



## GC-MS AND IR STUDIES OF ETHANOLIC EXTRACT OF COLONIAL ASCIDIAN- *POLYCLINUM MADRASENSIS* SEBASTIAN, 1952

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### ABSTRACT

Many marine origin organic molecules have entered clinical studies for their medicinal value. Ascidians, a marine sedentary urochordate has drawn the attention of researchers for its versatility as medicine and nutrition. *Polyclinum madrasensis* is a colonial ascidian found in abundance along Tuticorin coast. The ethanolic extract of *Polyclinum madrasensis* was subjected to GC-MS study to determine the possible bioactive components. The analysis revealed the presence of twenty two compounds. Chemical components which are biologically active include - n-Hexadecanoic acid, 4,8-dimethyl-Nonane-1-ol, 3-methyl-1,2-Cyclopentane-diol, 1,2-diisooctyl ester of Benzenedicarboxylic acid, 1-Iodo-Tridecane, 2-butyl-octan-1-ol, Squalene, Cholesterol, Cholestan-3-ol, Dihydrotachysterol, 3-hydroxy-, (3á,17á)-Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one and Diazoprogesterone. Major compounds are Cholesterol (23.01), Cholestan-3-ol (14.22), Squalene (10.50), n-Hexadecanoic acid (10.48) and Dihydrotachysterol (7.94). Of the twenty two compounds identified, thirteen compounds were reported for the first time from the ascidians of Indian water namely 4,8-dimethyl-Nonane-1-ol, (E)-5-Octadecene, 3-methyl-1,2-Cyclopentane-diol, 4,6,8-trimethyl-1-Nonane, Bis(2-ethylhexyl) ester of Hexanedioic acid, Methyl 2-hydroxydodecanoate, 1-Iodo-Tridecane, 2-butyl-octan-1-ol, (1,2-dimethylpropyl)-1-methyl-2-nonyl-cyclopropane, 6-methyl-octadecane, 2-oxo- methyl ester of Octadecanoic acid, Dihydrotachysterol and 1,8,15,22-Tricosatetrayne. These compounds exhibited many biological activities such as anticancer, antiarthritic, antiasthmatic, antimicrobial, antioxidant, antiandrogenic, pesticide and so on. Infrared (IR) spectral study of this species indicates the presence of primary free N-H stretching, alkanes -CH<sub>2</sub>- stretching, carboxylic acid and carbonyl compound.

**KEYWORDS:** Ascidians, *Polyclinum madrasensis*, GC-MS, IR Spectral studies, biological activities



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## INTRODUCTION

The search for marine derived products started 50 years ago and over the past few decades, about 3000 new compounds from various marine sources have been tested so far<sup>1</sup>. A large proportion of these have been extracted from marine animals, especially sponges, ascidians, bryozoans, molluscs and some of them are currently in clinical trials<sup>2</sup>. Ascidians are marine animals which ranks second having a wealth of interesting pharmacological substances<sup>3</sup> with promising source of drugs<sup>4</sup>. They are sessile, conspicuous and important members of shallow benthic communities having rich source of nitrogenous secondary metabolites which can act as chemical defense preventing the attachment of other bio fouling organisms on their surface<sup>5</sup>. Ascidians belong to phylum chordata and class Ascidiacea. *Polyclinum madrasensis* is a colonial ascidian (Polyclinidae) found from the littoral zone to deep sea. Review of literature indicates that work related to antitumour and antibacterial activity<sup>6,7</sup> has been carried out with *Polyclinum madrasensis*, but a systematic chemical screening has not been attempted at all. Hence the objective of the present investigation is to identify the possible chemical constituents with the aid of GC-MS and IR spectral studies.

## MATERIALS AND METHODS

### (i) Collection of animal

*Polyclinum madrasensis* Sebastian, 1952 was collected from the intertidal rocky area of Tuticorin Harbour during the low tide. The collected samples were rinsed with sterile seawater to remove associated debris.

### (ii) Preparation of animal extract

The whole animal was dried in shade, powdered and extracted using ethanol as solvent. 2 $\mu$ L of the ethanolic extract was employed for GC-MS and IR spectral study.

