

GC – MS Analysis of the Simple Ascidian *Microcosmus exasperatus* Heller, 1878

V.K.Meenakshi*, S.Gomathy and K.P.Chamundeswari

Department of Zoology, A.P.C.Mahalaxmi College for Women, Tuticorin – 628002,
Tamilnadu, India.

*Corres. Author: vkmeenakshi@yahoo.com

Abstract: An investigation was carried out to analyze the active constituents present in the simple ascidian *Microcosmus exasperatus*. Ethanolic extract of *Microcosmus exasperatus* has been subjected to GC – MS analysis. Twenty chemical constituents were identified of which n-hexadecanoic acid (21.66%), tetradecanoic acid (18.98%), trichloroacetic acid, hexadecyl ester (12.57%), 26-Nor-5-cholesten-3 α -ol-25-one (10.70%), 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)- (8.29%), Cholestan-3-ol (6.95%) and 2-piperidinone, N-[4,bromo-n-butyl]- (3.48%) were the main constituents with biological activity. All the 20 chemical constituents are being reported for the first time from the whole body ethanolic extract of the simple ascidian *Microcosmus exasperatus*.

Key words: Ascidian, *Microcosmus exasperatus*, GC – MS analysis.

Introduction

Ascidians are marine sedentary animals with cosmopolitan distribution. They are ciliary filter feeders found occurring from the littoral zone to the deep sea. They form major components of fouling community colonizing the surface of corals⁽¹⁾, harbor installations and almost all under water marine structures. Ascidians are capable of creating an unfavourable or toxic condition immediately above their surface as a chemical defense⁽²⁾. This may contain several secondary metabolites which can prevent the attachment of other bio-fouling organisms on their surface. *Microcosmus exasperatus* is a simple ascidian found widely distributed in all oceans. In India it was first reported by Krishan *et al.*, from Madras coast⁽³⁾. Later this species was found to occur throughout the year from Tuticorin coast⁽⁴⁾. A review of literature shows that work on the biodiversity⁽⁵⁾, antimicrobial⁽⁶⁾, antifouling⁽⁷⁾, nutritive value⁽⁸⁾ and isolation of bioactive compounds⁽⁹⁾ have been reported

from the ascidians of Indian waters by earlier workers. Chemical investigation of *Microcosmus exasperatus*, a commonly occurring bio-fouling ascidian of Tuticorin coast using GC – MS has not been carried out so far. This paper presents the results of the chemical constituents available in the whole body ethanolic extract of *Microcosmus exasperatus*.

Materials and Methods

Animal Material

Microcosmus exasperatus belongs to the Class: Ascidiacea, Order: Pleurogona, Suborder: Stolidobranchia and Family: Pyuridae. It has a hard leathery orange coloured tunic free from sand and other particles with two clearly visible siphons. The pharynx has 8-9 folds. There is one gonad in each side of the body, divided in to three portions. It has been found to breed throughout the year. Samples of *Microcosmus exasperatus* were collected from Tuticorin coast, cleaned with sea water, shade dried

and powdered. 5gm powder was soaked in 20ml of ethanol. The mixture was shaken well every 12hours. After three days it was filtered through Whatmann filter paper No.41. The extract was collected and evaporated to dryness using rotary vacuum evaporator. The final residue obtained was subjected to GC –MS analysis.

GC – MS Analysis

GC – MS analysis was carried out on a GC Clarus 500 Perkin Elmer comprising a AOC-20i auto-sampler 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 μ m df composed of 5% Diphenyl / 95% Dimethyl poly siloxane), 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 μ 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 2

minute 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.

Results and Discussion

GC – MS chromatogram of the ethanolic extract of *Microcosmus exasperatus* showed 20 peaks indicating the presence of 20 chemical constituents (Figure 1). The 20 active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the ethanolic extract of *Microcosmus exasperatus* are presented in Table 1. On comparison of the mass spectra of the constituents with the NIST library the 20 constituents were characterized and identified. The prevailing compounds were n-hexadecanoic acid (21.66%), tetradecanoic acid (18.98%), trichloroacetic acid, hexadecyl ester (12.57%), 26-Nor-5-cholesten-3 α -ol-25-one (10.70%) 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)- (8.29%), Cholestan-3-ol (6.95%) and 2-piperidinone, N-[4-bromo-n-butyl]- (3.48%).

