# 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)<sup>1</sup>. Algae are alternatives, especially marine algae, are least explored for their medicinal uses<sup>2</sup>.

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<sup>3</sup>. 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<sup>4</sup>.

#### 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)<sup>5,6,7</sup>.

#### **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:                 | Dalian Cala                                          | D 4      |
| 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:                   | r                                                    |          |
| 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:  |                                                      | 11000111 |
| Xanthoproteic test         | No formation yellow colour precipitate               | Absent   |
| Ninhydrin Test             | No formation of blue colour precipitate              | Absent   |

treated with few drops of conc. H<sub>2</sub>SO<sub>4</sub>, 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<sub>2</sub>SO<sub>4</sub> 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.<br>No | Retention time | Compound Name                                    | Molecular formula                                                                         | Molecular<br>weight | Percentage<br>area peak |
|-----------|----------------|--------------------------------------------------|-------------------------------------------------------------------------------------------|---------------------|-------------------------|
| 1.        | 7.112          | Acetamide,NN-diethyl-                            | C <sub>6</sub> H <sub>13</sub> NO                                                         | 115                 | 0.77                    |
| 2         | 7.379          | Acetamide, N- Ethyl-                             | $C_4H_0NO$                                                                                | 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                                | CHO.                                                                                      | 222                 | 1.79                    |
| 6         | 17.052         | Methyl tetradecanoate                            | $C_{15}H_{30}O_{2}$                                                                       | 242                 | 1.16                    |
| 7         | 17.817         | 1-Heptadecene                                    | $C_{15}H_{30}O_{2}$ $C_{17}H_{34}$ $C_{16}H_{32}O_{2}$ $C_{20}H_{38}$ $C_{18}H_{36}O_{3}$ | 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                                   | $C_{18}^{10}H_{36}^{30}O$                                                                 | 268                 | 0.64                    |
| 12        | 19.043         | Octadecane,1-Chloro-                             | $C_{18}^{13}H_{37}^{30}C1$                                                                | 288                 | 0.39                    |
| 13        | 19.176         | Hexadecanoic acid, Methyl ester                  | $C_{17}^{18}H_{34}^{37}O_2$                                                               | 270                 | 28.58                   |
| 14        | 19.513         | Dibutyl Phthalate                                | $C_{18}^{17}H_{37}^{34}CL$                                                                | 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        | $C_{17}^{21}H_{34}^{40}O_{3}$                                                             | 286                 | 1.36                    |
| 17        | 20.643         | 10-Nonadecanone                                  | $C_{19}^{17}H_{38}^{34}O$                                                                 | 282                 | 0.27                    |
| 18        | 20.872         | 9-Octadecanoic acid, methyl ester                | $C_{10}H_{11}O_{2}$                                                                       | 296                 | 7.20                    |
| 19        | 21.105         | Methyl stearate                                  | $C_{19}^{19}H_{38}^{36}O_{2}^{2}$                                                         | 298                 | 1.59                    |
| 20        | 21.217         | Cis-Vaccenic acid                                | $C_{18}^{19}H_{34}^{36}O_2^2$                                                             | 282                 | 0.92                    |
| 21        | 21.432         | Octadecanoic acid                                | $C_{10}H_{20}O_{2}$                                                                       | 284                 | 1.30                    |
| 22        | 21.730         | n-tetracosanol-1                                 | $C_{24}^{18}H_{50}^{30}O^{2}$                                                             | 354                 | 1.32                    |
| 23        | 21.855         | 2-Ethylbutyric acid,octadecyl ester              | $C_{24}^{24}H_{48}^{30}O_2$                                                               | 368                 | 12.74                   |
| 24        | 22.296         | 5, 8, 11, 14- Eicosatetraenoic acid. Ethyl ester | $C_{22}^{24}H_{36}^{40}O_{2}^{2}$                                                         | 332                 | 0.33                    |
| 25        | 23.137         | Silane,methylvinyl(4-methylpent-2-yloxy)ethoxy   | $C_{11}H_{42}O_{2}Si$                                                                     | 216                 | 0.57                    |
| 26        | 23.444         | n-tetracosanol                                   | $C_{11}^{21}H_{42}^{2}O_{2}^{2}Si$<br>$C_{24}^{2}H_{50}^{2}O$                             | 354                 | 1.11                    |
| 27        | 24.558         | 1,2 Benzendicarboxylic acid                      | $C_{24}H_{28}O_4$                                                                         | 390                 | 4.92                    |
| 28        | 25.027         | Octacosanol                                      | $C_{24}^{24}H_{38}^{38}O_4^4$                                                             | 390                 | 0.55                    |