### (iii) GC – MS Analysis

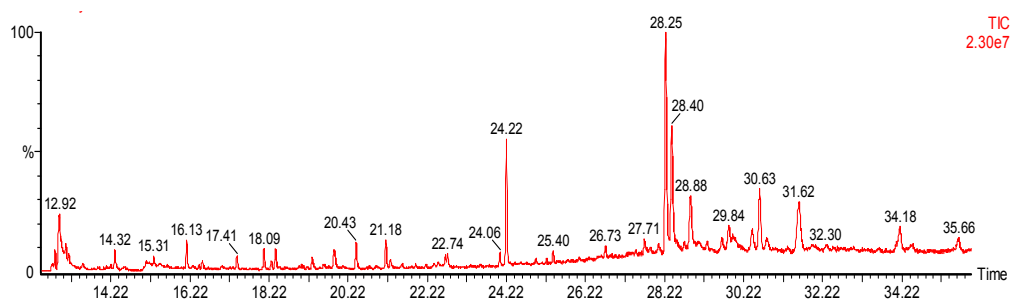
GC – MS analysis was carried out on a GC Clarus 500 Perkin Elmer comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC – MS) instrument employing the following conditions: column Elite-5MS fused silica capillary column (30mm x 0.25mm x 0.25 $\mu$ m df, composed of 5% Diphenyl / 95% Dimethyl polysiloxane), operating in electron impact mode at 70eV. Helium (99.999%) was used as carrier gas at a constant flow rate of 1ml per minute and an injection volume of 2 $\mu$ l (split ratio 10:1). An injector temperature of 250 °C and an ion-source temperature of 280 °C were employed. The oven temperature was programmed from 110 °C for 2 minutes with an increase of 10 °C / minute to 200 °C, then 5 °C / minute, ending with a 9 minute isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. The total MS running time was 36 minutes. The percentage of each chemical constituent was calculated by comparing the average peak area to the total areas. Turbomass 5.2 was the software used<sup>7</sup>.

### (iv) Identification of Components

Interpretation of mass spectrum 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 formula and structure of the components of the test material were ascertained.

### (v) IR Spectral Analysis

Infrared spectral study was carried out with the ethanol extract. One mg of the dried extract was mixed with about 100 mg of dried potassium bromide (IR) grade powder. The mixture was then pressed in a special dye to yield a transparent disc. The disc was then held in the instrument beam for spectroscopic examination and the resulting IR spectrum was recorded.



**Figure 1**  
GC-MS Chromatogram of the ethanolic extract of *Polyclinum madrasensis*

**Table 1**  
**Activity of Components identified in the ethanol extract of *Polyclinum madrasensis***

S. No.	RT	Name of the compound	Molecular formula	Peak Area %	Nature of the Compound	**Activity
1	12.92	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	10.48	Palmitic acid	Pesticide Nematicide Antioxidant Flavor 5 Alpha reductase inhibitor Antiandrogenic Lubricant
2	13.08	4,8-dimethyl-Nonane-1-ol	C <sub>11</sub> H <sub>24</sub> O	2.71	Alcoholic compound	Antimicrobial
3	14.32	(E)-5-Octadecene	C <sub>18</sub> H <sub>36</sub>	1.96	Alkene compound	No activity reported
4	16.13	1,7-Dimethyl-4-(1-methylethyl)cyclodecane	C <sub>15</sub> H <sub>30</sub>	2.25	Cyclic compound	No activity reported
5	16.54	3-methyl-1,2-Cyclopentane-diol	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	0.68	Alcoholic compound	Antimicrobial
6	17.41	Nonyl ester of nitric acid	C <sub>9</sub> H <sub>19</sub> NO <sub>3</sub>	1.03	Ester compound	No activity reported
7	18.09	4,6,8-trimethyl-1-Nonane	C <sub>12</sub> H <sub>24</sub>	1.88	Alkene compound	No activity reported
8	18.38	Bis(2-ethylhexyl) ester of Hexanedioic acid	C <sub>22</sub> H <sub>42</sub> O <sub>4</sub>	1.86	Ester compound	No activity reported
9	19.31	Methyl 2-hydroxydodecanoate	C <sub>13</sub> H <sub>26</sub> O <sub>3</sub>	1.30	Hydroxy compound	No activity reported
10	20.43	1,2-diisooctyl ester of Benzenedicarboxylic acid	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	2.29	Plasticizer compound	Antimicrobial Antifouling
11	21.29	1-Iodo-Tridecane	C <sub>13</sub> H <sub>27</sub> I	0.85	Iodine compound	Antimicrobial
12	22.69	2-butyl-octan-1-ol	C <sub>12</sub> H <sub>26</sub> O	1.05	Alcoholic compound	Antimicrobial
13	22.74	(1,2-dimethylpropyl)-1-methyl-2-nonyl-cyclopropane	C <sub>18</sub> H <sub>36</sub>	1.20	Propane compound	No activity reported
14	24.06	6-methyl-octadecane	C <sub>19</sub> H <sub>40</sub>	1.20	Alkane compound	No activity reported
15	24.22	Squalene	C <sub>30</sub> H <sub>50</sub>	10.50	Triterpene compound	Antioxidant Anticancer Pesticide Sunscreen Perfumery Chemo preventive
16	25.40	2-oxo- methyl ester of Octadecanoic acid	C <sub>19</sub> H <sub>36</sub> O <sub>3</sub>	1.07	Stearic acid compound	No activity reported
17	28.25	Cholesterol	C <sub>27</sub> H <sub>46</sub> O	23.01	Steroid	Antimicrobial Anticancer Antiarthritic Antiasthma Diuretic Antiinflammatory
18	28.40	Cholestan-3-ol	C <sub>27</sub> H <sub>48</sub> O	14.22	Steroid	Antimicrobial Anticancer Antiarthritic Antiasthma Diuretic Antiinflammatory

