



Research Article

## INFRA RED AND GAS CHROMATOGRAM-MASS SPECTRAL STUDIES OF THE ETHANOLIC EXTRACT OF *ASCIDIA SYDNEIENSIS*

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**Abstract:** Ascidiaceans have tremendous potential in pharmaceutical and biomedical field. *Ascidia sydneiensis* is commonly called as crevice ascidian. It has been subjected to Infrared (IR) spectral study which indicates the presence of aromaticity, hydroxyl and carbonyl group in the ethanolic extract. GC-MS studies revealed the presence of ten chemical constituents –Tetradecanoic acid, Bis-(2-methylpropyl)ester of 1,2-benzenedicarboxylic acid, n-Hexadecanoic acid, 9-Hexyl-heptadecane, Diisooctyl ester of 1,2-benzenedicarboxylic acid, 3-ethyl-5-(2-ethylbutyl)-octadecane, Squalene, Cholest-5-en-3-ol(3 $\alpha$ )-carbonochloridate, Cholesterol, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-methylester of cyclopropanoic acid.

**Key word:** *Ascidia sydneiensis*, IR Spectral studies, GC-MS studies.

### 1. Introduction

Marine organisms are a rich source of structurally novel and biologically active compounds. Primary and secondary metabolites are produced by these microorganisms, may have potential bioactive properties of interest in pharmaceutical industry<sup>1</sup>. Ascidiaceans are marine, sedentary filter feeding organisms found world wide occurring as the major components of fouling community<sup>2,3</sup>. They have been screened in a variety of pharmacological bioassays. Biological activities of ascidiaceans which have been frequently observed in ascidian crude extracts include antibiosis against both human microbial pathogens and marine microorganisms<sup>4</sup>. Antimicrobial activity of *Ascidia sydneiensis* against *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Alcaligenes* has been studied<sup>5</sup>. Biochemical components of various species of ascidiaceans have been reported by earlier workers<sup>3,6,7,8</sup>. IR spectral and GC-MS studies on the ethanolic extract of *Ascidia sydneiensis* has not been carried out so far. Hence the present attempt has been made.

### Materials and Methods

#### 2.1. Collection of animal material

*Ascidia sydneiensis* (Family: Ascidiidae) was collected from Tuticorin coast in the month of April 2013 by SCUBA diving (Plate -1). Molluscan shell, calcareous pieces and rock fragments attached to the specimen was carefully removed. They were identified using key to identification of Indian ascidiaceans<sup>6</sup>. A voucher specimen AS 2252 has been submitted in the ascidian collection of museum of the Department of Zoology, A. P. C. Mahalaxmi College for Women, Tuticorin – 628 002, Tamilnadu, India.



**Plate-1. *Ascidia sydneiensis* Savigny, 1816**

#### 2.2. Preparation of extract

The whole animal was dried in shade and homogenized to get a coarse powder which was extracted with ethanol, concentrated in a rotary evaporator under reduced pressure. 2  $\mu$ l of the extract of *Ascidia sydneiensis* was employed for IR Spectral studies and GC-MS studies<sup>7</sup>.

#### 2.3. Instruments and chromatographic conditions

##### 2.3.1. IR Spectral studies

Infra red spectral study was made for the ethanol dried extract. One mg of finely powdered 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. The following conditions were employed; Perkin Elmer Model spectrum RXI; Range 4000nm-400nm; Resolution 4; Transmittance test mode.