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

| No.      | Chemical compounds                                                | Molecular<br>formula                                             | Pharmacological activity                                                                                                                                                                        |
|----------|-------------------------------------------------------------------|------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.<br>2. | Hexadecanoic acid, methyl ester 9-octadecanoic acid, methyl ester | $\begin{matrix} C_{16}H_{32}O_2 \\ C_{19}H_{36}O_2 \end{matrix}$ | Anti-bacterial and antifungal activity [18]. 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.

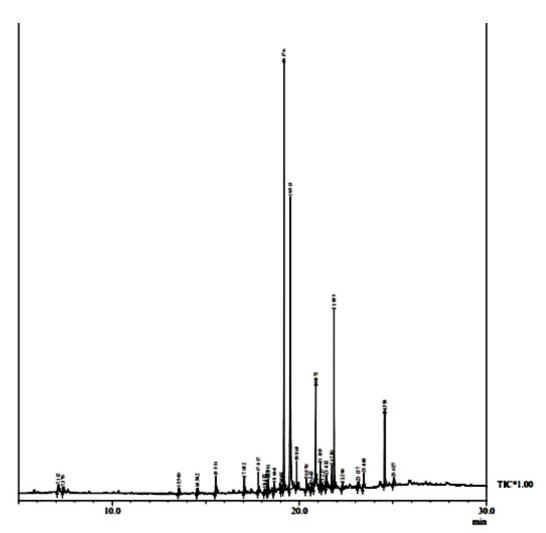


Fig.1. GC-MS Analysis of Methanolic Extract of Acoathophora Deilei

Hittl:1 Entry:4421 Library:NIST14.lib SI:94 Formula:C6H13NO CAS:685-91-6 MolWeight:115 RetIndex:819 CompName:Acetamide, N,N-diethyl-SS N,N-Diethylacetamide SS CH3CON(C2H5)2 SS

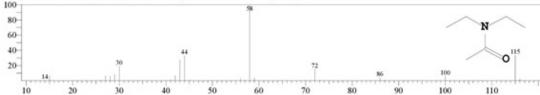


Figure. 2.1. Acetamide N,N diethyl-

Hit#:1 Entry:3902 Library:WILEY8.LIB

SI:86 Formula:C4H9NO CAS:625-50-3 MolWeight:87 RetIndex:0

CompName: ACETAMIDE, N-ETHYL-SS N-ETHYLACETAMIDE SS ACETAMIDE, N-ETHYL SS ACETAMIDOETHANE SS ACETOETHYLAMID E SS A13-02181 SS BRN 0969292 SS EINECS 210-896-7 SS ETHYLACETAMIDE SS N-ACETYLETHYLAMINE SS N-AETHYLACETAMID SS N-A ETHYLACETAMID [GERMAN] SS NSC 406307

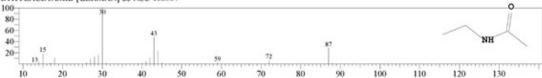


Figure. 2.2. Acetamide, N -Ethyl

Hit#:1 Entry:19033 Library:NIST14s.lib

SI:92 Formula:C15H24 CAS:87-44-5 MolWeight:204 RetIndex:1494

CompName:Caryophyllene \$\$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R\*,4E,9S\*)]- \$\$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (E)-(1R,9S)-(-)-\$\$ beta.-Caryophyllene \$\$ trans-Caryophyllene \$\$ trans-Caryophyllene \$\$ L.-Caryophyllene \$\$ Bicyclo(7,2.0)undec-4-ene, 8-methylene-4,11,11-trimethyl-, (E)-(1R,9S)-(-)-\$\$ 8-Methylene-4,11,11-(trimethyl)bicyclo(7,2.0)undec-4-ene, (1R,4E,9S)-\$\$ beta-Caryophyllene \$\$

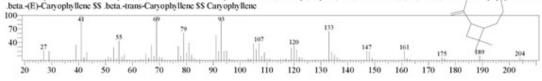
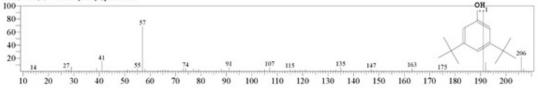