19	28.88	Dihydrotachysterol	C <sub>28</sub> H <sub>46</sub> O	7.94	Steroid	Antimicrobial Anticancer Antiarthritic Antiasthma Diuretic Antiinflammatory
20	29.84	3-hydroxy-, (3 $\alpha$ ,17 $\alpha$ )- Spiro[androst-5-ene-17,1'- cyclobutan]-2'-one	C <sub>22</sub> H <sub>32</sub> O <sub>2</sub>	3.78	Ketone compound	Antimicrobial Diuretic Antiinflammatory Antiasthma Antiarthritic Anticancer
21	30.63	Diazoprogestosterone	C <sub>21</sub> H <sub>30</sub> N <sub>4</sub>	4.36	Nitrogen compound	Antimicrobial Antiinflammatory
22	34.18	1,8,15,22-Tricosatetrayne	C <sub>23</sub> H <sub>32</sub>	4.36	Alkynes	No activity reported

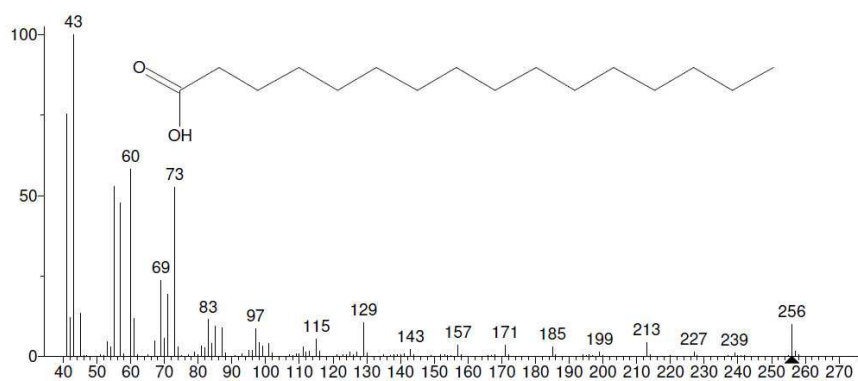
\*\*Activity source: Dr Duke's Phytochemical and Ethnobotanical data bases.

## RESULTS AND DISCUSSION

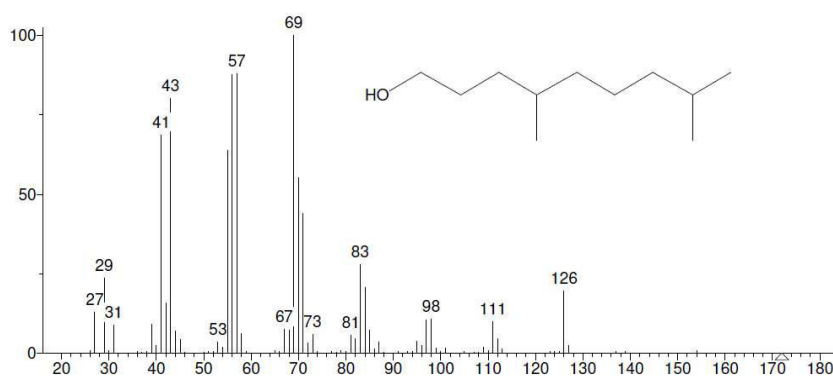
GC-MS chromatogram of the ethanolic extract of *Polyclinum madrasensis* given in Figure 1 showed twenty two peaks indicating the presence of twenty two compounds. The chemical compounds identified with their retention time (RT), molecular formula and concentration (peak area %) are presented in Table-1. The results revealed the presence of compounds like n-Hexadecanoic acid (10.48), 4,8-dimethyl-Nonane-1-ol (2.71), (E)-5-Octadecene (1.96), 1,7-Dimethyl-4-(1-methylethyl)cyclodecane (2.25), 3-methyl-1,2-Cyclopentane-diol (0.68), Nonyl ester of nitric acid (1.03), 4,6,8-trimethyl-1-Nonane (1.88), Bis(2-ethylhexyl) ester of Hexanedioic acid (1.86), Methyl 2-hydroxydodecanoate (1.30), 1,2-diisooctyl ester of Benzenedicarboxylic acid (2.29), 1-Iodo-Tridecane (0.85), 2-butyl-octan-1-ol (1.05), (1,2-dimethylpropyl)-1-methyl-2-nonyl-cyclopropane (1.20), 6-methyl-octadecane (1.20), Squalene (10.50), 2-oxo-methyl ester of Octadecanoic acid (1.07), Cholesterol (23.01), Cholestan-3-ol (14.22), Dihydrotachysterol (7.94), 3-hydroxy-, (3 $\alpha$ ,17 $\alpha$ )-Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one (3.78), Diazoprogestosterone (4.36) and 1,8,15,22-Tricosatetrayne (4.36). Of these twenty two compounds, thirteen compounds - 4,8-dimethyl-Nonane-1-ol, (E)-5-Octadecene, 3-methyl-1,2-Cyclopentane-diol, 4,6,8-trimethyl-1-Nonane, Bis(2-ethylhexyl)

