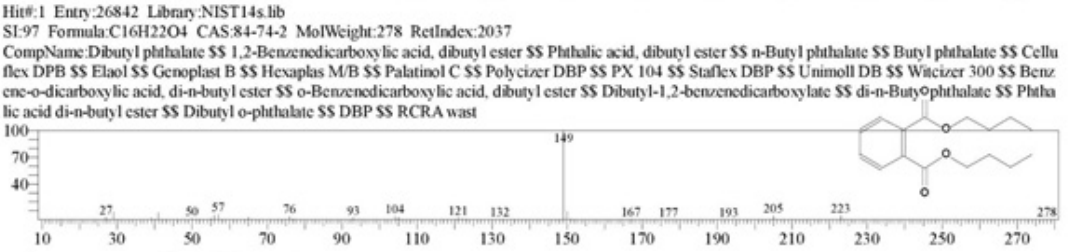

Figure. 2.14. Dibutyl phthalate

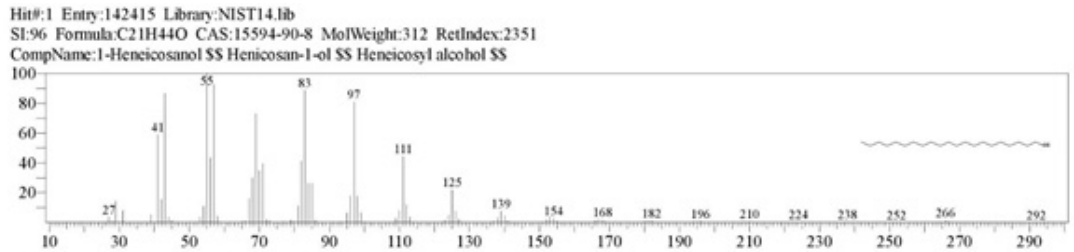

Figure. 2.15. 1-Heneicosanol

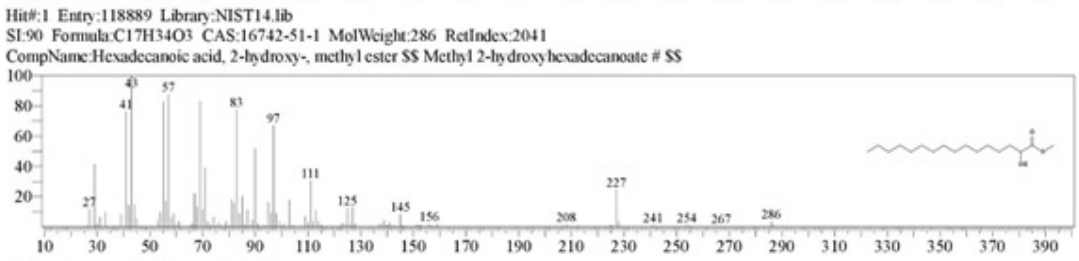

Figure. 2.16. Hexadecanoic acid,2-hydroxy-,methyl ester

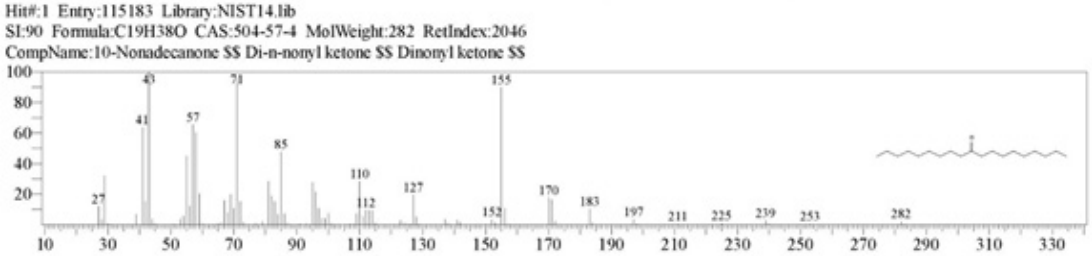

Figure. 2.17. 10-Nonadecanone

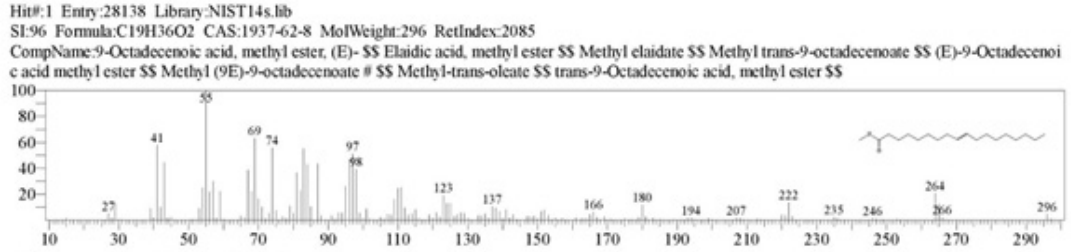

Figure. 2.18. 9-Octadecenoic acid, methyl ester,(E)-

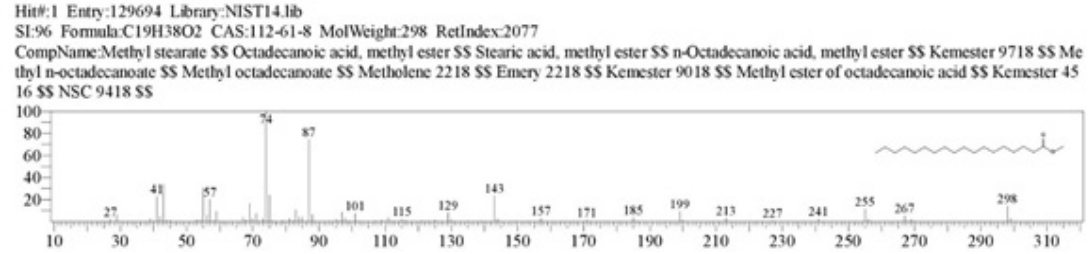

Figure. 2.19. Methyl stearate

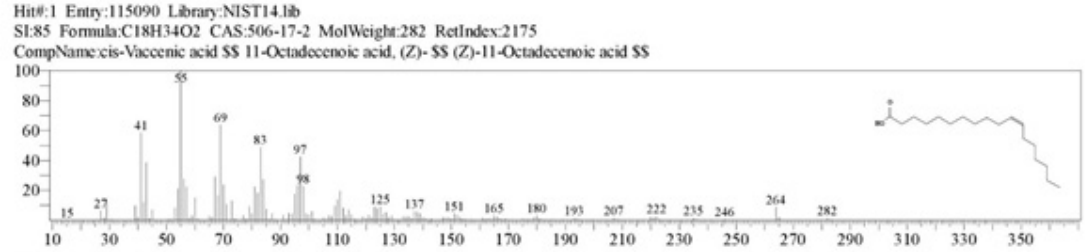

Figure. 2.20. Cis-Vaccenic acid

Hit#:1 Entry:117078 Library:NIST14.lib
SI:92 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:2167
CompName:Octadecanoic acid $$ Stearic acid $$ n-Octadecanoic acid $$ Humko Industrene R $$ Hydrofol Acid 150 $$ Hystrene S-97 $$ Hystrene T-70 $$ Hystrene 80 $$ Industrene R $$ Kam 1000 $$ Kam 2000 $$ Kam 3000 $$ Neo-Fat 18 $$ Neo-Fat 18-53 $$ Neo-Fat 18-54 $$ Neo-Fat 18-55 $$ Neo-Fat 18-59 $$ NAA 173 $$ PD 185 $$ Stearex Beads $$ Stearophanic acid $$ Steric acid $$ Vanicol $$ 1-Heptadecanecarboxylic acid $$ Heptadecanecarboxylic acid $$ Neo-fat 18-61 $$ Pearl stearic $$ Century 1240 $$ Dar-chem 14 $$ Emersol 120 $