Figure. 2.3. Caryophyllene

Hit#:1 Entry:19487 Library:NIST14s.lib

SI:90 Formula:C14H22O CAS:1138-52-9 MolWeight:206 RetIndex:1555

CompName:Phenol, 3,5-bis(1,1-dimethylethyl)- \$\$ Phenol, 3,5-di-tert-butyl- \$\$ 3,5-Di-tert-butylphenol \$\$ Phenol, 3,5-bis(t-butyl) \$\$ 3,5-Di-t-butylphenol \$\$ 3,5-bis(1,1-Dimethylethyl)phenol \$\$



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

Hit#:1 Entry:21543 Library:NIST14s.lib

SI:94 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:1639

CompName:Diethyl Phthalate SS 1,2-Benzenedicarboxylic acid, diethyl ester SS Phthalic acid, diethyl ester SS o-Benzenedicarboxylic acid, diethyl ester SS Anozol SS Ethyl phthalate SS Neantine SS Patatinol A SS Phthalol SS Placidol E SS Solvanol SS Unimoll DA SS Diethyl-1,2-benzenedicarboxylate SS Diethyl-1,2-benzenedicarboxylate SS Diethyl-1,2-benzenedicarboxylate SS Diethyl ester of 1,2-Benzenedicarboxylic acid SS Diethyl 1,2-benzenedicarboxylate S

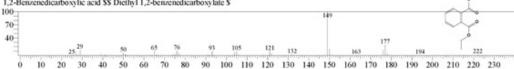


Figure. 2.5. Diethyl

Hit#:1 Entry:80645 Library:NIST14.lib

SI:96 Formula:C15H30O2 CAS:124-10-7 MolWeight:242 RetIndex:1680

CompName:Methyl tetradecanoate \$\$ Tetradecanoic acid, methyl ester \$\$ Myristic acid, methyl ester \$\$ Metholeneat 2495 \$\$ Methyl myristate \$\$ Methyl n-tetradecanoate \$\$ Uniphat A50 \$\$ Emery 2214 \$\$ Acide myristique methyl ester \$\$ Methyl myristylate \$\$

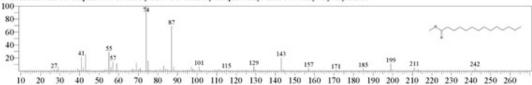


Figure. 2.6. Methyl tetradecanoate

Hit#:1 Entry:23441 Library:NIST14s.lib

SI:96 Formula:C17H34 CAS:6765-39-5 MolWeight:238 RetIndex:1701

CompName:1-Heptadecene \$\$ Hexalty droaplotaxene \$\$ Heptadec-1-ene \$\$ NSC 77132 \$\$ Heptadecene (Related) \$\$

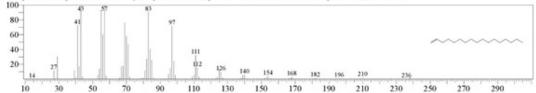


Figure. 2.7. 1-Heptadecene

Hit#:1 Entry:183039 Library:WILEY8.LIB

SI:90 Formula:C16H32O2 CAS:7132-64-1 MolWeight:256 RetIndex:0

CompName:PENTADECANOIC ACID, METHYL ESTER \$\$ METHYL PENTADECANOATE \$\$ PENTADECANOIC ACID METHYL ESTER \$\$ AI 3-36452 \$\$ EINECS 230-430-6 \$\$ METHYL 2-ETHYLTRIDECANOATE \$\$ METHYL N-PENTADECANOATE \$\$ METHYL PENDECANOATE \$\$ N-PENTADECANOIC ACID METHYL ESTER \$\$ PENTADECANOIC ACID METHYL ESTER \$\$ PENTADECANOIC ACID.

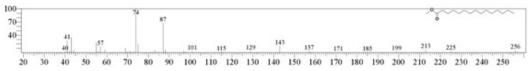


Figure. 2.8. Pentadecanoic acid, Methyl ester

Hit#:1 Entry:111732 Library:NIST14.lib

SI:94 Formula:C20H38 CAS:504-96-1 MolWeight:278 RetIndex:0

CompName:Neophytadiene \$\$ 7,11,15-Trimethyl-3-methylenehexadec-1-ene \$\$ 1-Hexadecene, 7,11,15-trimethyl-3-methylene-\$\$ 1,3-Butadiene, 2-(4,8,12-trimethyltridecyl)-\$\$ 2-(4,8,12-Trimethyltridecyl)-\$\$ 2-(4,8,12-Trimethyltridecyl)-\$\$ 3-Methylene-7,11,15-trimethyltridecyl-ene \$\$