**2.3.2. GC-MS studies**

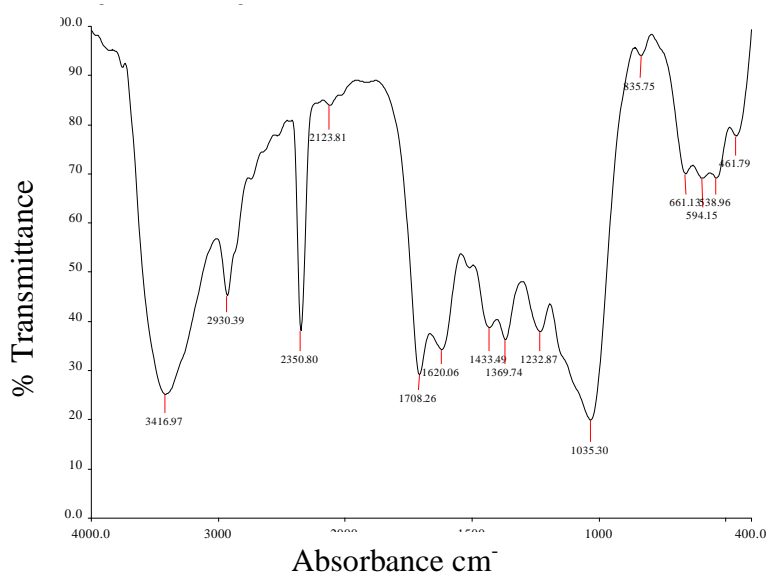
GC-MS studies was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite -1 fused silica capillary column (30 × 0.25 mm 1D × 1EM df, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70 ev; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C to 280°C/min, ending

with a 9 min isothermal at 280°C. Mass spectra were taken at 70 ev; a scan interval of 0.5 s and fragments from 40 to 550 Da.

**2.4. Identification of compounds**

Interpretation of mass spectrum of GC-MS was conducted using the data base of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of unknown compounds was compared with the spectrum of the known stored in the NIST library. The Name, Molecular weight and Structure of the compounds of the test materials were ascertained.

**3.Results and Discussion**



**3.1. IR spectral studies**

Infra red spectrum for ethanolic extract of *Ascidia sydneiensis* (Figure-1) shows broad band at 3416.97 cm<sup>-1</sup> which is due to the presence of moisture or hydroxyl groups in the compound and that at 2930.39 cm<sup>-1</sup> is characteristic for C-H stretching vibration indicating aliphatic chain. The band at 2350.80 cm<sup>-1</sup> for O-H stretching gives evidence for the presence of carboxylic acid<sup>8</sup> and the band at 1620.06 cm<sup>-1</sup> for C=O stretching vibration indicates carbonyl group. The strong bands above 3000 cm<sup>-1</sup> shows evidence for aromatic rings<sup>9</sup>.

**3.2. GC-MS Studies**

GC-MS Chromatogram of the ethanolic extract of *Ascidia sydneiensis* gives 10 prominent peaks indicating the

**Identification for *Ascidia sydneiensis***

presence of 10 compounds which is given in Table 01. The mass of these compounds are presented in Figures 2 to 12. The mass spectra of these compounds were compared with those of the compiled data for the known compounds. Here the peak with retention time 10.70 corresponds to Tetradecanoic acid, 11.60 to Bis-(2-methylpropyl)ester of 1,2-benzenedicarboxylic acid, 12.79 to n-Hexadecanoic acid, 19.66 to 9-Hexyl-heptadecane, 20.22 to Diisooctyl ester of 1,2-benzenedicarboxylic acid, 21.07 to 3-ethyl-5-(2-ethylbutyl)octadecane, 23.99 to Squalene, 27.53 to Cholest-5-en-3-ol (3 α)-carbonochloridate, 28.03 to Cholesterol, 32.93 to 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-methylester of cyclopropaneoctanoic acid.

**Table 01 - Chemical compounds identified in the ethanolic extract of *Ascidia sydneiensis***

No	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	10.70	Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228	15.0
2	11.60	Bis-(2-methylpropyl)ester of 1,2-benzenedicarboxylic acid	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	6.1
3	12.79	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	21.7

