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Infrared and gas chromatogram-mass spectral studies of the ethanolic extract of *Phallusia arabica* Savigny, 1816

Kohila Subathra Christy H.^{1*}, Jothibai Margret R.² and Meenakshi V. K.³

¹Department of Chemistry, A. P. C. Mahalaxmi College for Women, Tuticorin, Tamil Nadu, India

²Department of Chemistry, Pope's College, Sawyerpuram, Tuticorin, Tamil Nadu, India

³Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin, Tamil Nadu, India

ABSTRACT

Ascidians are reported to be a rich source of extremely potent bioactive compounds. *Phallusia arabica*, is a simple ascidian belonging to the family Ascidiidae found in plenty throughout the year along the Tuticorin coast of India. Infrared (IR) spectral study indicates the presence of hydroxyl, carbonyl group and aromatic compounds. GC-MS studies revealed the presence of fourteen chemical constituents *n*-Decanoic acid, *n*-Hexadecanoic acid, 7-Nonen-4,8-dimethyl-2-one, 7-Tridecanone, 1-Iodoundecane, 1,2-diisooctyl ester of Benzene dicarboxylic acid, 2,6,10,15-tetramethylheptadecane, 1-Iodotridecane, 1-Iodododecane, Squalene, 1-Iodo octadecane, 26-Nor-5-cholesten-3 α -ol-25-one, cholestan-3 α -ol, 6,22-dien,3,5-dedihydrostigmastan.

Keywords: *Phallusia arabica*, IR Spectral studies, GC-MS studies.

INTRODUCTION

The ascidians, commonly called "sea squirts" (Subphylum: Urochordata, Class: Ascidiacea) are dominant organisms in many marine communities, having a wide geographic distribution [1]. One of the reasons for this ecological success is the ability of these animals to synthesize secondary metabolites with important defensive roles, including antimicrobial peptides [2], cytosine like compounds, lectins [3] and antileukemic compounds. Ascidians contain a wealth of interesting pharmacological substances [4]. Sac-like filter feeding ascidians have been reported to be an important source in drug discovery. *Phallusia arabica* is a simple ascidian belonging to the family Ascidiidae found to occur in plenty along the Tuticorin coast. The objective of the present investigation is to identify the possible chemical constituents with the aid of IR spectral and GC-MS studies.

MATERIALS AND METHODS

Collection of animal material

Phallusia arabica (Family : Ascidiidae) was collected from Tuticorin coast in the month of April 2013 by SCUBA diving (Plate -1). Molluscan shell and calcrete rock fragments attached to the test of the animal was carefully removed. They were identified using key to identification of Indian ascidians [5]. A voucher specimen AS 2276 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. *Phallusia arabica* Savigny, 1816

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 μ l of the extract of *Phallusia arabica* was employed for IR Spectral studies and GC-MS studies [6].

Instruments and chromatographic conditions

IR Spectral studies

Infrared 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.

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 \times 0.25 mm ID \times 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 μ l 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.

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.