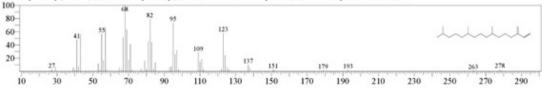


Figure. 2.9. Neophytadiene

Hit#:1 Entry:26084 Library:NIST14s.lib

SI:95 Formula:C18H36O CAS:502-69-2 MolWeight:268 RetIndex:1754

CompName:2-Pentadecanone, 6,10,14-trimethyl- \$\$\text{ Hexahydrofarnesyl acetone \$\$}6,10,14-Trimethyl-2-pentadecanone \$\$}6,

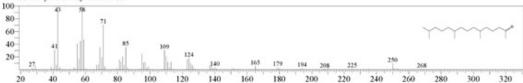


Figure. 2.10. 2-Pentadecanone,6,10,14-trimethyl-

Hit#;1 Entry:102853 Library:NIST14.lib SI:88 Formula:C18H36O CAS:79246-41-6 MolWeight:268 RetIndex:1946 CompName:8-Octadecanone

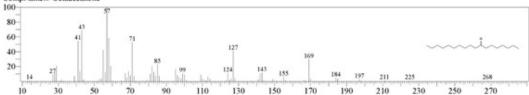


Figure. 2.11. 8-Octadecanone

Hit#:1 Entry:225120 Library:WILEY8.LIB

SI:77 Formula:C18H37Cl CAS:3386-33-2 MolWeight:288 RetIndex:0

CompName:OCTADECANE, 1-CHLORO-SS 1-CHLOROOCTADECANE SS 1-CHLOROCTADECAN SS A13-28591 SS CHLOROOCTADECANE SS EINECS 222-207-7 SS N-OCTADECYL CHLORIDE SS NSC 5543 SS OCTADECYL CHLORIDE

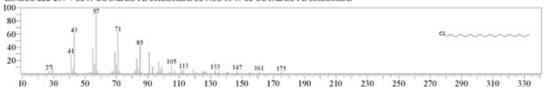


Figure. 2.12. Octadecane, 1-Chloro-

Hit#:1 Entry:104648 Library:NIST14.lib SI:97 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878

CompName:Hexadecanoic acid, methyl ester \$\$ Palmitic acid, methyl ester \$\$ n-Hexadecanoic acid methyl ester \$\$ Metholene 2216 \$\$ Methyl hexadecano ate SS Methyl n-hexadecanoate SS Methyl palmitate SS Uniphat A60 SS Emery 2216 SS Radia 7120 SS

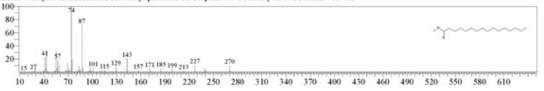


Figure. 2.13. Hexadecanoic acid, methyl ester

Hit#:1 Entry:26842 Library:NIST14s.lib

SI:97 Formula:C16H22O4 CAS:84-74-2 MolWeight:278 RetIndex:2037

CompName:Dibutyl phthalate \$\$ 1,2-Benzenedicarboxylic acid, dibutyl ester \$\$ Phthalic acid, dibutyl ester \$\$ n-Butyl phthalate \$\$ Butyl phthalate \$\$ Cellu flex DPB \$\$ Elaol \$\$ Genoplast B \$\$ Hexaplas M/B \$\$ Palatinol C \$\$ Polycizer DBP \$\$ PX 104 \$\$ Staflex DBP \$\$ Unimoll DB \$\$ Witeizer 300 \$\$ Benzened-o-dicarboxylic acid, di-n-butyl ester \$\$ o-Benzenedicarboxylic acid, dibutyl ester \$\$ Dibutyl-1,2-benzenedicarboxylate \$\$ di-n-ButyOphthalate \$\$ Phtha lic acid di-n-butyl ester \$\$ Dibutyl o-phthalate \$\$ DBP \$\$ RCRA wast

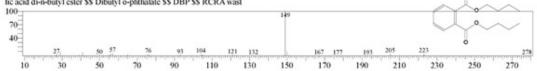


Figure. 2.14. Dibutyl phthalate

Hit#:1 Entry:142415 Library:NIST14.lib

SI:96 Formula:C21H44O CAS:15594-90-8 MolWeight:312 RetIndex:2351 CompName:1-Heneicosanol \$\$ Henicosan-1-ol \$\$ Heneicosyl alcohol \$\$

