The Eco-friendly Marine Gastropod *Turbo brunneus* (L. 1758) and its Vital role in Future Pharmaceutical Industry Through GC-MS Study

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Abstract: Molluscs form valuable fisheries in various parts of the coast of India providing shell fish as food and as source of lime, pearls and decorative shells, as constituents of medicinal preparations etc. In the present study, the eco-friendly *Turbo brunneus* and its vital role in future pharmaceutical industry through GC-MS analysis was carried out. Almost eighteen compounds are obtained through GC-MS which might be responsible for antimicrobial, pharmaceutical, insect repellent, anti-inflammatory, anticancer, antiasthmatic, diuretic and antiarthritic activities.

Keywords: *Molluscs, pharmaceutical industry, GC-MS analysis, antimicrobial, anticancer*

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I. INTRODUCTION

Among the marine organisms, molluscs are one of the most successful forms of animal life and they have conquered almost every habitat and exist in all the oceans (from shallow tidal pools to the deepest trenches). Many studies on bioactive compounds from molluscs exhibiting antibacterial, antitumour, antileukemic and antiviral activities have been reported worldwide (Pettit *et al.,* 1987; Kamiya *et al.,* 1989; Prem Anand *et al.,* 2002; Rajaganapathy *et al.,* 2002; Thilaga, 2005; Kathiresan *et al.,* 2008; Dhinakaran *et al.,* 2011 and Ashok Kumar 2011).

Today most infectious diseases can be brought under control with natural or synthetic drugs. We are still in great need of safer, cheaper and effective drugs. Some marine molluscs have shown pronounced activities, useful in the biomedical arena. The potential of marine molluscs as a source of biologically active products is largely unexplored in India. Hence, a broad based screening of marine molluscs for bioactive compounds is necessary. A thorough understanding of chemical structure and biological activity will lead to the formulation of novel drugs with specific actions. Considering the above facts the present study has been undertaken to test the molluscan extracts against human pathogens and to isolate the bioactive substance from the most potent extract that purify and characterize it.

II. MATERIAL AND METHODS

In the present study, the whole body tissues of *Turbo brunneus* were used. The freshly collected samples were cleaned and washed with fresh sea water to remove all impurities. The shells were removed and the tissues were then sun dried. From this dried tissues, the crude methanol extract was prepared.

A. Fractionation

Crude methanol extract was fractioned by silica gel column chromatography. The extract was fractionated with five solvent systems. Elutions with Hexane: Chloroform (1:1), Chloroform (100%), Benzene (100%), Methanol (100%) and Distilled water in order of their polarity afford five fractions F1, F2, F3, F4 and F5. A known amount of extract was taken and their organic solvents were removed by vaccum evaporation, solids were dissolved in deionised water and concentration series of 1mg/ml, 10 mg/ml and 100 mg/ml were prepared and the antibacterial activity of each extract was tested thrice for confirmation.

B. GC-MS

The more potent fraction was characterized to know the functional groups through GC-MS study at Indian institute of crop processing Technology-Thanjavur.

GC – MS Analysis

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC 20i auto sampler and gas chromatography interfaced to a mass spectrophotometer (GC-MS) instrument employing the following conditions such as Column Elite – 5 MS fused silica capillary column (30 x 0.25 mm ID x 0.25 µm df, composed of 5% Diphenyl/95% Dimethyl poly siloxane), operating in electron impact mode at 70eV: Helium (99.999%) was used as a carrier gas at constant flow of 1ml/min and an injection volume of 3 µl (split ratio of 10:1) injector temperature 250^0C. The oven temperature was programmed from 110^0C (isothermal for 2 min), with an increase of 10^0C/min to 200^0C, then 5^0C/min to 280^0C. Mass spectra were taken at 70eV; a scan interval of 0.5s and fragments from 45 to 450 Da.

C. Identification of compounds

Interpretation on mass spectrum was conducted using the data base of National Institute Standard and Technology (NIST 08s), WILEY 8 and FAME. The unknown components found in the methanol fraction of the internal bone were matched with the spectrum of the known components stored in NIST, WILEY and FAME, the MS library and predicted from Dukes ethno botanical database.

III. RESULTS

Antibacterial activity was tested against eight bacterial pathogens with respect to five different fractions of the crude methanol extracts of body tissues of *Turbo brunneus.* As fraction 4 exhibited more potent antibacterial activity than other fractions, it was further purified with TLC and characterized through GC –MS.