4	19.66	9-hexyl-heptadecane	C <sub>23</sub> H <sub>48</sub>	324	2.5
5	20.22	Diisooctyl ester of 1,2-benzenedicarboxylic acid	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390	3.0
6	21.07	3-ethyl-5-(2-ethylbutyl)-octadecane	C <sub>26</sub> H <sub>54</sub>	366	1.7
7	23.99	Squalene	C <sub>30</sub> H <sub>50</sub>	410	5.2
8	27.53	Cholest-5-en-3-ol (3α)-carbonochloridate	C <sub>28</sub> H <sub>45</sub> ClO <sub>2</sub>	448	8.8
9	28.03	Cholesterol	C <sub>27</sub> H <sub>46</sub> O	386	12.0
10	32.93	2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-methyl ester of cyclopropaneoctanoic acid	C <sub>22</sub> H <sub>38</sub> O <sub>2</sub>	334	24.0

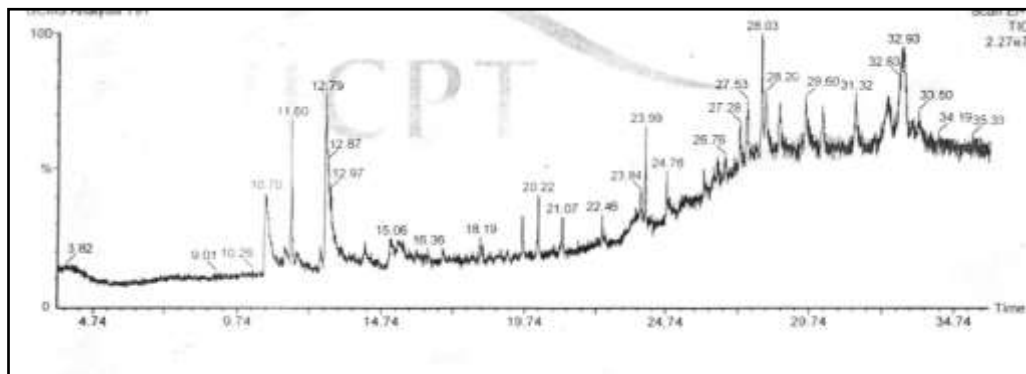


Figure 2. GC-MS Chromatogram of the ethanolic extract of *Ascidia sydneiensis*

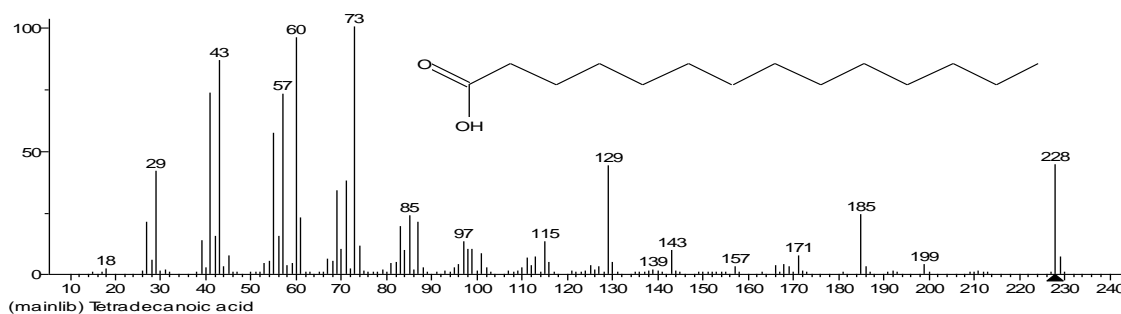


Figure 3. Mass spectrum of Tetradecanoic acid (RT: 10.70)

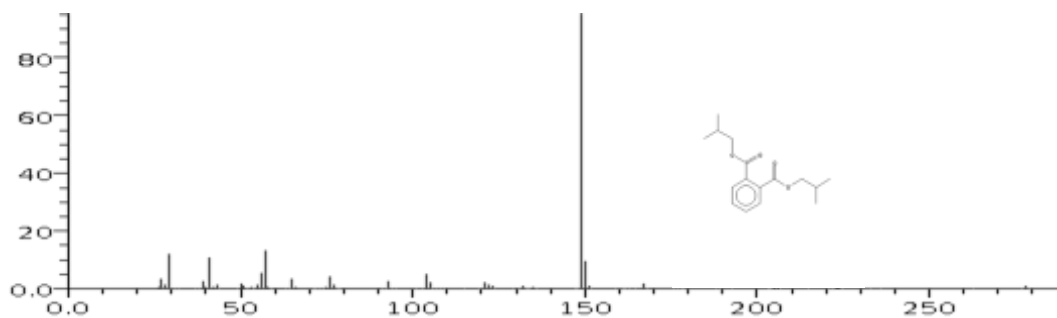


Figure 4. Mass spectrum of Bis-(2-methylpropyl) ester of 1,2-benzenedicarboxylic acid (RT: 11.60)