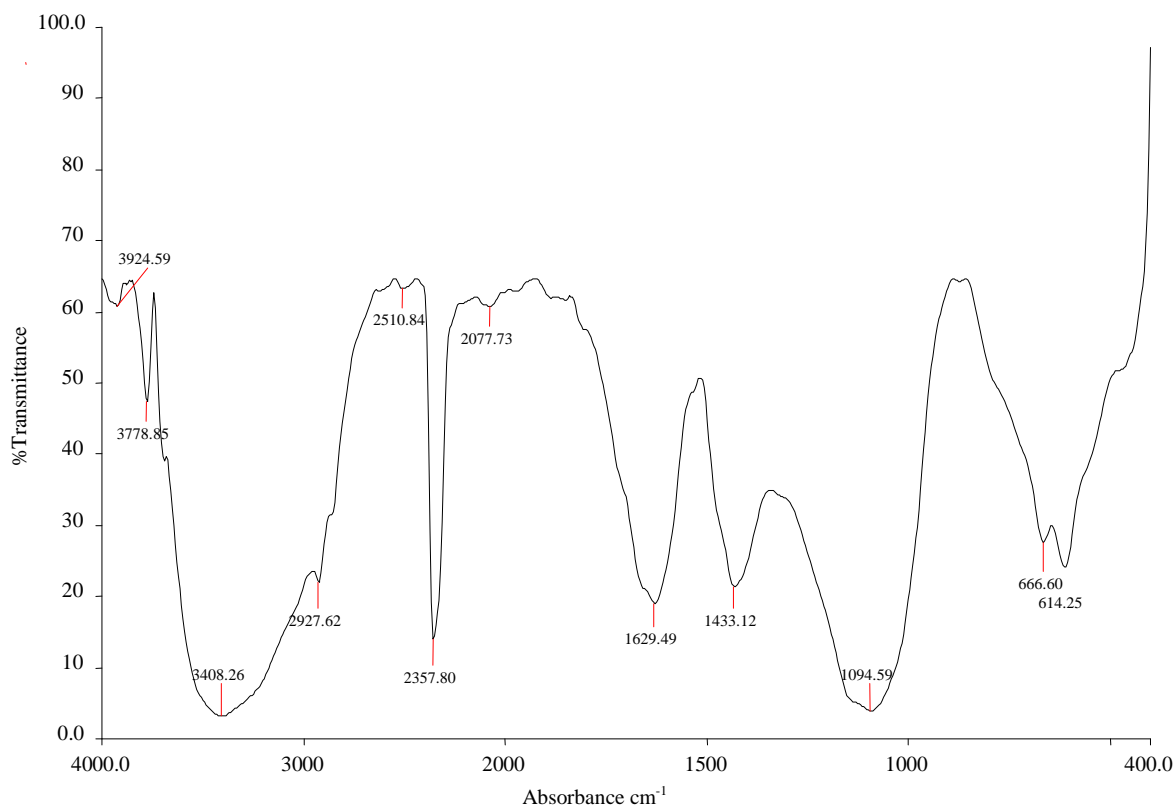
RESULTS AND DISCUSSION

IR spectral studies

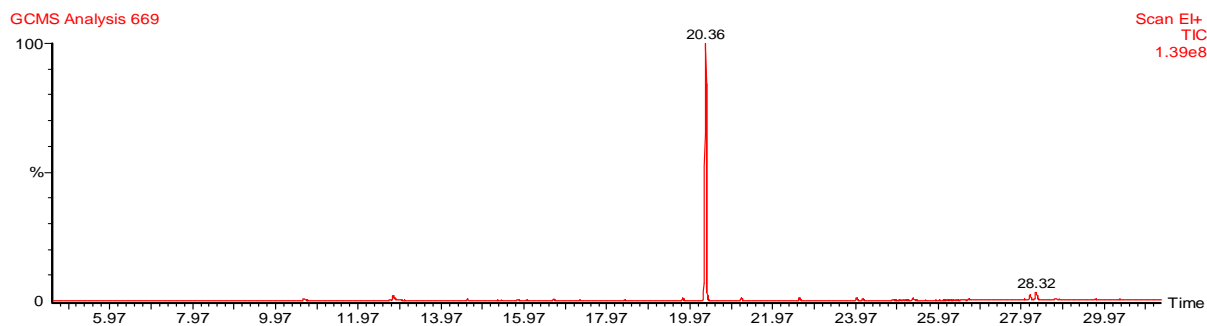
Infrared spectrum for ethanolic extract of *Phallusia arabica* (Figure-1) shows broad band at 3408.26 cm^{-1} due to the presence of moisture or hydroxyl groups in the compound and that at 2927.62 cm^{-1} is characteristic for C-H stretching vibration which gives evidence for the presence of aliphatic chain. The band at 1629.49 cm^{-1} is characteristic of carbonyl group (7) and that at 1433.12 cm^{-1} shows the presence of CH bending vibration. The presence of strong bands above 3000 cm^{-1} indicates the presence of aromatic ring (8).

GC-MS Studies

GC-MS Chromatogram of the ethanolic extract of *Phallusia arabica* gives 14 prominent peaks indicating the presence of 14 compounds which is given in Table 1. The mass of these compounds are presented in Figures 2 to 16. The mass spectra of these compounds were compared with those of the compiled data for the known compounds. Here the peak with retention 10.66 corresponds to n-Decanoic acid, 12.81 to n-Hexadecanoic acid, 14.61 to 4,8-7-Nonen-dimethyl-2-one, 16.69 to 7-Tridecanone, 19.81 to 1-Iodoundecane, 20.36 to 1,2-diisooctyl ester of Benzenedicarboxylic acid, 21.22 to 2,6,10,15-tetramethylheptadecane, 22.62 to 1-Iodotridecane, 24.00 to 1-Iodododecane, 24.15 to Squalene, 25.36 to 1-Iodooctadecane, 28.18 to 26-Nor-5-cholesten-3 α -ol-25-one, 28.32 to cholestan-3 α -ol and 28.80 to 3,5-dedihydro-6,22-dienstigmastan.

