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GC-MS DETERMINATION OF THE BIOACTIVE COMPONENTS OF *MICROCOSMUS EXASPERATUS* HELLER, 1878

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ABSTRACT

Bioactive components of the methanolic extract of simple ascidian *Microcosmus exasperatus* was investigated using Perkin Elmer Gas chromatography-Mass Spectrometry. The mass spectra of the components were matched with the data available in the National Institute of Standards and Technology (NIST) library. The analysis revealed the presence of nine chemical constituents of which the major are (Z)-9-Octadecenamide (47.31%), 3, 5-bis-trimethylsilyl-2, 4, 6-cycloheptatrien-1-one, (15.50%), Trimethylsilyl ether 1-monolinoleoyl glycerol (10.54%). Of the nine components identified, seven components are being reported for the first time from ascidians. 2-Isopropyl-5-methyl-1-heptanol (RT : 5.33), Isotridecanol (RT : 5.43), 2-methyl-2-undecanethiol (RT : 5.57), 9,9-dimethoxybicyclo[3.3.1]nona-2, 4-dione (RT : 13.13), Mono (2-ethylhexyl) ester-1,2-benzenedicarboxylic acid (RT : 20.91), (Z)-9-Octadecenamide (RT : 24.46), Trimethylsilyl ether 1-monolinoleoylglycerol (RT : 28.34) showed antimicrobial, anti-inflammatory, antioxidant and antifouling activities.

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

INTRODUCTION

Ascidians are marine sessile filter feeders. They contain toxic secondary metabolites which can act as chemical defense preventing the attachment of other bio-fouling organisms on their surface[1]. *Microcosmus exasperatus* is a simple ascidian belonging to the family Pyuridae found widely distributed in all oceans. Krishnan et al.[2] reported its occurrence from Madras coast of India. A review of literature shows that report on the GC-MS analysis of *Phallusia nigra*[3] and *Microcosmus exasperatus*[4] using ethanolic extract is avaliable from Indian waters. In the present study GC-MS determination of the bioactive components of methanolic extract of *Microcosmus exasperatus* was carried out.

EXPERIMENTAL

Animal material

Microcosmus exasperatus were collected from Tuticorin coast and identified using key to identification of Indian ascidians[5]. A voucher specimen AS 2240 has been deposited in the museum, Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin for further reference.

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Preparation of extract

The whole animal was dried under shade and then homogenized to get a coarse powder. The powder was extracted with methanol using Soxhlet apparatus. The extract was cooled to room temperature, evaporated in a rotary evaporator to get a residue. 3 µL of the methanolic extract of *Microcosmus exasperatus* was employed for 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 (30 mm x 0.25 mm x 0.25 µm df composed of 5% Diphenyl / 95% Dimethyl poly siloxane), operating in electron impact mode at 70 eV. Helium (99.999%) was used as carrier gas at a constant flow rate of 1 mL per minute and an injection volume of 3 µL (split ratio 10 : 1). An injector temperature of 250^{o}C and an ion-source temperature of 280^{o}C were employed.

The oven temperature was programmed from 110^{o}C for 2 minutes with an increase of 10^{o}C / minute to 200^{o}C, then 5^{o}C / minute, ending with a 9 minute isothermal at 280^{o}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 software was used.

RESULTS AND DISCUSSION

GC–MS chromatogram of the methanolic extract of *Microcosmus exasperatus* (Fig. 1) showed nine peaks indicating the presence of nine chemical constituents. On comparison of the mass spectra of the constituents with the NIST library the nine constituents were characterized and identified. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the methanolic extract of *Microcosmus exasperatus* are presented in Table 1. Fig. 2 to 10 show the mass spectrum and structure of the nine compounds N-[4-bromo-n-butyl]-2-piperidinone (RT : 4.12), 2-Iso propyl-5-methyl-1-heptanol (RT : 5.33), Isotridecanol (RT : 5.43), 2-methyl-2-undecanethiol (RT : 5.57), 9, 9-dimethoxybicyclo[3.3.1]nona-2,4-dione (RT: 13.13), Mono(2-ethylhexyl) ester-1,2-benzenedicarboxylic acid (RT : 20.91), (Z)-9-Octadecenamide (RT: 24.46), Trimethylsilyl ether 1-monolinoleoylglycerol (RT: 28.34) and 3,5-bis-trimethylsilyl-2,4,6-cycloheptatrien-1-one (RT: 28.94), respectively. Among the nine components 3,5-bis-trimethylsilyl-2,4,6-cycloheptatrien-1-one has been reported earlier from the methanolic extract of *Phallusia nigra*[3] and N-[4-bromo-n-butyl]-2-Piperidinone from the ethanolic extract of *Microcosmus exasperatus*[4] where as the remaining seven components are being reported for the first time from ascidians. 20 different compounds have already been identified by GC-MS analysis from the ethanolic extract of *Microcosmus exasperatus* compared to only nine compounds from methanolic extract indicating that the solvent selected for extraction plays an important role in identifying the chemical constituents.