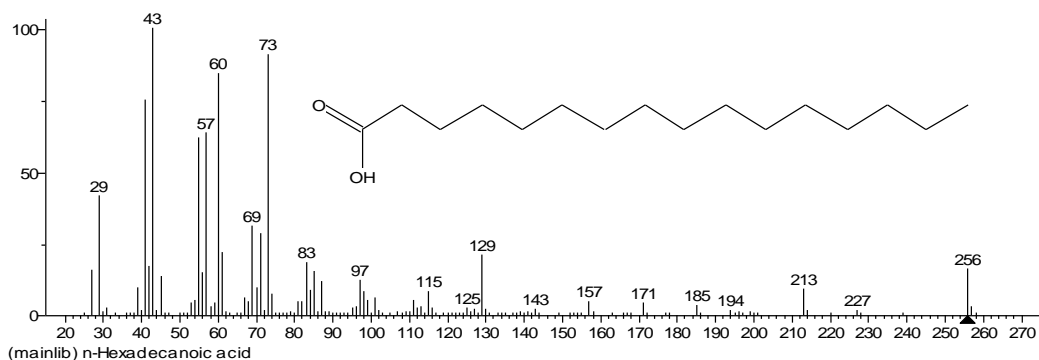


Figure 5. Mass spectrum of n-Hexadecanoic acid (RT: 12.79)

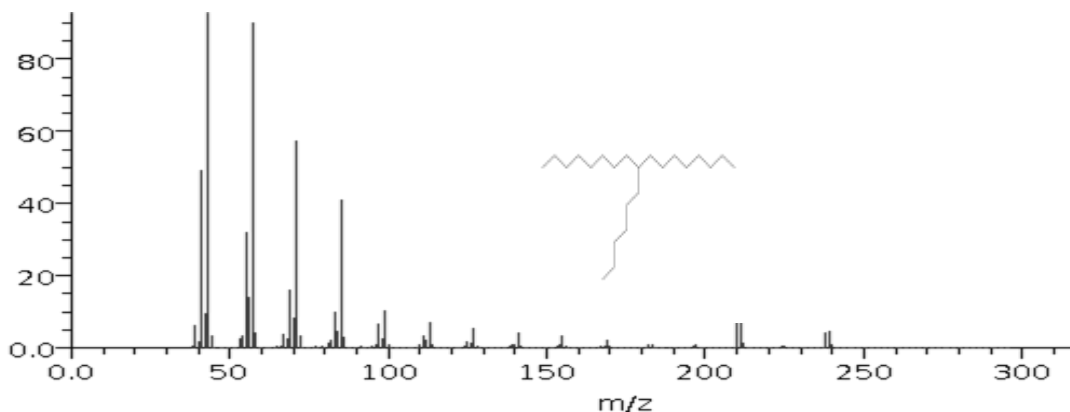


Figure 6. Mass spectrum of 9-hexylheptadecane (RT: 19.66)