ester of Hexanedioic acid, Methyl 2-hydroxydodecanoate, 1-Iodo-Tridecane, 2-butyl-octan-1-ol, (1,2-dimethylpropyl)-1-methyl-2-nonyl-cyclopropane, 6-methyl-octadecane, 2-oxo-methyl ester of Octadecanoic acid, Dihydrotachysterol and 1,8,15,22-Tricosatetrayne are reported for the first time from ascidians of Indian water.

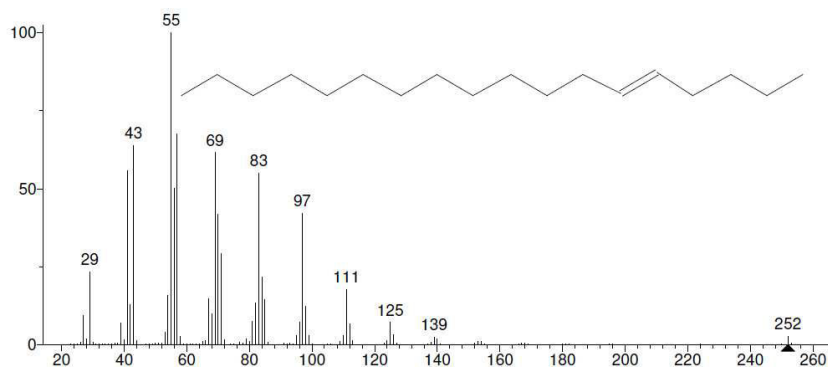
According to the result, steroid compounds exhibited anticancer, antiarthritic, antiasthma, diuretic, antimicrobial and antiinflammatory activities. On the other hand, antimicrobial activity was found in alcoholic, iodine and nitrogen compounds. Squalene, which is triterpene in nature showed anticancer, antioxidant, pesticide, sunscreen, perfumery and chemo preventive properties. In addition to antioxidant and pesticide activity, palmitic compound showed antiandrogenic, nematocidal, lubricant, flavor and 5 alpha reductase inhibitor effects. Infrared spectrum of ethanolic extract of *Polyclinum madrasensis* shows broad band at 3394.43 cm<sup>-1</sup> indicating the presence of primary free N-H stretching, 2920.40 cm<sup>-1</sup> is characteristic for C-H stretching vibration which gives evidence of the presence of aliphatic chain. Carboxylic acid and carbonyl compound was identified at the bands 2352.81 cm<sup>-1</sup> and 1637.50 cm<sup>-1</sup> respectively<sup>9</sup>.



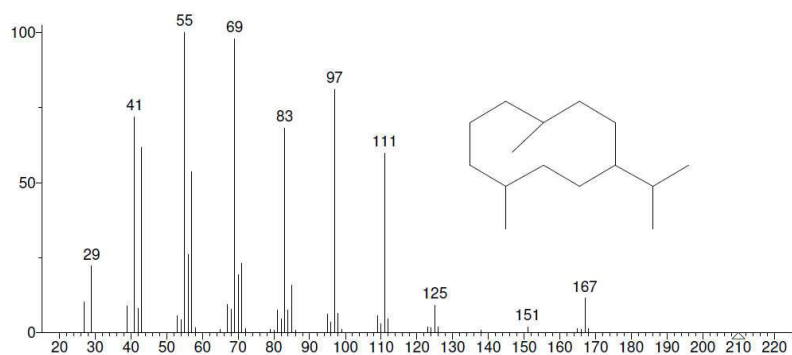
**Figure 2**  
**Mass spectrum of n-Hexadecanoic acid**