100 80-60-40 20-10 30 50 70 90 110 130 150 170 190 210 230 250 270 290

Figure. 2.15. 1-Heneicosanol

Hit#:1 Entry:118889 Library:NIST14.lib SI:90 Formula:C17H34O3 CAS:16742-51-1 MolWeight:286 RetIndex:2041 CompName:Hexadecanoic acid, 2-hydroxy-, methyl ester \$\$ Methyl 2-hydroxyhexadecanoate # \$\$ 100 80-60 40 20 254 110 150 210 290 10 30 50 70 90 130 170 190 230 250 270 310 330 350 370 390

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

Hit#:1 Entry:115183 Library:NIST14.lib

SI:90 Formula:C19H38O CAS:504-57-4 MolWeight:282 RetIndex:2046

CompName:10-Nonadecanone SS Di-n-nonyl ketone SS Dinonyl ketone SS

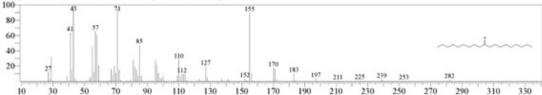


Figure. 2.17. 10-Nonadecanone

Hit#:1 Entry:28138 Library:NIST14s.lib

SI:96 Formula:C19H36O2 CAS:1937-62-8 MolWeight:296 RetIndex:2085

CompName:9-Octadecenoic acid, methyl ester, (E)- \$\$ Elaidic acid, methyl ester \$\$ Methyl elaidate \$\$ Methyl trans-9-octadecenoate \$\$ (E)-9-Octadecenoi c acid methyl ester \$\$ Methyl (9E)-9-octadecenoate # \$\$ Methyl-trans-oleate \$\$ trans-9-Octadecenoic acid, methyl ester \$\$

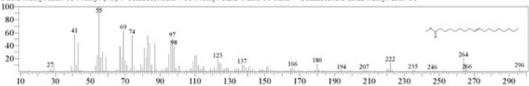


Figure. 2.18. 9-Octadecenoic acid, methyl ester, (E)-

Hit#:1 Entry:129694 Library:NIST14.lib SI:96 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077

CompName:Methyl stearate \$\$ Octadecanoic acid, methyl ester \$\$ Stearic acid, methyl ester \$\$ n-Octadecanoic acid, methyl ester \$\$ Kemester 9718 \$\$ Me thyl n-octadecanoate \$\$ Methyl octadecanoate \$\$ Metholene 2218 \$\$ Emery 2218 \$\$ Kemester 9018 \$\$ Methyl ester of octadecanoic acid \$\$ Kemester 45 16 \$\$ NSC 9418 \$\$

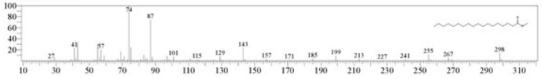


Figure. 2.19. Methyl stearate

Hit#:1 Entry:115090 Library:NIST14.lib SI:85 Formula:C18H34O2 CAS:506-17-2 MolWeight:282 RetIndex:2175

CompName:cis-Vaccenic acid \$\$ 11-Octadecenoic acid. (Z)-\$\$ (Z)-11-Octadecenoic acid \$\$

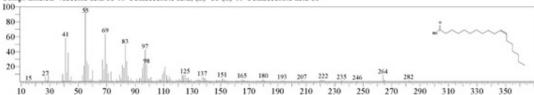


Figure. 2.20. Cis-Vaccenic acid

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 18-54 \$\$ Neo-Fat 18-54 \$\$ Neo-Fat 18-55 \$\$ Neo-Fat 18-54 \$\$ Neo-Fat 18-55 \$\$ Neo-Fat 18-55 \$\$ Neo-Fat 18-55 \$\$ Neo-Fat 18-54 \$\$ Neo-Fat 18-54 \$\$ Neo-Fat 18-54 \$\$ Neo-Fat 18-54 \$\$ Neo-Fat 18-55 \$\$