Figure -1. IR Spectrum for *Phallusia arabica*Table 1 Chemical compounds identified in the ethanolic extract of *Phallusia arabica*

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	10.66	n-Decanoic acid	C ₁₀ H ₂₀ O ₂	172	1.17
2	12.81	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	3.23
3	14.61	7-Nonen-4,8-dimethyl-2-one	C ₁₁ H ₂₀ O	168	0.59
4	16.69	7-Tridecanone	C ₁₃ H ₂₆ O	198	0.59
5	19.81	1-Iodoundecane	C ₁₁ H ₂₃ I	282	0.88
6	20.36	Diisooctyl ester of 1,2-Benzenedicarboxylic acid	C ₂₄ H ₃₈ O ₄	390	82.82
7	21.22	2,6,10,15-tetramethyl-Heptadecane	C ₂₁ H ₄₄	296	0.88
8	22.62	1-Iodotridecane	C ₁₃ H ₂₇ I	310	1.03
9	24.00	1-Iodododecane	C ₁₂ H ₂₅ I	296	0.88
10	24.15	Squalene	C ₃₀ H ₅₀	410	0.73
11	25.36	1-iodooctadecane	C ₁₈ H ₃₇ I	380	0.88
12	28.18	26-Nor-5-cholesten-3 α -ol-25-one	C ₂₆ H ₄₂ O ₂	386	2.06
13	28.32	Cholestan-3 α -ol	C ₂₇ H ₄₈ O	388	3.52
14	28.80	3,5-dedihydro -6,22-dien, Stigmastan	C ₂₉ H ₄₆	394	0.73

Figure - 2. GC-MS Chromatogram of the ethanolic extract of *Phallusia arabica*

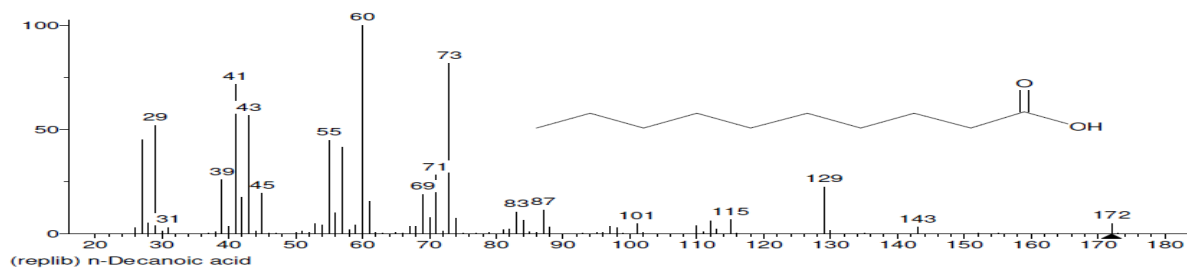


Figure - 3. Mass spectrum of n-Decanoic acid (RT :10.66)

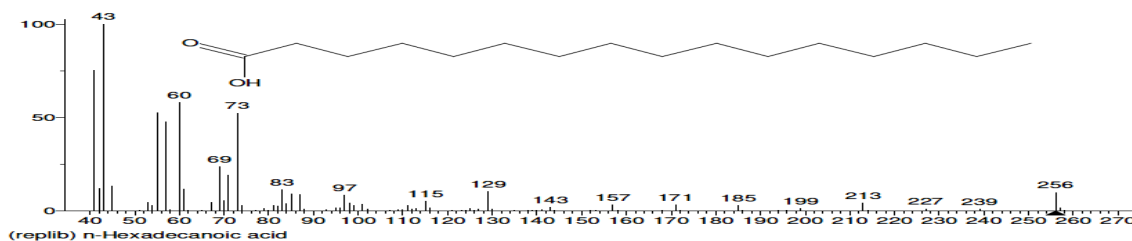


Figure - 4. Mass spectrum of n-Hexadecanoic acid (RT :12.81)