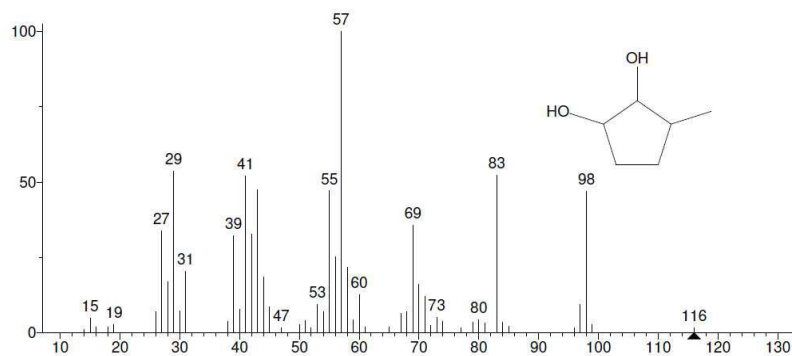
**Figure 3**  
**Mass spectrum of 4,8-dimethyl-Nonane-1-ol**



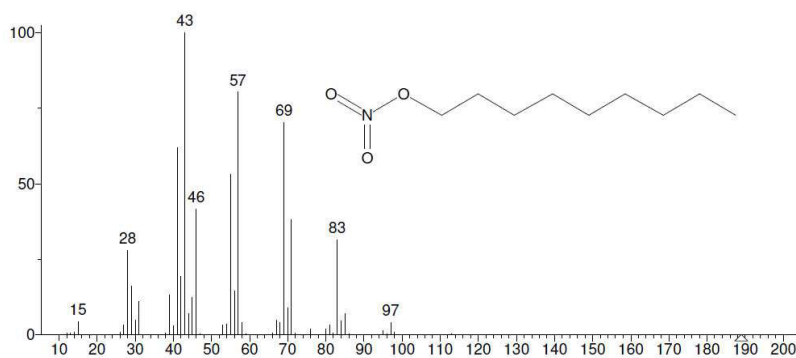
**Figure 4**  
**Mass spectrum of (E)-5-Octadecene**



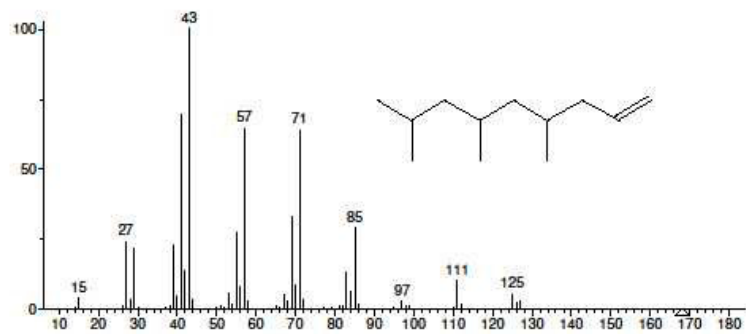
**Figure 5**  
**Mass spectrum of 1, 7-Dimethyl-4-(1-methylethyl)cyclododecane**



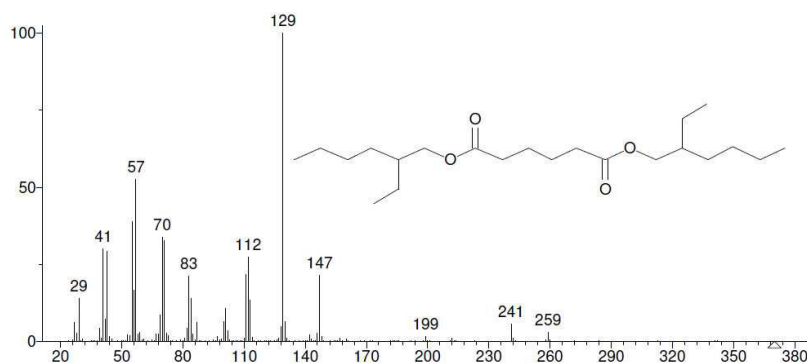
**Figure 6**  
**Mass spectrum of 3-methyl-1, 2-Cyclopentane-diol**