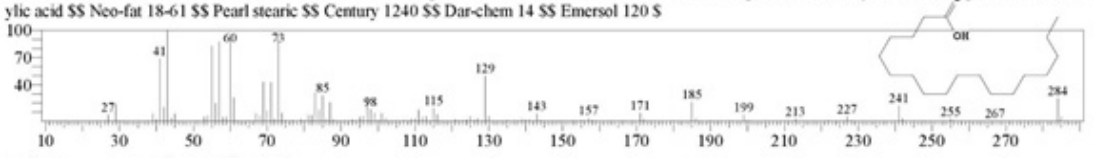

Figure. 2.21. Octadecanoic acid

Hit#:1 Entry:178097 Library:NIST14.lib
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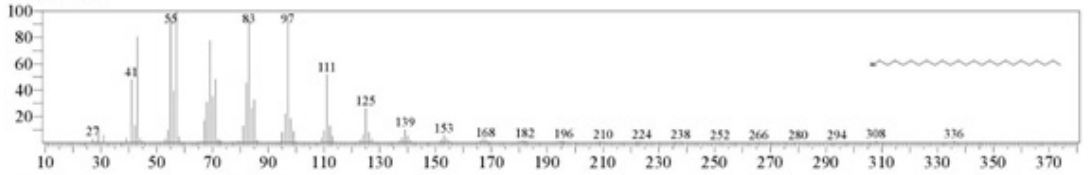

Figure. 2.22. N-tetracosanol-1

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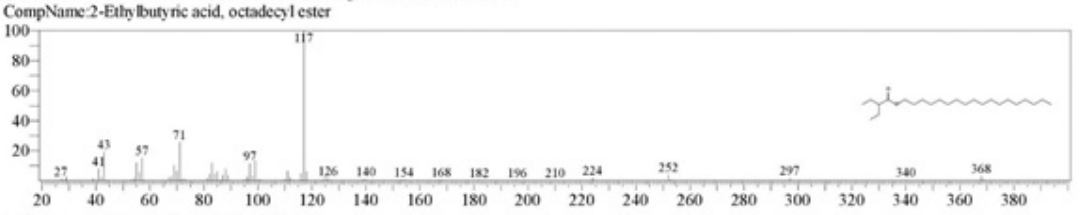

Figure. 2.23. 2-Ethylbutyric acid, Octadecyl ester

Hit#:1 Entry:277385 Library:WILEY8.LIB
SI:86 Formula:C22H36O2 CAS:1808-26-0 MolWeight:332 RetIndex:0
CompName:5,8,11,14-EICOSATETRAENOIC ACID, ETHYL ESTER, (ALL-Z)- $$ ETHYL (5Z,8Z,11Z,14Z)-5,8,11,14-ICOSATETRAENOATE # $$ 5,8,11,14-EICOSATETRAENOIC ACID, ETHYL ESTER $$ 5,8,11,14-EICOSATETRAENOIC ACID, ETHYL ESTER, (ALL Z)- $$ ARACHIDONIC ACID, ETHYL ESTER $$ ARACHIDONIC ACID, ETHYL ESTER (6CI,7CI,8CI) $$ ETHYL (5Z,8Z,11Z,14Z)-5,8,11,14-ICOSATETRAENOATE $$ ETHYL (5Z,8Z,11Z,14Z)-5,8,11,14-ICOSATETRAENOATE (COMPUTER-GENERATED NAME) $$ ETHYL 5,8,11,14-EICOSATETRAENOATE $$ ETHYL 5,8,11,14-EICOSATETRAENOATE (ALL Z) $$ E