Sample 56

GC-MS Analysis274

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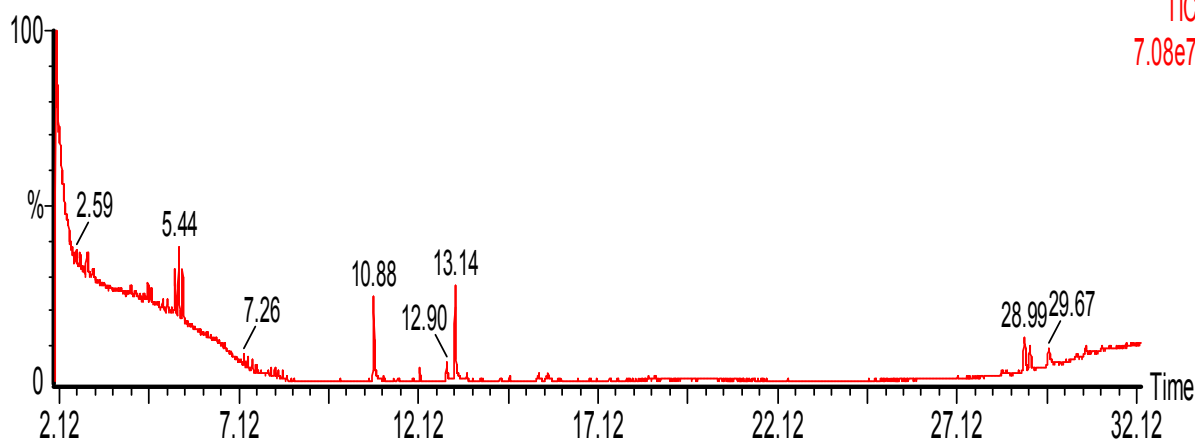


Fig. 1: GC - MS Chromatogram of the Ethanolic extract of *Microcosmus exasperatus*