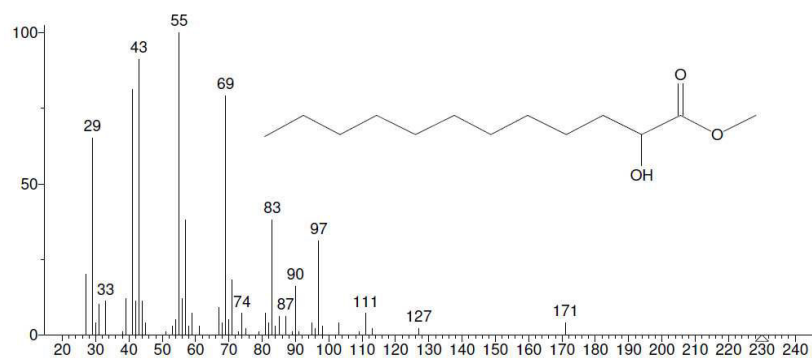
**Figure 7**  
**Mass spectrum of Nonyl ester of nitric acid**



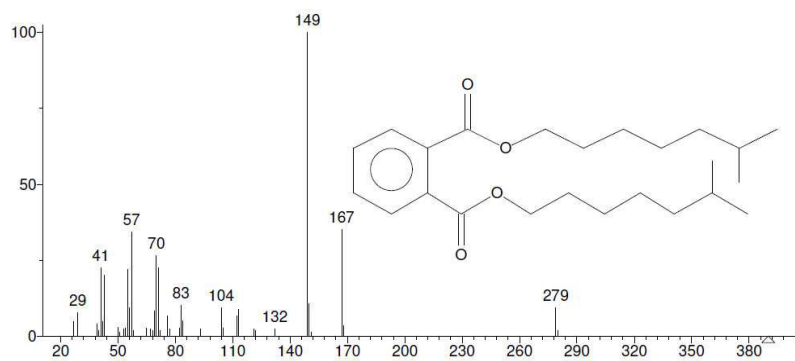
**Figure 8**  
**Mass spectrum of 4, 6, 8-trimethyl-1-Nonane**



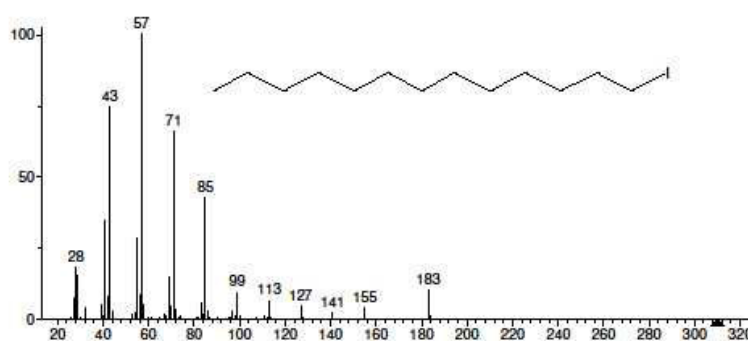
**Figure 9**  
**Mass spectrum of Bis(2-ethylhexyl) ester of Hexanedioic acid**



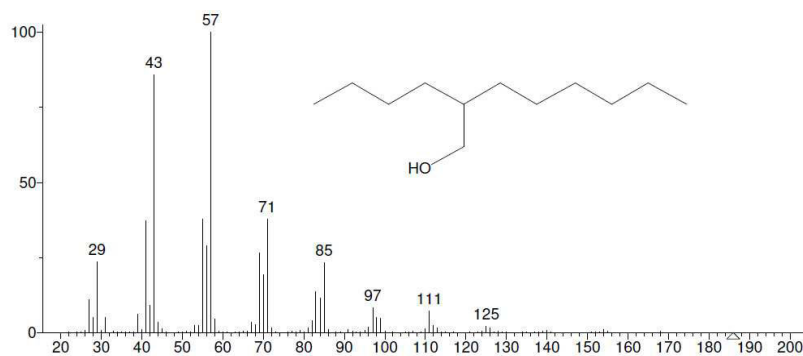
**Figure 10**  
**Mass spectrum of Methyl 2-hydroxydodecanoate**