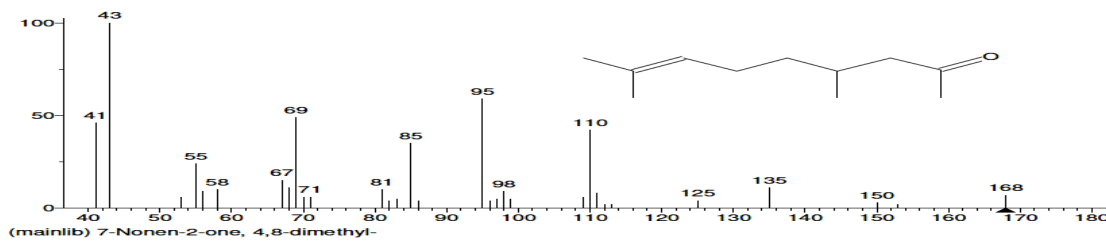


Figure - 5. Mass spectrum of 7-Nonen-4,8-dimethyl 2-one (RT :14.61)

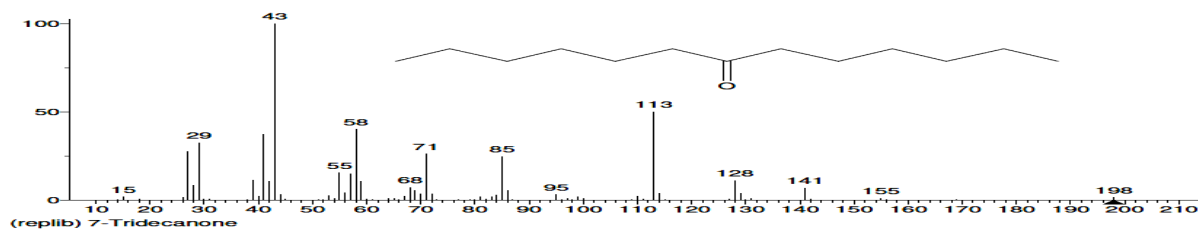


Figure - 6. Mass spectrum of 7-Tridecanone (RT :16.69)

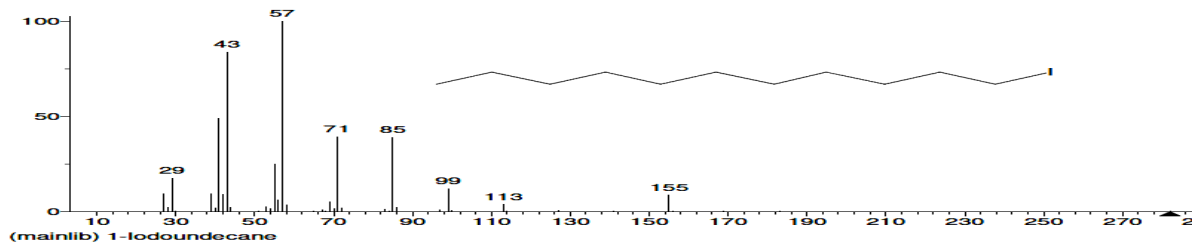


Figure - 7. Mass spectrum of 1-Iodoundecane (RT :19.81)

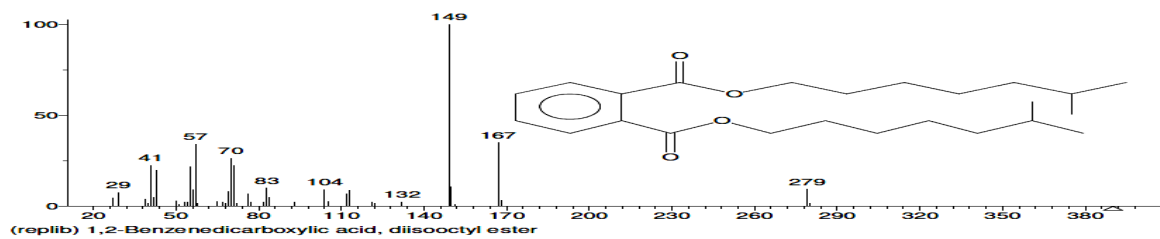


Figure - 8. Mass spectrum of diisooctyl ester of 1,2-Benzenedicarboxylic acid(RT 20.36)