GC-MS

GC – MS study of fraction 4 from *Turbo brunneus* revealed the presence of the following 18 compounds: Aziridine, 1-methyl, 5-Methyl-2-hexanone oxime, Cyclohexanol,2-amino-,trans, Azocine, octahydro, Butanal, O-methyloxime, L-Homocitrulline, Pentanal, oxime, E-2-Tetradecen-1-ol, Z-10-Pentadecen-1-ol, Lysine, Trimethylamine, compd. with borane (11), 1,1-Cyclopropanedicarbonitrile, 2,2-dimethyl, Cyclopentanol, 2-(aminomethyl)-, cis, Tricyclo[4.2.1.1(2,5)]decan-3-ol, n-Nonanoylmorpholine, Thiomorpholine, Semioxamazide and Cholan-24-oic acid, 3-oxo-, methyl ester, (5á). Of which fifteen antimicrobial compounds were conferred by GC-MS analysis, when F4 fraction (Methanol 100%) of *Turbo brunneus* extract was injected viz: Aziridine, 1-methyl, Cyclohexanol,2-amino-,trans, Azocine, octahydro, Butanal, O-methyloxime, L-Homocitrulline, Pentanal, oxime, E-2-Tetradecen-1-ol, Z-10-Pentadecen-1-ol, Trimethylamine, compd. with borane (11), 1,1-Cyclopropanedicarbonitrile, 2,2-dimethyl, Cyclopentanol, 2-(aminomethyl)-, cis, Tricyclo[4.2.1.1(2,5)] decan-3-ol, Thiomorpholine, Semioxamazide and Cholan-24-oic acid, 3-oxo-, methyl ester, (5á) (Table 21). A steroid compound Cholan-24-oic acid, 3-oxo-, methyl ester, (5a)-with maximum percentage (50.06%) and a Ketone compound 5-methyl-2-hexanone oxime with minimum percentage (0.33%) were identified as antimicrobial compounds (Fig 1– 19 and Table 1).

IV. DISCUSSION

In the present study, a pronounced antibacterial activity has been observed against some bacterial strains. The F1, F2, F3, F4 and F5 and crude extracts of *Turbo brunneus* showed activity against all bacterial strains tested.

4 of *Turbo brunnues* was found to be more potent and it was purified and characterized through GC-MS study. F4 revealed the presence of 18 active compound viz: Aziridine, 1-methyl, 5-Methyl-2-hexanone oxime, Cyclohexanol,2-amino-,trans, Azocine, octahydro, Butanal, O-methyloxime, L-Homocitrulline, Pentanal, oxime, E-2-Tetradecen-1-ol, Z-10-Pentadecen-1-ol, Lysine, Trimethylamine, compd. with borane (11), 1,1-Cyclopropanedicarbonitrile, 2,2-dimethyl, Cyclopentanol, 2-(aminomethyl)-, cis, Tricyclo[4.2.1.1(2,5)]decan-3-ol, n-Nonanoylmorpholine, Thiomorpholine, Semioxamazide and Cholan-24-oic acid, 3-oxo-, methyl ester, (5á).

The present study based on various analysis revealed the presence of alkaloid, Ketone, amino, nitrogen, aldehyde, alcoholic, sulphur and steroid compounds (Table 1). These identified probable antimicrobial compounds which might be responsible for the inhibition of antimicrobial activity in various fractions during the study. These above identified compounds were already been established as an antimicrobial agent by so many authors (De Vries and Beast, 1995; De Lucca, 2000; Hancock, 2000; Welling *et al.,* 2000 and Selitrennikoff, 2001).

The GC-MS study of *Turbo brunneus* reveals the probable antimicrobial compounds such as Aziridine, 1-methyl, Cyclohexanol,2-amino-,trans, Azocine, octahydro, Butanal, O-methyloxime, L-Homocitrulline, Pentanal, oxime, E-2-Tetradecen-1-ol, Z-10-Pentadecen-1-ol, Trimethylamine, compd. with borane (11), 1,1-Cyclopropanedicarbonitrile, 2,2-dimethyl, Cyclopentanol, 2-(aminomethyl)-, cis, Tricyclo[4.2.1.1(2,5)]decan-3-ol, Thiomorpholine, Semioxamazide and Cholan-24-oic acid, 3-oxo-, methyl ester, (5á) which are responsible for the inhibition of *A. hydrophilla, E. coli* and *S.typhi.* The present findings are in agreement with Emiliano Manzo *et al.,* (2007), who reported that two novel triterpenoids, aplyosils A and B BEtzionin a tyrosin derived compound exhibited antibacterial activity against *Bacillus subtilis.* (Lindquist and Fenical, 1990). High concentrations of macrolides, extracted from the egg of Spanish dancer nuetibranch, *Hexabranchus sanguineous* prevented the growth of pathogenic micro organism (Pawlik, 1992).