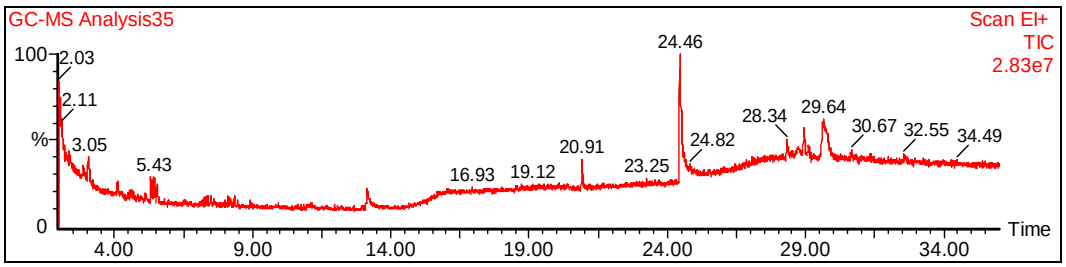

Fig. 1: GC-MS Chromatogram of the Methanolic extract of *Microcosmus exasperatus*

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

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area%
1.	4.12	N-[4-bromo-n-butyl]-2-Piperidinone	$C_9H_{16}BrNO$	233	3.10
2.	5.33	Isopropyl-5-methyl-1-heptanol	$C_{11}H_{24}O$	172	3.72
3.	5.43	Isotridecanol	$C_{13}H_{28}O$	200	3.72
4.	5.57	2-methyl-2-undecanethiol	$C_{12}H_{26}S$	202	3.51
5.	13.13	9,9-dimethoxybicyclo[3.3.1]nona-2, 4-dione	$C_{11}H_{16}O4$	212	7.64
6.	20.91	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	4.96
7.	24.46	(Z)-9-Octadecenamide	$C_{18}H_{35}NO$	281	47.31
8.	28.34	Trimethylsilyl ether 1-monolinoleoylglycerol	$_{27}H_{54}O_4Si_2$	498	10.54
9.	28.94	3,5-bis-trimethylsilyl-2, 4, 6-cycloheptatrien-1-one	$C_{13}H_{22}OSi2$	250	15.50

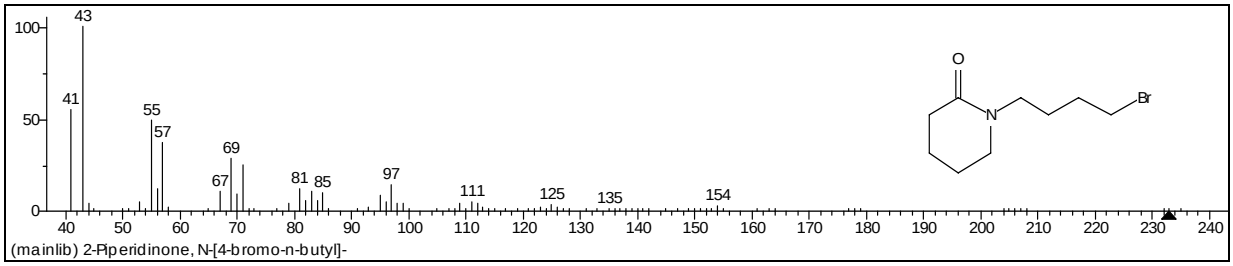

Fig. 2: Mass spectrum of N-[4-bromo-n-butyl] - 2-Piperidinone (RT: 4.12)

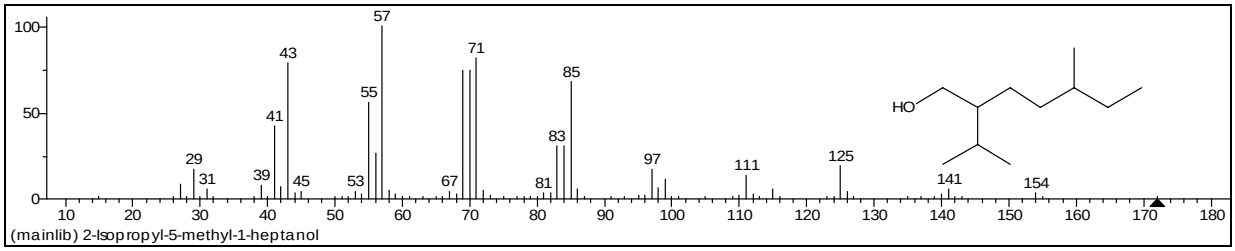

Fig. 3: Mass spectrum of 2-Isopropyl-5-methyl-1-heptanol (RT: 5.33)