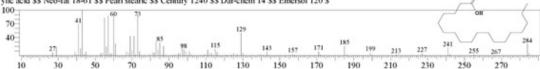


Figure. 2.21. Octadecanoic acid

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

SI:96 Formula:C24H50O CAS:506-51-4 MolWeight:354 RetIndex:2650

CompName:n-Tetracosanol-1 \$\$ Lignoceric alcohol \$\$ Lignoceryl alcohol \$\$ 1-Tetracosanol \$\$ Tetracosan-1-ol \$\$ Tetracosyl alcohol \$\$ Lignocerol \$\$ Lignocerol

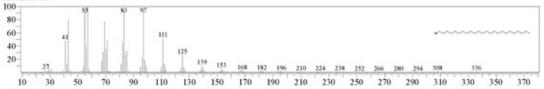


Figure. 2.22. N-tetracosanol-1

Hit#:1 Entry:187855 Library:NIST14.lib

SI:82 Formula:C24H48O2 CAS:0-00-0 MolWeight:368 RetIndex:2510

CompName:2-Ethylbutyric acid, octadecyl ester

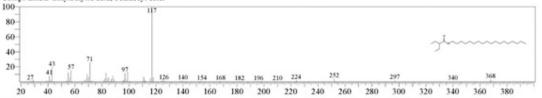


Figure. 2.23. 2-Ethylbutyric acid, Octadecyl ester

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)- SS ETHYL (5Z,8Z,11Z,14Z)-5,8,11,14-ICOSATETRAENOIC #SS 5,8,11,14-EICOSATETRAENOIC ACID, ETHYL ESTER, (ALL Z)- SS ARACHIDONIC ACID, ETHYL ESTER (6CL,7CL,8Cl) SS ETHYL (5Z,8Z,11Z,14Z)-5,8,11,14-ICOSATETRAENOATE SS ETHYL (5Z,8Z,11Z,14Z)-5,8,11,14-ICOSATETRAENOATE SS ETHYL 5Z,811,14-EICOSATETRAENOATE (COMPUTER-GENERATED NAME) SS ETHYL 5,8,11,14-EICOSATETRAENOATE (ST. ELL) SS ETHYL 5Z,811,14-EICOSATETRAENOATE (ALL Z) SS ETHYL 5Z,811,14-EICOSATETR

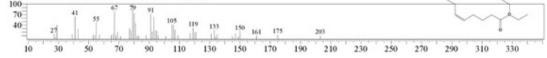


Figure. 2.24. 5,8,11,14-Eisosatetraenoic acid,Ethyl ester

Hit#:1 Entry:58865 Library:NIST14.lib

SI:75 Formula:C11H24O2Si CAS:0-00-0 MolWeight:216 RetIndex:0

CompName:Silane, methylvinyl(4-methylpent-2-yloxy)ethoxy-100 80-115 Si-0 60-40 20-10 30 40 100 110 120 130 140 150 170 210 160 180 190 200

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

380

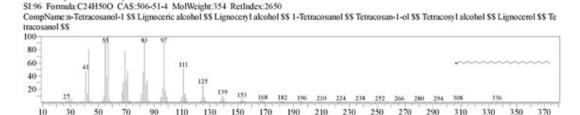


Figure. 2.26. N-tetracosanol

Hit#:1 Entry:328368 Library:WILEY8.LIB SI:97 Formula:C24H38O4 CAS:117-81-7 MolWeight:390 RetIndex:0 CompName: 1, 2-BENZENEDIC ARBOXYLIC ACID SS 1, 2-BENZENEDIC ARBOXYLIC ACID, BIS(2-ETHYLHEXYL) ESTER SS 1, 2-BENZENEDIC ARBOXYLIC ACID, BIS (2-ETHYLHEXYL) ESTER \$\$ 1, 2-BENZENEDICARBOXYLIC ACID, BIS(ETHYLHEXYL) ESTER \$\$ 1, 2-BENZENEDIC ARBOXYLIC ACID BIS(2-ETHYLHEXYL) ESTER \$\$ 1,2-BENZENEDICARBOXYLIC ACID, BIS(ETHYLHEXYL) ESTER \$\$ 2-ETHYLHEXYL P HTHALATE \$\$ A13-04273 \$\$ BEHP \$\$ BENZENEDICARBOXYLIC ACID, BIS(2-ETHYLHEXYL) ESTER \$\$ BI(2-ETHYLHEXYL) INMELLITAT E ESTER \$\$ BIS (2-ETHYLHEXYL) PHTHALATE \$\$ BIS(2-ETHYLHEXY) PHTHALATE 70 40 104 113 168

200 Figure. 2.27. 1,2Benzenedi Carboxy acid

220

240

260

280 300 320

160 180

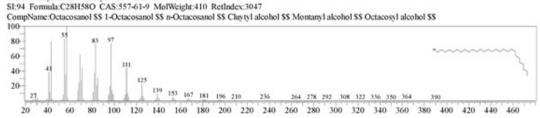


Figure. 2.28. Octacosanol

# **Detection of diterpenes** Copper acetate Test

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

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.