The GC-MS spectra from *Turbo brunneus* extract provided a complete carbon skeleton of Aziridine, 1-methyl, 5-Methyl-2-hexanone oxime, Cyclohexanol,2-amino-,trans, Azocine, octahydro, Butanal, O-methyloxime, L-Homocitrulline, Pentanal, oxime, E-2-Tetradecen-1-ol, Z-10-Pentadecen-1-ol, Lysine, Trimethylamine, compd. with borane (11), 1,1-Cyclopropanedicarbonitrile, 2,2-dimethyl, Cyclopentanol, 2-(aminomethyl)-, cis, Tricyclo[4.2.1.1(2,5)]decan-3-ol, n-Nonanoylmorpholine, Thiomorpholine, Semioxamazide and Cholan-24-oic acid, 3-

oxo-, methyl ester, (5á) which might be responsible for the inhibition of bacterial growth in the present study, similar finding was reported by way on Mudianta *et al.,* (2010) in an Indonesian sponge *Helichondria sps.* has provided 3-akyl piperidine alkaloids tetradehydrohaliclona-cyclamine A, the mono-N-oxide and a C-2 epimer. Brominated indoles 6-bromo 2-methylthio indolin -3-one extracted from Australian muricid *Dicathdis orbita* has been identified as anticancer drug indole derivatives of 6,6' dibromoindigo have been antimicrobial activity (Benkendorff *et al.,* 2001). An alkaloid Batzelladine L and M isolated by Hua *et al.,* (2007) inhibits *S. aureus.*

In the present study, the potent antibacterial compounds could be detected from *Turbo brunneus.* Since, *Turbo brunneus* is surviving in intertidal rocky shore with coral reef area, either to protect from their enemies or through food the animal might synthesis the chemicals which might be responsible for the inhibition of bacterial growth. These antibacterial compounds can be relatively synthesized, chemically modified, analyzed and manipulated. However, these compounds are also primarily translational products of genes with potent biological activity and can be manipulated by techniques of modern molecular genetics confers the antibacterial compounds from *Turbo brunneus* an important role in expanding bridge between bioactive drug and molecular genetics.

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Table 1 Activity of Components identified in the methanol column fraction (F4) of extract of *Turbo brunneus* [GC MS study]

No.	RT	Name of the compound	Molecular formula	Peak Area %	Compound Nature	**Activity
1.	3.15	Aziridine, 1-methyl-	C_3H_7N	4.75	Alkaloid	Antimicrobial Antiinflammatory
2.	10.37	5-Methyl-2-hexanone oxime	$C_7H_{15}NO$	0.33	Ketone compound	No activity reported
3.	10.85	Cyclohexanol,2-amino-,trans-	$C_6H_{13}NO$	1.73	Amino compound	Antimicrobial
4.	11.41	Azocine, octahydro-	$C_7H_{15}N$	0.85	Nitrogen compound	Antimicrobial
5.	12.59	Butanal, O-methyloxime	$C_5H_{11}NO$	7.29	Aldehyde compound	Antimicrobial
6.	13.20	L-Homocitrulline	$C_7H_{15}N_3O_3$	22.39	Nitrogen compound	Antimicrobial
7.	14.35	Pentanal, oxime	$C_5H_{11}NO$	0.88	Aldehyde compound	Antimicrobial
8.	14.76	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	1.77	Alcoholic compound	Antimicrobial
9.	15.47	Z-10-Pentadecen-1-ol	$C_{15}H_{30}O$	1.62	Alcoholic compound	Antimicrobial
10.	15.71	Lysine	$C_6H_{14}N_2O_2$	1.73	Amino acid	Nutrient
11.	15.97	Trimethylamine, compd. with borane (11)	$C_3H_{12}BN$	0.59	Amino compound	Antimicrobial
12.	16.89	1,1-Cyclopropane dicarbonitrile, 2,2-dimethyl-	$C_7H_8N_2$	1.18	Nitrogen compound	Antimicrobial
13.	17.46	Cyclopentanol, 2-(aminomethyl)-, cis-	$C_6H_{13}NO$	0.41	Amino compound	Antimicrobial
14.	19.80	Tricyclo[4.2.1.1(2,5)]decan-3-ol	$C_{10}H_{16}O$	0.77	Alcoholic compound	Antimicrobial
15.	20.44	n-Nonanoylmorpholine	$C_{13}H_{25}NO_2$	0.63	Nitrogen compound	Insect repellent
16.	21.01	Thiomorpholine	C_4H_9NS	0.88	Sulphur compound	Antimicrobial used in Pharmaceuticals
17.	21.31	Semioxamazide	$C_2H_5N_3O_2$	2.14	Amino compound	Antimicrobial
18.	28.85	Cholan-24-oic acid, 3-oxo, methyl ester, (5á)-	$C_{25}H_{40}O_3$	50.06	Steroid	Antimicrobial Antiinflammatory Anticancer Antiasthma Diuretic Antiarthritic