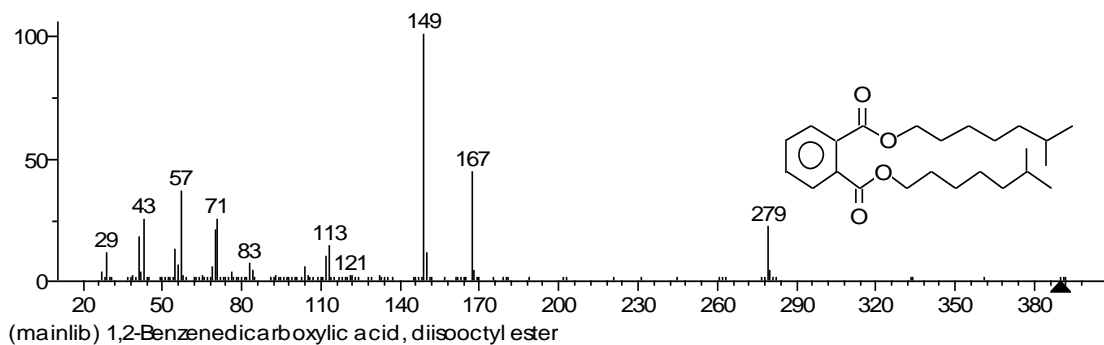


Figure 7. Mass spectrum of Diisooctyl ester of 1,2-Benzenedicarboxylic acid (RT: 20.22)

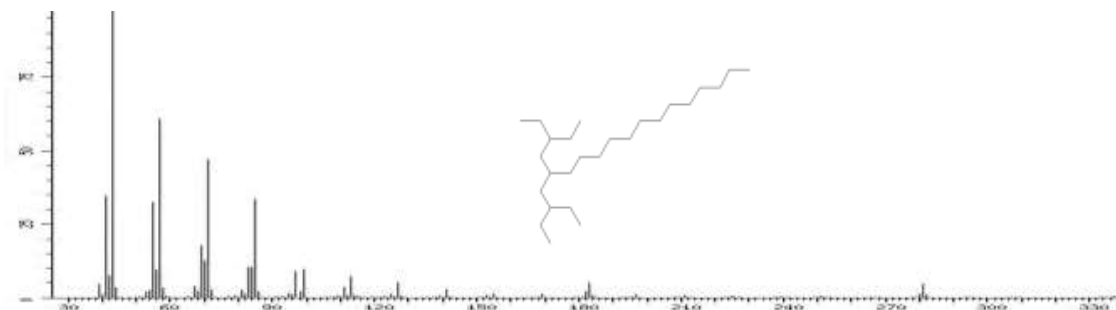


Figure 8. Mass spectrum of 3-ethyl-5-(2-ethylbutyl)-octadecane (RT:21.07)

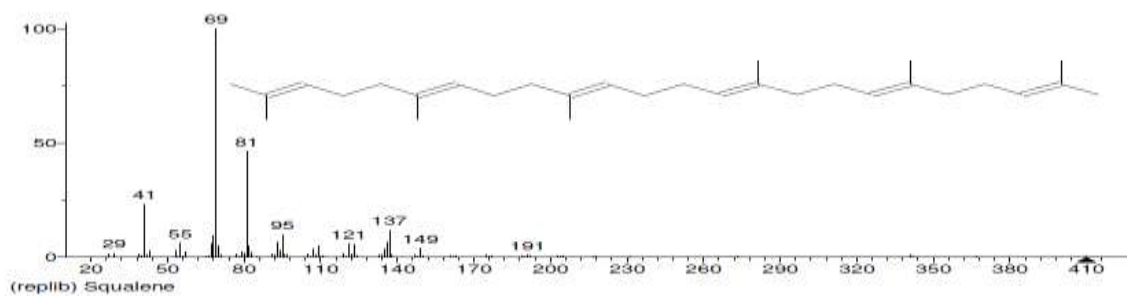


Figure 9. Mass spectrum of Squalene (RT: 23.99)