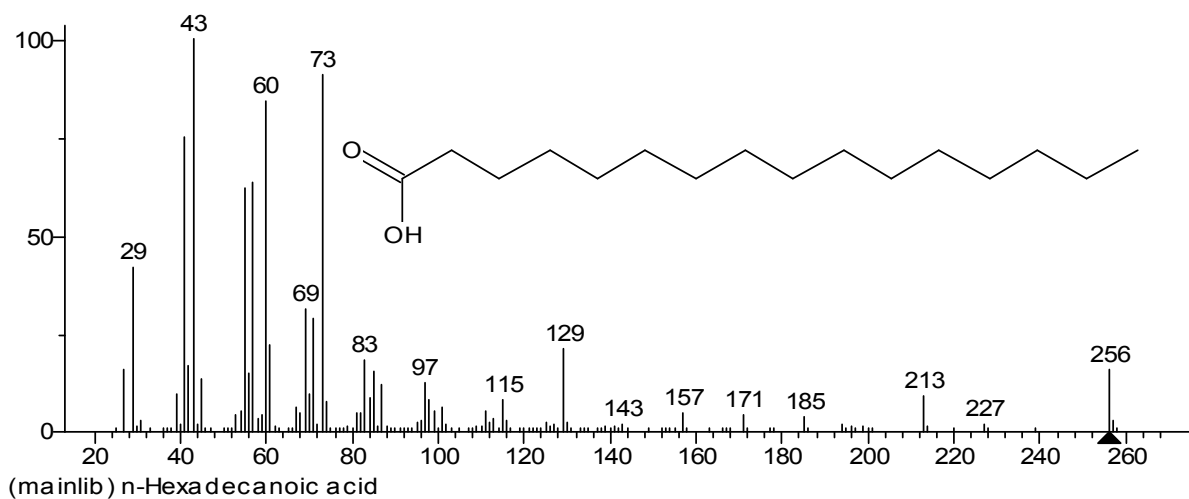
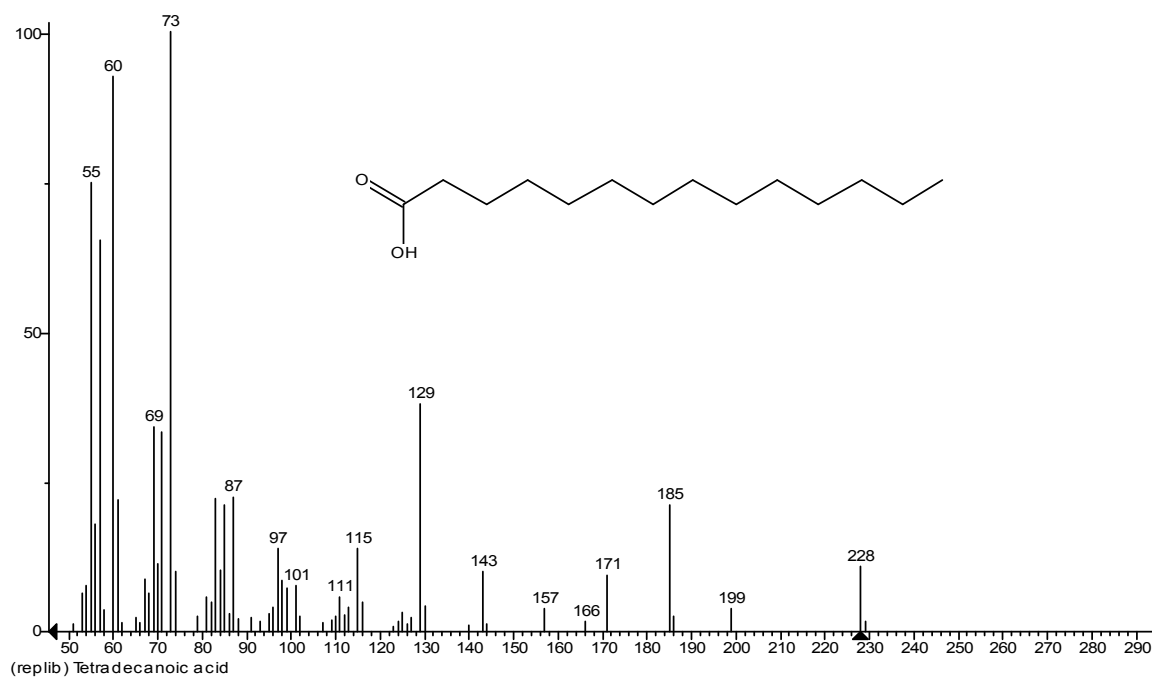
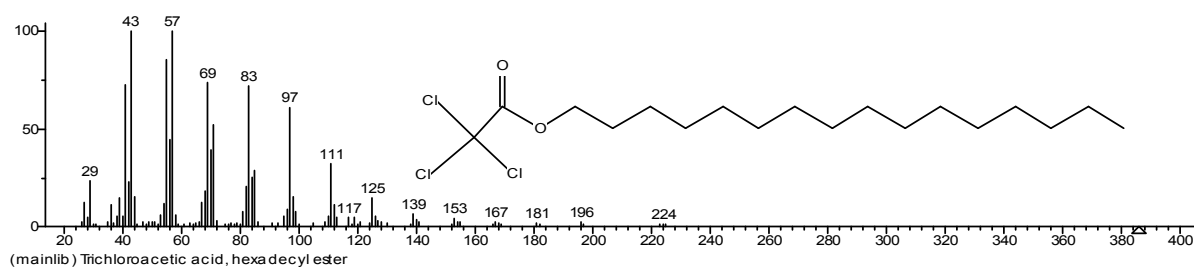
Table 1: Chemical constituents identified in the Ethanolic extract of *Microcosmus exasperatus*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	5.44	Trichloroacetic acid, hexadecyl ester	C ₁₈ H ₃₃ Cl ₃ O ₂	386	12.57
2.	7.26	Octadecanoic acid, 2-oxo-, methyl ester	C ₁₉ H ₃₆ O ₃	312	1.60
3.	7.49	Nitric acid, nonyl ester	C ₉ H ₁₉ NO ₃	189	1.87
4.	8.12	Cyclohexane, 1,2,4,5-tetraethyl-, (1à,2à,4à,5à)-	C ₁₄ H ₂₈	196	1.34
5.	10.88	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	18.98
6.	12.16	1-Dodecanol	C ₁₂ H ₂₆ O	186	1.60
7.	12.90	7-Tetradecene, (E)-	C ₁₄ H ₂₈	196	4.28
8.	13.14	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	21.66
9.	13.46	Heptanoic acid, 2-ethyl-	C ₉ H ₁₈ O ₂	158	0.80
10.	14.40	2,3-Epoxyhexanol	C ₆ H ₁₂ O ₂	116	0.53
11.	14.66	Nitric acid, nonyl ester	C ₉ H ₁₉ NO ₃	189	0.53
12.	15.46	4-Undecene, 3-methyl-, (E)-	C ₁₂ H ₂₄	168	1.60
13.	15.73	(2S,3S)-(-)-3-Propyloxiranemethanol	C ₆ H ₁₂ O ₂	116	1.60
14.	16.02	Cyclohexane, (ethoxymethoxy)-	C ₉ H ₁₈ O ₂	158	0.53
15.	18.71	Acetic acid, trifluoro-, 2,2-dimethylpropyl ester	C ₇ H ₁₁ F ₃ O ₂	184	0.53
16.	18.92	Hydroperoxide, 1-ethylbutyl	C ₆ H ₁₄ O ₂	118	0.53
17.	28.99	26-Nor-5-cholesten-3à-ol-25-one	C ₂₆ H ₄₂ O ₂	386	10.70
18.	29.15	Cholestan-3-ol	C ₂₇ H ₄₈ O	388	6.95
19.	29.66	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	C ₂₅ H ₃₆ O ₂	368	8.29
20.	30.70	2-Piperidinone, N-[4-bromo-n-butyl]-	C ₉ H ₁₆ BrNO	233	3.48