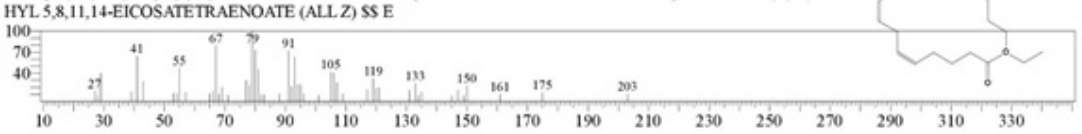

Figure. 2.24. 5,8,11,14-Eisosatetraenoic acid,Ethyl ester

Hit#:1 Entry:58865 Library:NIST14.lib
SI:75 Formula:C11H24O2Si CAS:0-00-0 MolWeight:216 RetIndex:0
CompName:Silane, methyl vinyl(4-methylpent-2-yloxy)ethoxy-

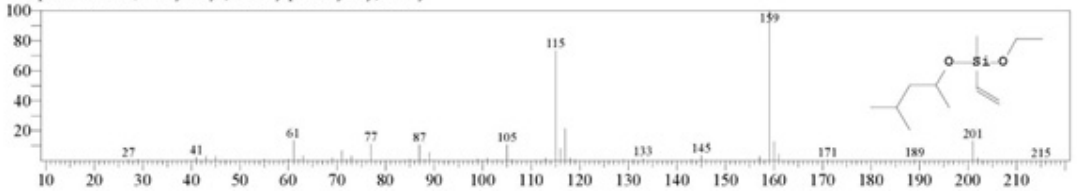

Figure. 2.25. Silane, methyl vinyl(4-methylpent-2-yloxy) ethoxy-

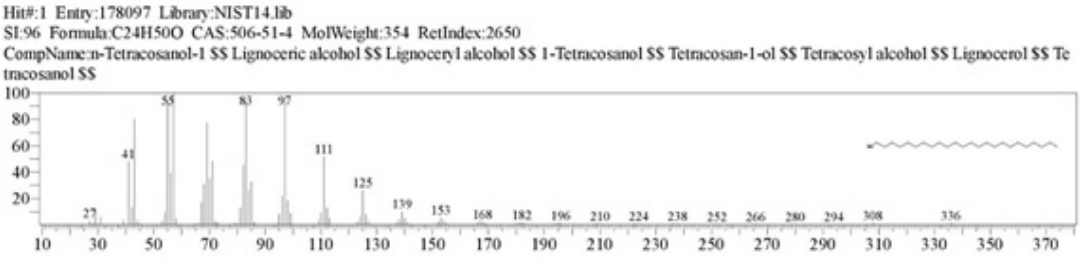

Figure. 2.26. N-tetracosanol

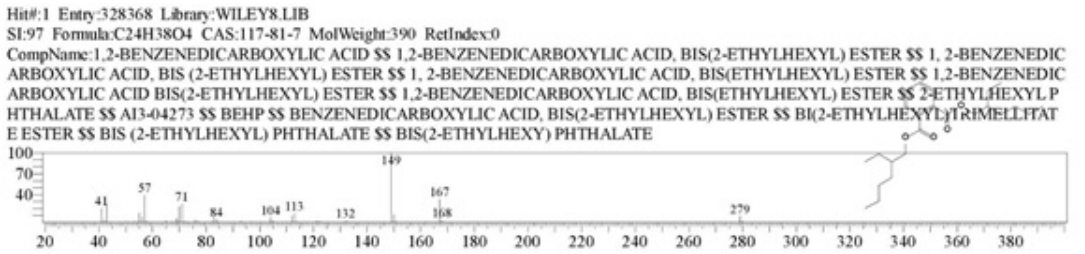

Figure. 2.27. 1,2Benzenedi Carboxy acid

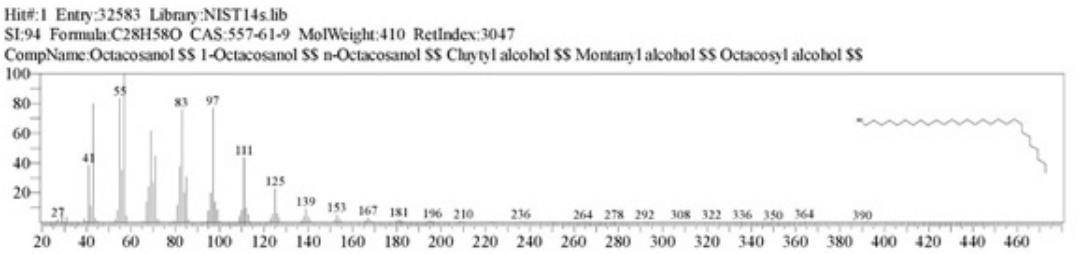

Figure. 2.28. Octacosanol

Detection of diterpenes

Copper acetate Test

Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Appearance of emerald green colour showed the presence of diterpenes.

Gas Chromatography- Mass Spectroscopy

Column Chromatography and Analysis

Derivatization procedure

GC-MS analysis of the active fractions of *A.deilei seaweed* was performed using GC SHIMADZU QP2010system and gas chromatograph interfaced to a mass spectrometer (GC-MS) *Acoathophora deilei* (Red algae) was extracted and concentrated by using rotary evaporator. The 1.5 ml upper layer of extract was taken in funnel and added 100µl N, O-Bis (trimethylsilyl) trifluoroacetamide, trimethyl chlorosilane (BSTFA+TMCS) and 20µl pyridine and heated at 60°c for 30 minutes. To this aceto nitrile was added and filtered into a conical flask. 50µl BSTFA+TMCS was added to the filtrate and heated at 60°c in a water bath for 30 minutes. Filtered using 0.45µ membrane filter to a vial[8].

Identification of components

Interpretation of mass spectrum GC-MS was done using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The name, molecular weight, molecular formula and structure of the component of the test material was as certained.

RESULTS AND DISCUSSION

The phytochemical screening showed that methanolic extract of *Acoathophora deilei*