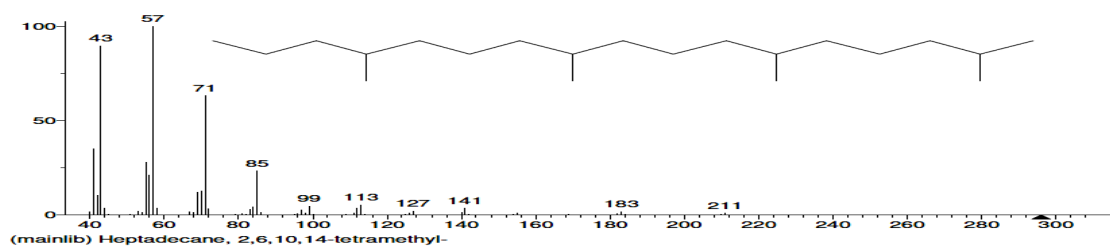


Figure - 9. Mass spectrum of 2,6,10,14-tetramethylheptadecane (RT :21.22)

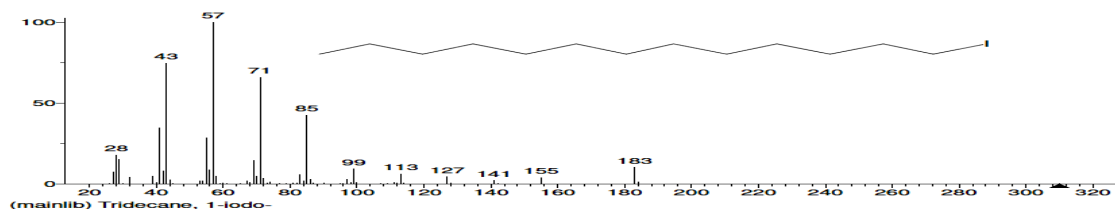


Figure - 10. Mass spectrum of 1-Iodotridecane (RT :22.62)

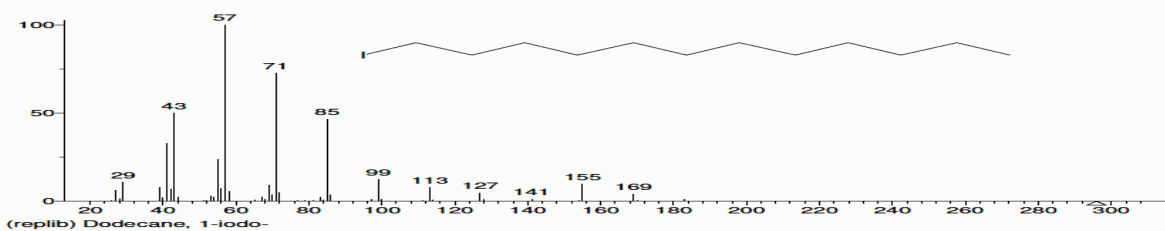


Figure - 11. Mass spectrum of 1-Iodododecane (RT :24.00)

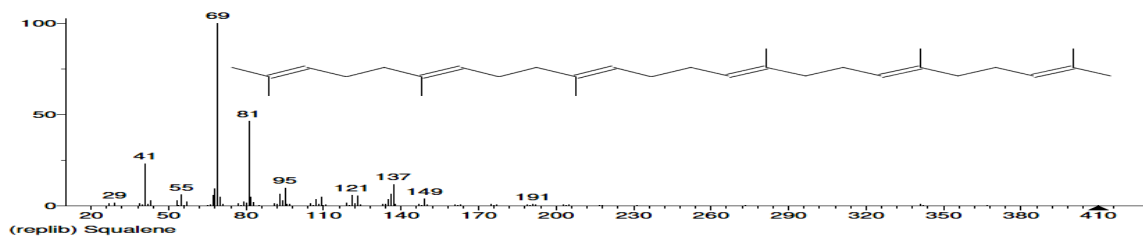


Figure - 12. Mass spectrum of Squalene (RT :24.15)

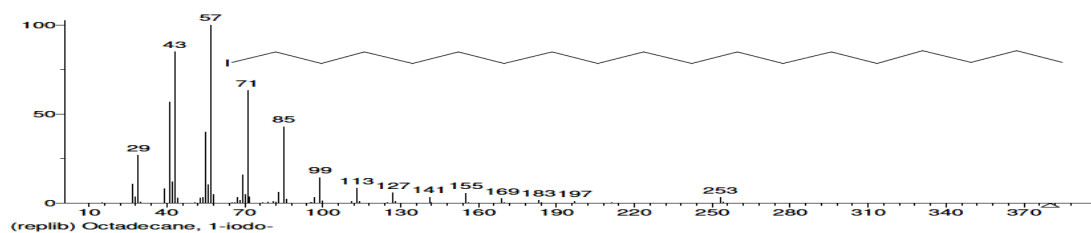


Figure - 13. Mass spectrum of 1-Iodooctadecane (RT :25.36)