Table 2: Activity of Chemical constituents identified in the Ethanolic extract of *Microcosmus exasperatus*

S. No.	Name of the compound	Compound Nature	**Activity
1.	Trichloroacetic acid, hexadecyl ester	Chlorine compound	Antimicrobial
2.	Octadecanoic acid, 2-oxo-, methyl ester	Ester compound	No activity reported
3.	Nitric acid, nonyl ester	Ester compound	No activity reported
4.	Cyclohexane, 1, 2, 4,5-tetraethyl-, (1à, 2à, 4à, 5à)-	Aromatic hydrocarbon	No activity reported
5.	Tetradecanoic acid	Myristic acid	Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic
6.	1-Dodecanol	Alcoholic compound	Antimicrobial
7.	7-Tetradecene, (E)-	Alkene compound	No activity reported
8.	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor,

			Hemolytic 5-Alpha reductase inhibitor
9.	Heptanoic acid, 2-ethyl-	Fatty acid compound	No activity reported
10.	2,3-Epoxyhexanol	Epoxy compound	No activity reported
11.	Nitric acid, nonyl ester	Ester compound	No activity reported
12.	4-Undecene, 3-methyl-, (E)-	Alkene compound	No activity reported
13.	(2S,3S)-(-)-3-Propyloxiranemethanol	Oxirane compound	No activity reported
14.	Cyclohexane, (ethoxymethoxy)-	Aromatic compound	No activity reported
15.	Acetic acid, trifluoro-, 2,2-dimethylpropyl ester	Fluro compound	Antimicrobial
16.	Hydroperoxide, 1-ethylbutyl	Peroxide compound	No activity reported
17.	26-Nor-5-cholesten-3-ol-25-one	Steroid	Antimicrobial Anti-inflammatory Antiasthma Anticancer Antioxidant Hepatoprotective Hypoglycemic Antipyretic Estrogenic
18.	Cholestan-3-ol	Steroid	Antimicrobial Anti-inflammatory Antiasthma Anticancer Antioxidant Hepatoprotective Hypoglycemic Antipyretic Estrogenic
19.	6,9,12-Octadecatrienoic acid, phenyl methyl ester, (Z,Z,Z)-	Linolenic acid ester compound	Cancer preventive Anti-inflammatory, Hypocholesterolemic, Hepatoprotective Nematicide, Insectifuge Antihistaminic, Antieczemic Antiacne, Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
20.	2-Piperidinone, N-[4-bromo-n-butyl]-	Alkaloid	Antimicrobial Anti-inflammatory Antioxidant

**Fig. 2: Mass spectrum of n-Hexadecanoic acid (RT. 13.14)****Fig. 3: Mass spectrum of Tetradecanoic acid (RT. 10.88)****Fig. 4: Mass spectrum of Trichloroacetic acid, hexadecyl ester (RT. 5.44)**