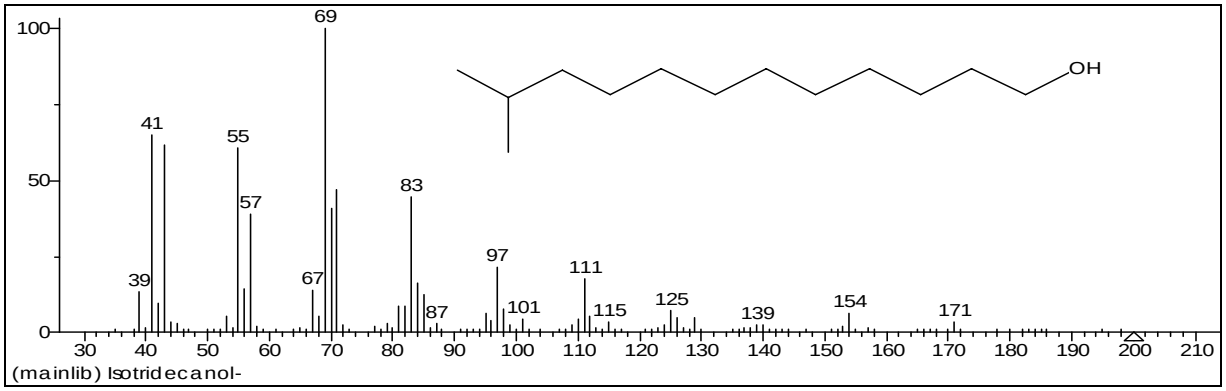

Fig. 4: Mass spectrum of Isotridecanol- (RT: 5.43)

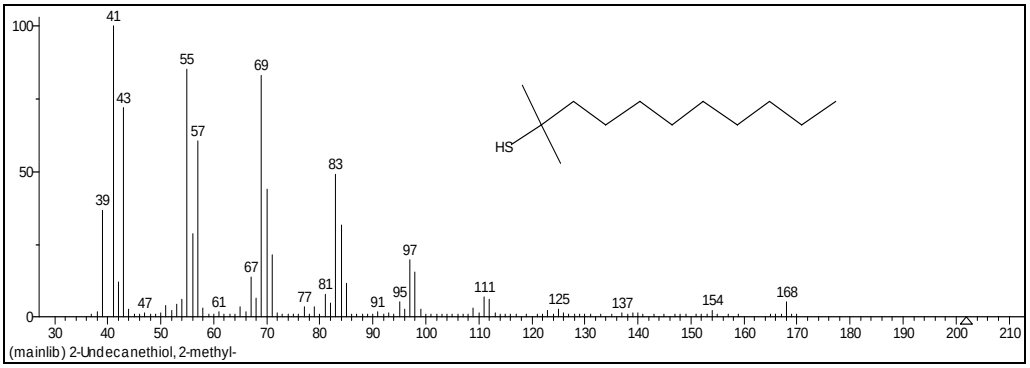

Fig. 5: Mass spectrum of 2-methyl-2-Undecanethiol (RT: 5.57)

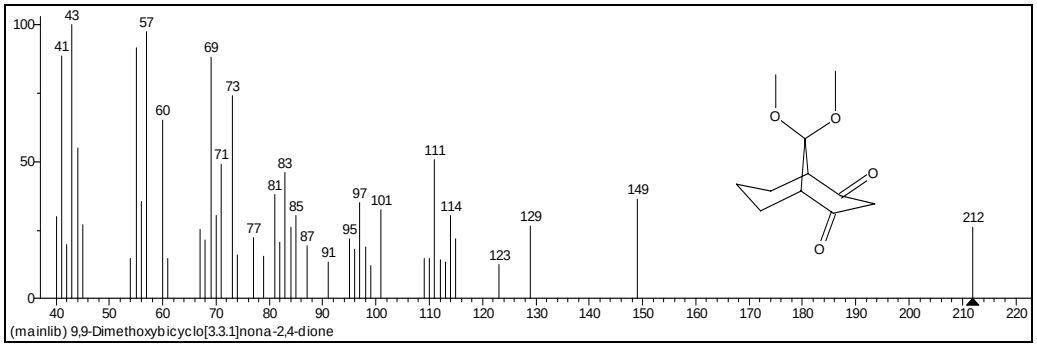

Fig. 6: Mass spectrum of 9, 9-Dimethoxybicyclo [3.3.1] nona-2, 4-dione (RT: 13.13)