80

Hit#:1 Entry:32583 Library:NIST14s.lib

100 120 140

# **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 20ul 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<sup>8</sup>.

# **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<sup>5</sup>. Previous studies reported the presence of different phytochemical compounds of seaweeds collected from the coastal regions of the world<sup>9,10</sup>. Flavonoids have been proved with antitumor and anti-oxidant properties<sup>11</sup>. Alkaloids was found to have antimicrobial<sup>12,13,14</sup>, cytotoxic<sup>15</sup> and antispasmodic properties<sup>16,17</sup>.

### **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 Acoathophora deilei (red algae).

#### **ACKNOWLEDGEMENT**

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#### REFERENCES

- Murugeshamuthaliyar Gunapadam thathu Geevan 2<sup>nd</sup>, Directorate of Indian Medicine & homeopathy Thurai Publication, third edition 2008
- 2. Rizvi SI,Mishra N.Traditional Indian medicines used for the management of diabetes mellitus. *J Diabetes Res*, **13**;pp1-11 (2013). http://dx.doi.org/10.1155/2013/712092.
- 3. Kaliaperumal. N., Seaweeds diversity, resources, utilization conservation and cultivation. In: Abstracts of the National seminar on Marine resources: Sustainable utilization and Conservation, organized by department of Plant Biology and Biotechnology. St. Mary's College Thooothukudi. P.1; (2009).
- 4. Prashant T, Kumar B, Mandeep K, Gurpreet K, Harleen K: Phytochemical Screening and Extraction: A Review; *Internationale Pharmaceutica Sciencia*; 1(1); 98-106 (2011).
- Sofowara A . Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289 (1993).
- Trease GE and Evans WC; Pharmacognosy 11<sup>th</sup> edition; Brailiar Tiridel can.Macmilian publishers (1989).
- Harborne J B: Phytochemical methods, chapman and hall, London (1973).
- 8. Aneesh. T.P,Elizabeth T, Della G T, Anandan R : GC-MS analysis of Phytochemical compounds present in the rhizomes of Nervilia Aragoana GAUD; *Asian Journal of Pharmaceutical and Clinical Research*, **6**(3), pp-68-74 (2013).
- 9. Sachithananthan K., Sivapalan A. Antibacterial properties of some marine algae of Sri Lanka. Bulletin of Fisheries Research Station, Sri Lanka.; 26:5-9; (1975).
- 10. Vidhyavathi, N and Sridhar, K.R. Seasonal and Geographical variations in the antimicrobial activity of seaweeds from the Mangalore coast in India. *Bot. Mar;* **34**: 279-284 (1991).

- 11. Cody V; Middleton E; Harborne JB; Baretze A. Plant flavonoids. In: Biology and medicine II: Biochemical, cellular and medicinal properties. Alan R. Liss Inc, New York, 330 (1988).
- Omulokoli E, Khan B, Chhabra SC. Antiplasmodial activity of four Kenyan medicinal plants. J. Ethnopharmacol. 56: 133-137 (1997).
- Cowan, M.M. Plant products as antimicrobial agents. Clinical Microbiology Reviews; 12: 564-582; (1999).
- Srivastava, N., K. Saurav, V. Mohanasrinivasan, K. Kannabiran and M. Singh. Antibacterial Potential of Macroalgae Collected from the Mandapam Coast, *India British J. Pharmacol.* and Toxicol., 1(2): 72-76 (2010).
- 15. Nobori T; Miurak K; Wu DJ; Takabayashik CA; Carson DA. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers *Nature*: **46**; 753-756 (1994).
- Stray F. The natural guide to medicinal herbs and plants. Tiger Books International, London:12-16; (1998).
- 17. DE Okwu and ME Okwu. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. J. Sustain. *Agric. Environ.*, **6**(2): 140-147; (2004).
- 18. Krishnamoorthy.K, Subramaniam .P; Phytochemical profiling of leaf, stem an tuber parts of solena amplexicaulis (lami) Gandhi using GC-MS: International scholarly research notices, vol pp1-13; (2014).