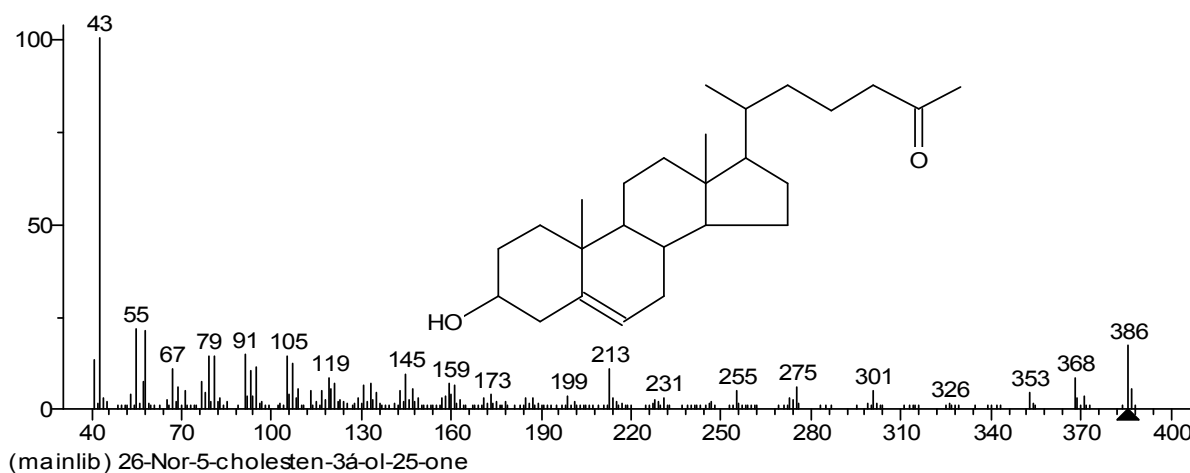


Fig. 5: Mass spectrum of 26-Nor-5-cholesten-3-ol-25-one (RT. 28.99)

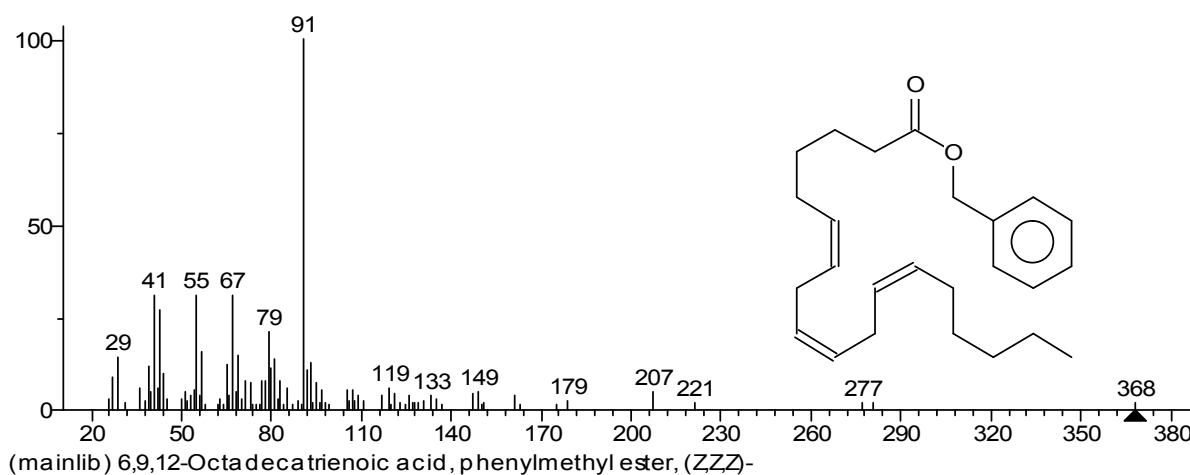


Fig. 6: Mass spectrum of 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)- (RT. 29.66)