**Figure 11**  
*Mass spectrum of 1, 2-diisooctyl ester of Benzenedicarboxylic acid*

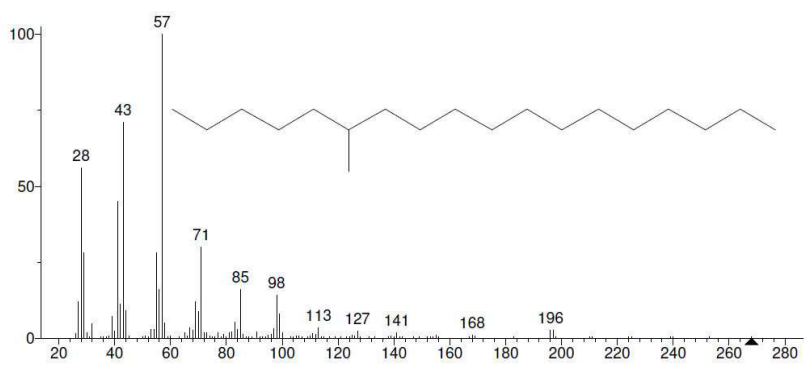


**Figure 12**  
*Mass spectrum of 1-Iodo-Tridecane*

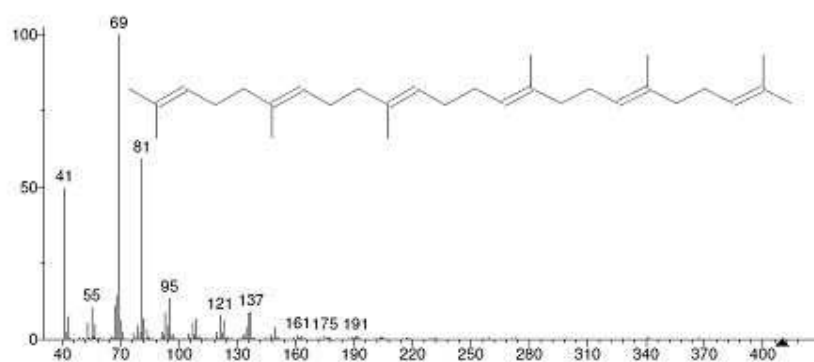


**Figure 13**  
*Mass spectrum of 2-butyl-octan-1-ol*

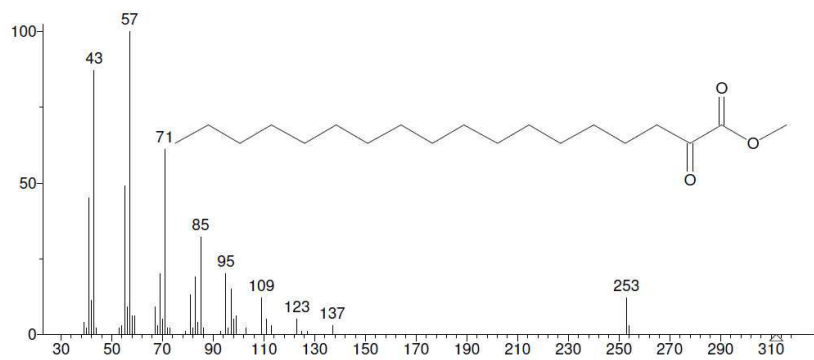




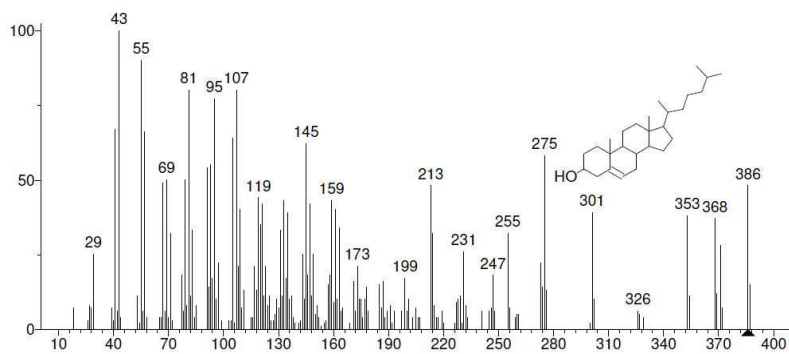
**Figure 14**  
*Mass spectrum of 6-methyl-octadecane*



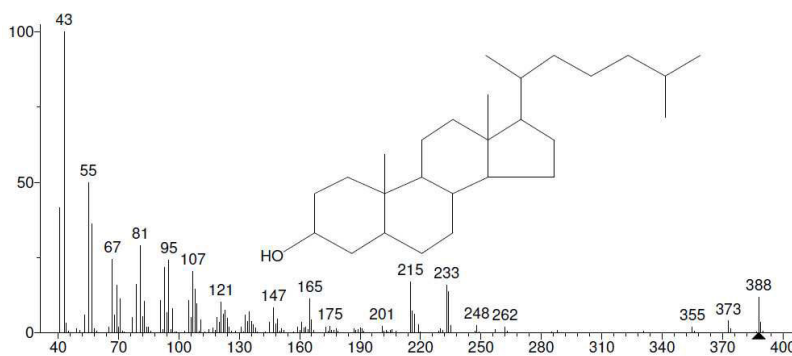
**Figure 15**  
*Mass spectrum of Squalene*