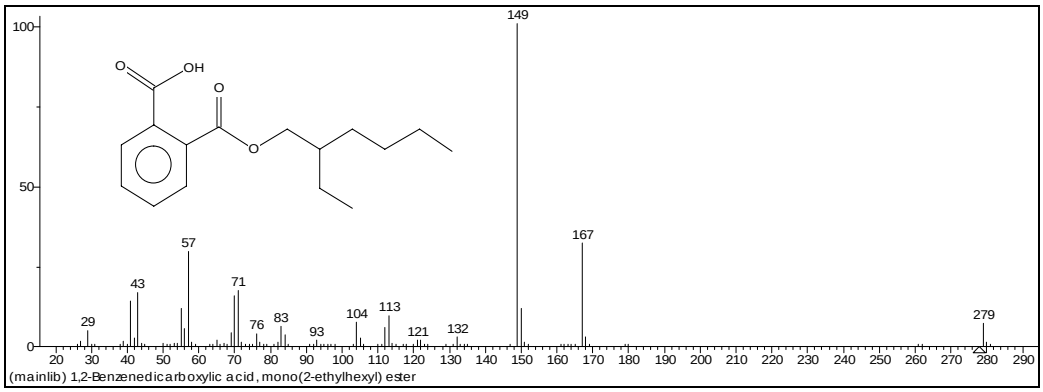

Fig. 7: Mass spectrum of 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (RT: 20.91)

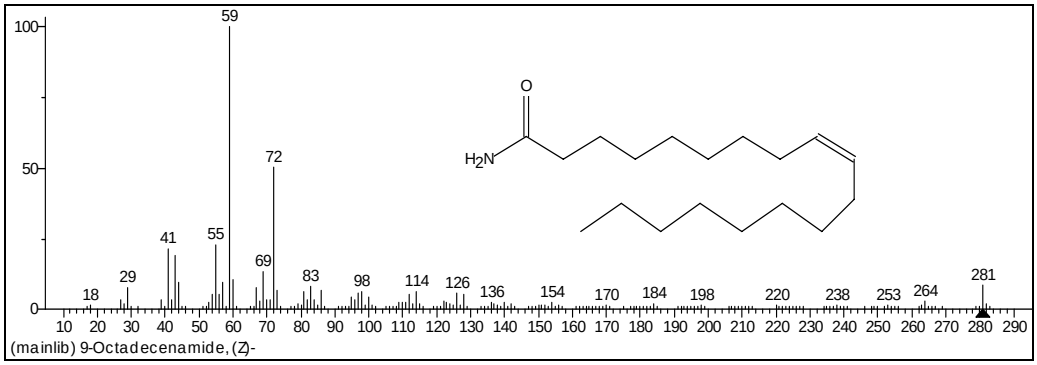

Fig. 8: Mass spectrum of 9-Octadecenamide, (Z)- (RT: 24.46)

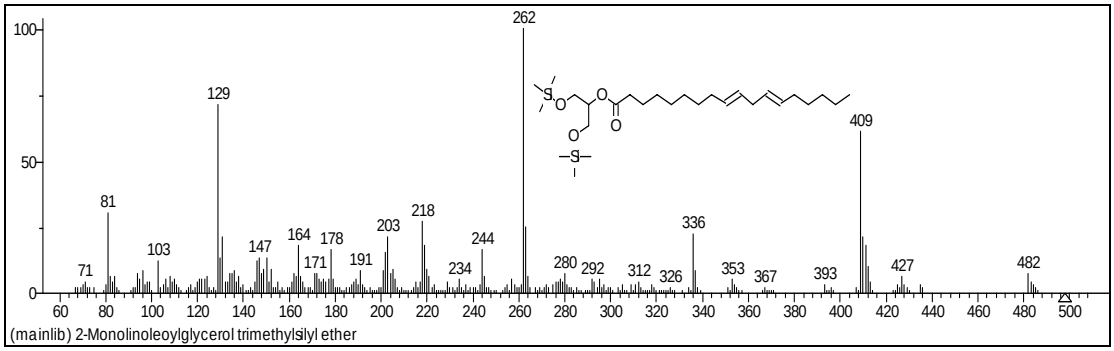

Fig. 9: Mass spectrum of 2-Monolinoleoylglycerol trimethylsilyl ether (RT: 28.34)