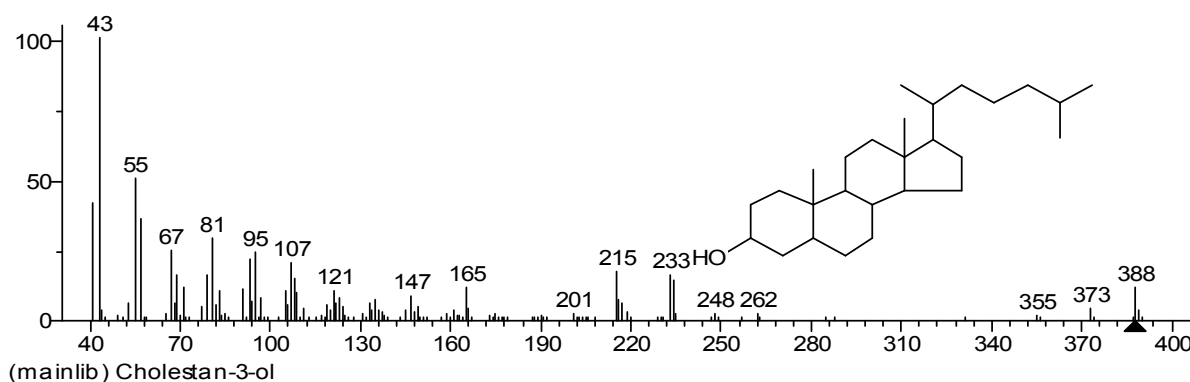


Fig. 7: Mass spectrum of Cholestan-3-ol (RT. 29.15)

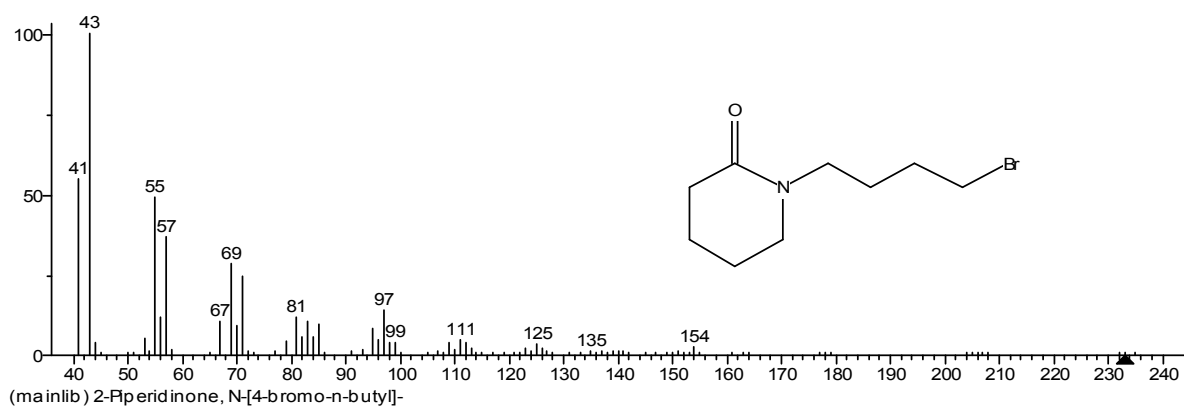


Fig. 8: Mass spectrum of 2-Piperidinone, N-[4-bromo-n-butyl]- (RT. 30.70)

Figures 2 to 8 shows the mass spectrum and structure of these compounds. Table 2 shows the biological activities of the major components obtained through the GC – MS study of *Microcosmus exasperatus*. In the present study 20 different compounds were identified from the ethanolic extract of *Microcosmus exasperatus*. These compounds exhibited the following biological activities such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, hypoglycemic, antiasthmatic, anticancer, antipyretic and antiandrogenic. Similar observations on the presence of antimicrobial⁽⁶⁾, larvicidal⁽¹⁰⁾, antifouling⁽⁷⁾, antioxidant⁽¹¹⁾ and bioactive compounds⁽¹²⁾ have been reported from other species of Indian ascidians.

It can be concluded that *Microcosmus exasperatus* contains various bioactive compounds. Hence, it is a simple ascidian of pharmaceutical importance from Tuticorin coast. Further studies on the isolation, purification and structure determination of the chemical constituents responsible for the biological activity is needed.

Acknowledgement

The authors express their deep sense of gratitude to the University Grants Commission, New Delhi for financial assistance and Dr. S. Kumaravel, Senior scientist, Indian Institute of Crop Processing Technology, Thanjavur, India for providing facilities for GC - MS studies.

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