was found to contain alkaloids, carbohydrates, phytosterols, flavonoids and diterpenes (Table -1). They were known to exhibit various medicinal and pharmacological activities[5]. Previous studies reported the presence of different phytochemical compounds of seaweeds collected from the coastal regions of the world[9,10]. Flavonoids have been proved with antitumor and anti-oxidant properties[11]. Alkaloids was found to have antimicrobial[12,13,14], cytotoxic[15] and antispasmodic properties[16,17].

GC-MS Interpretation

The components present in the crude extract of Acoathophora deilei (Red algae) were identified by GC-MS analysis. The Chromatograph is shown in figure-1. The various components with their retention time (RT), molecular formula, molecular weight (MW) and percentage composition in the crude extract of the drug was presented in Table -2

28 components were identified in the drug. The major compounds were hexadecanoic acid methyl ester (28.58%), followed by Dibutyl phthalate (26.93%), 2-Ethyl butyric acid, octadecyl ester (12.74%) ,9-Octadecanoic acid, methyl ester (7.20%), and 1,2- Benzendicarboxylic acid (4.92%) and their pharmacological importance activity in Table 3 .All other components were less than 4% and hence found to be less significance as their bioavailability is negligible. Figure (2.1 to 2.28) shows the mass spectrum and structures of the major phytol compounds. The biological activities listed are based on Dr.Dukes phytochemical and ethnobotanical databases by Dr. Jim Duke of the agricultural Research service, USDA.

Figures (2.1 To 2.28) Mass Spectrum And Structures Of The Major Phytol Compounds Of *Acoathophora Delilei.*

CONCLUSION

Phytochemical analysis of *Acoathophora deilei*. shown the presence of various metabolites such as flavonoids, alkaloids, phytosterols and Diterpenes are present. GC-MS analysis showed the presence of 28 different compounds of varied nature in methanolic extract of *Acoathophora deilei*. The present study is the first report to be published till date related to the GC-MS analysis of *Acoathophora deilei,* and it is a significant source for novel bio active compounds. Future studies (in vitro and in vivo) are required to prove the potential medicinal properties of the A*coathophora deilei* (red algae).

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