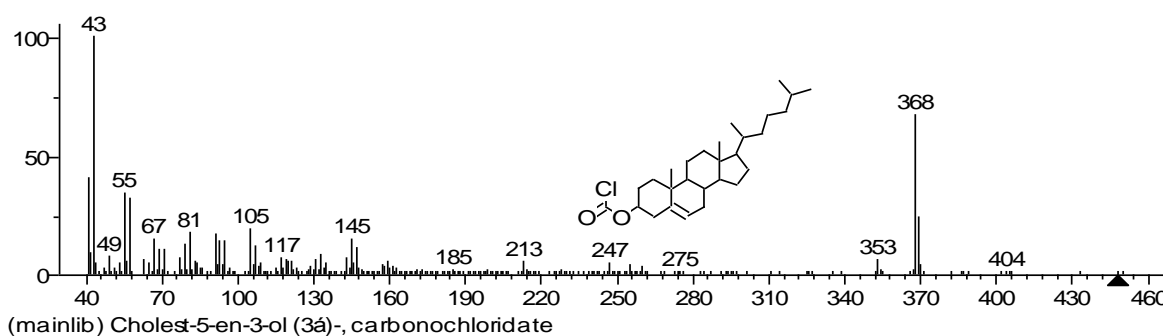


Figure 10. Mass spectrum of Cholest-5-en-3-ol (3 $\alpha$ )-Carbonochloridate (RT: 27.53)

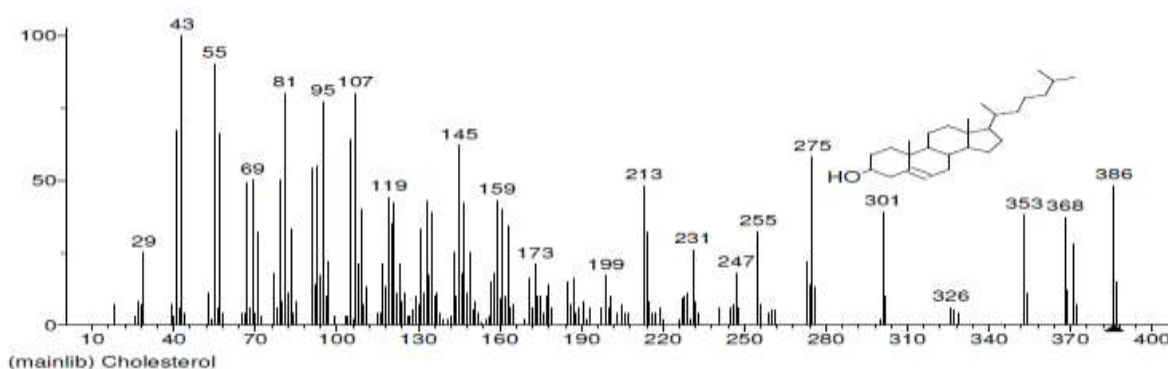


Figure 11. Mass spectrum of cholesterol (RT: 28.03)

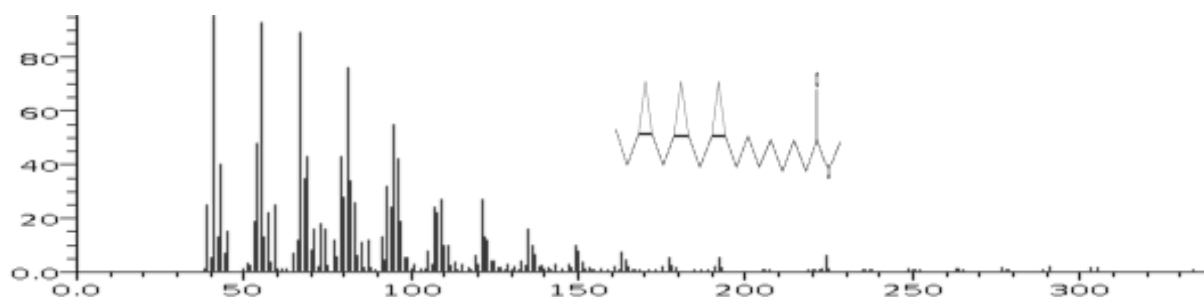


Figure 12. Mass spectrum of 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]methyl ester of cyclopropaneoctanoic acid (RT: 32.93)

## Conclusion

In the present study ten chemical constituents have been identified from the ethanolic extract of *Ascidia sydneiensis* by IR and GC-MS studies. It has been found that the natural products derived from ascidians have tremendous potential in pharmaceutical and biomedical field. Further studies such as isolation, purification and structure determination is required for the development of a new drug.

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