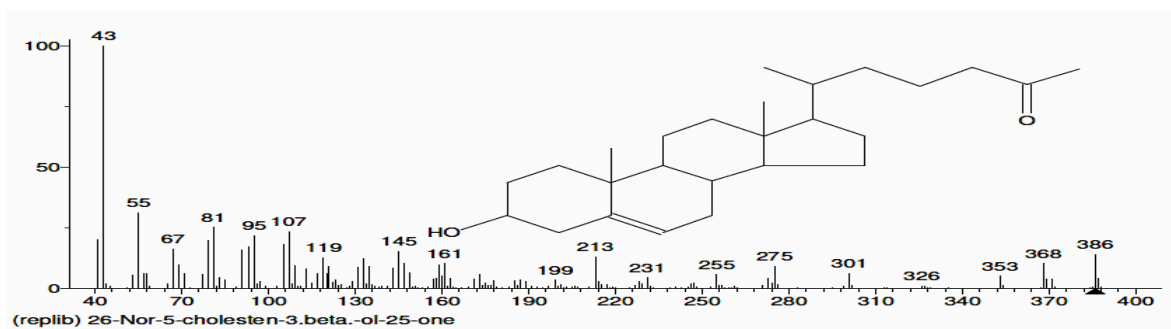


Figure -14. Mass spectrum of 26-Nor-5-cholesten-3-beta-ol-25-one (RT :28.18)

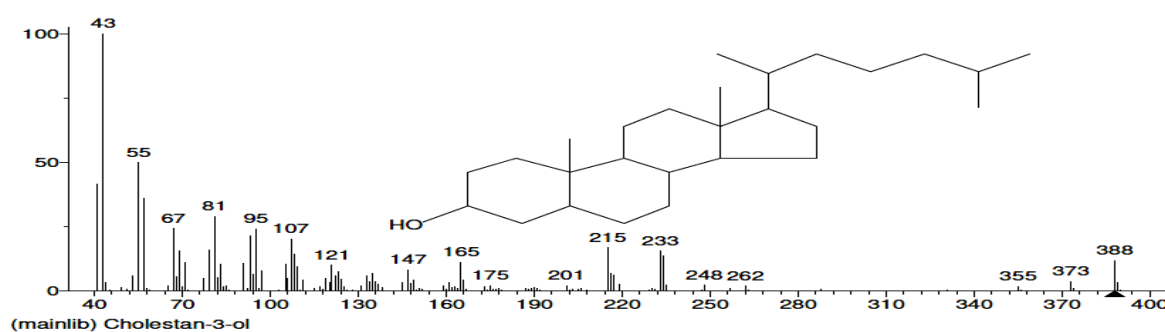
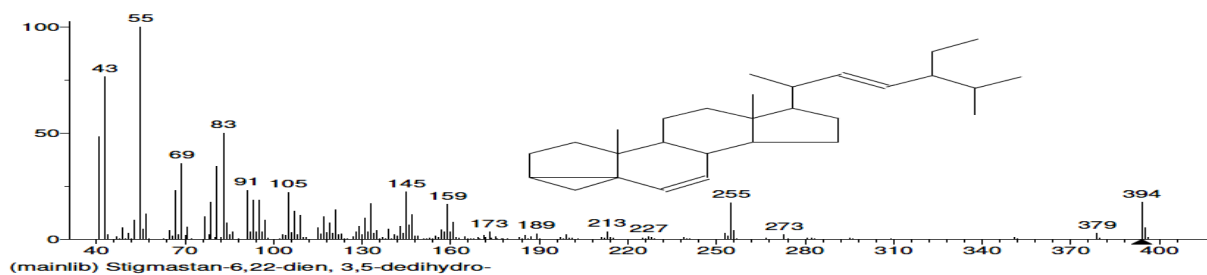
Figure - 15. Mass spectrum of Cholestan-3 α -ol (RT :28.32)

Figure - 16. Mass spectrum of 3,5-dedihydro-6,22-dien-stigmastan (RT :28.80)

CONCLUSION

The study clearly indicates that the ethanolic extract of *Phallusia arabica* is rich in many bioactive chemical compounds. However further studies such as isolation, purification and structure determination is required for the development of a new drug.

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