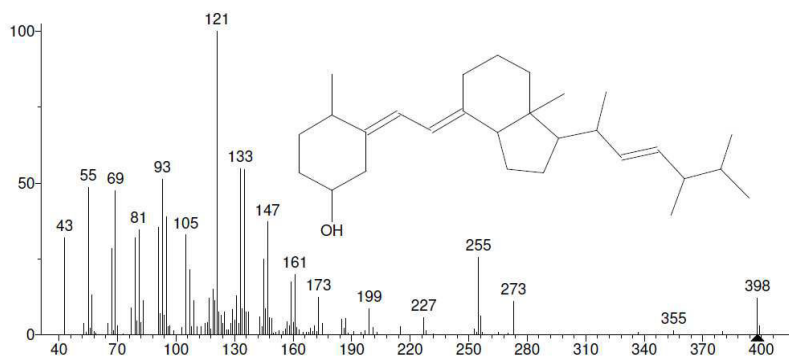
**Figure 16**  
*Mass spectrum of 2-oxo- methyl ester of Octadecanoic acid*



**Figure 17**  
**Mass spectrum of Cholesterol**



**Figure 18**  
**Mass spectrum of Cholestan-3-ol**



**Figure 19**  
**Mass spectrum of Dihyrotachysterol**

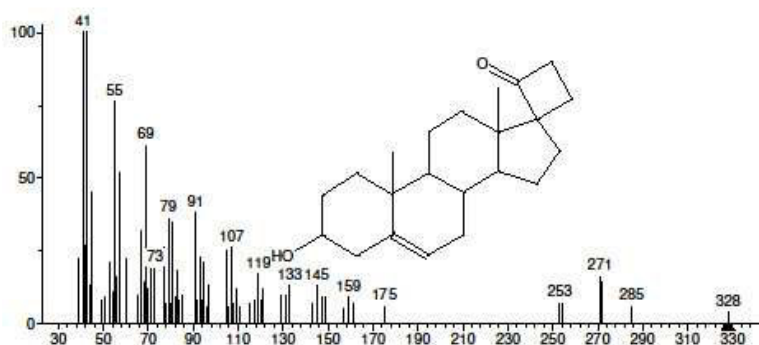


Figure 20

Mass spectrum of 3-hydroxy-, (3á, 17á)-Spiro [androst-5-ene-17, 1'-cyclobutan]-2'-one

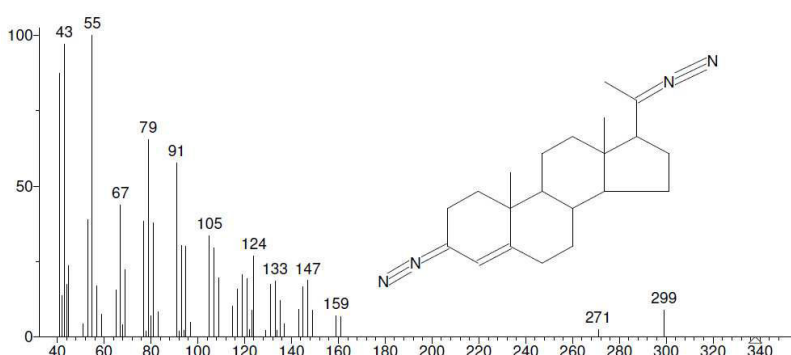


Figure 21

Mass spectrum of Diazoprogestosterone

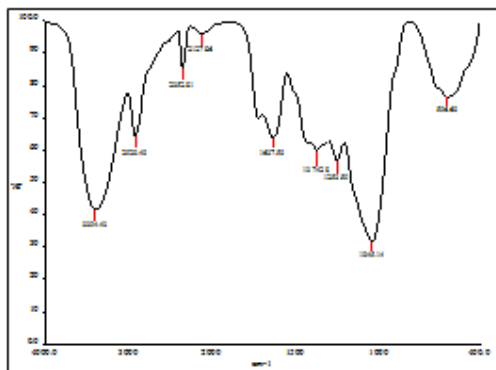


Figure 22

IR spectrum for the Ethanolic extract of *Polyclinum madrasensis*

## CONCLUSION

The present study has been found to be useful in the identification of several constituents present in the ethanolic extract of *Polyclinum madrasensis*. The presence of various bioactive compounds identified as steroids, Ketone, Palmitic acid, Alcoholic, Alkene, Cyclic, Ester, Hydroxy, Plasticizer, Iodine, Propane, Alkane, Triterpene, Triterpene, Stearic acid, Nitrogen and Alkynes. It could be concluded that, *Polyclinum madrasensis* is pharmacologically important.

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