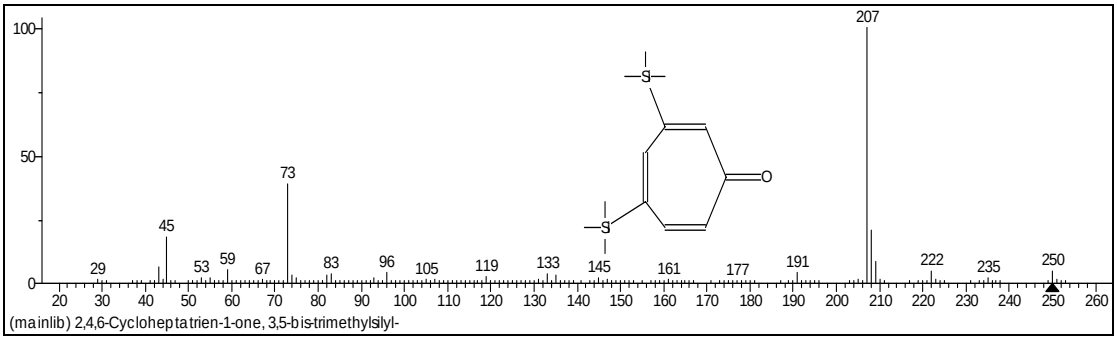

Fig. 10: Mass spectrum of 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl- (RT: 28.94)

The biological activities of the major components obtained through the GC-MS study of *Microcosmus exasperatus* is given in Table 2. Of the nine constituents identified, seven of them exhibited antimicrobial where as only one constituent showed anti-inflammatory, anti oxidant and antifouling activities. Similar observations on the presence of antimicrobial[5,6], antifouling[7] and antioxidant[8] compounds have been reported from other species of Indian ascidians.

Table 2: Activity of chemical constituents identified in the methanolic extract of *microcosmus exasperatus*

S. No.	Name of the compound	Nature of compound	**Activity
1	N-[4-bromo-n-butyl] - 2-Piperidinone	Alkaloid	Antimicrobial. antioxidant, anti-inflammatory
2	Isopropyl-5-methyl-1-heptanol	Alcoholic	Antimicrobial
3	Isotridecanol-	Alcoholic	Antimicrobial
4	2-methyl-2-undecanethiol	Sulfur	Antimicrobial
5	9,9-dimethoxybicyclo [3.3.1] nona-2,4-dione	Ketone	No activity
6	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	Plasticizer	Antimicrobial antifouling
7	(Z)-9-octadecenamide	Amide	Antimicrobial
8	Trimethylsilyl ether 1-monolinoleoylglycerol	Steroid	Antimicrobial
9	3,5-bis-trimethylsilyl-2,4,6-cycloheptatrien-1-one	Ketone	No activity

**Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

CONCLUSION

Microcosmus exasperatus contains various bioactive compounds in both ethanolic and methanolic extract. An evaluation of the pharmacological and biomedical properties is suggested, which may lead to the discovery of drug molecules as chemotherapeutic agents in combating various diseases of mankind

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REFERENCES

1. M. Wahl, Mar. Ecol. Prog. Ser., **58**, 175-189 (1989).
2. R. Krishnan, M. R. Chandran and T. K. Renganathan, Geobios New Reports, **8**, 70-74 (1989).
3. S. Gopalakrishnan, V. K. Meenakshi and D. Shanmuga Priya, Int. J. Pharma and Biosci., **2**, 382-387 (2011).
4. V. K. Meenakshi, S. Gomathy and K. P. Chamundeswari, Int. J. Chem. Tech. Res., **4**, 55-62 (2012).
5. B. K. Amutha, V. K. Meenakshi and S. Senthamarai, Int. J. Pharm. Sci., **2**, 750-758 (2010).
6. N. Sri Kumaran, S. Bragadeeswaran and V. K. Meenakshi, Asian Pacific J. Tropical Biomedicine, S90-S99 (2011).
7. M. Santhana Ramasamy and A. Murugan, SDMRI Research Publications, **3**, 165-167 (2003).
8. P. Krishnaiah, V. L. Reddy, G. Venkataramana, K. Ravinder, M. Srinivasulu, T. V. Raju, K. Ravikumar, D. Chandrasekar, S. Ramakrishna and Y. Venkateswarlu, J. Nat. Prod., **67**, 1168-1171 (2004).