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Fig 1 Chromatogram of column extract (F4, Methanol 100%) of *Turbo brunneus* by GC-MS

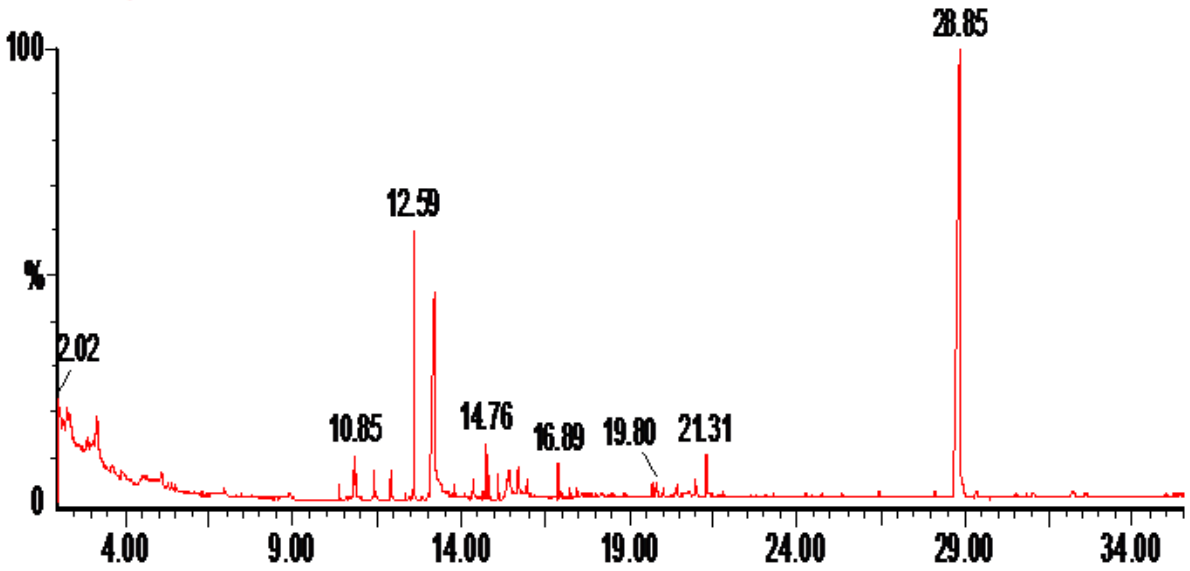

Fig 2 Chromatogram

Name: 5-Methyl-2-hexanone oxime
Formula: C_3H_7N

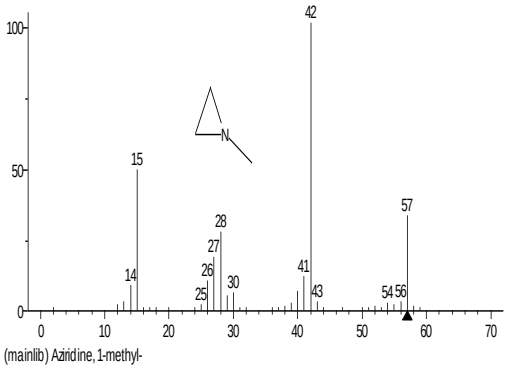

Fig 3 Chromatogram

Name: Aziridine, 1-methyl-
Formula: $C_7H_{15}NO$

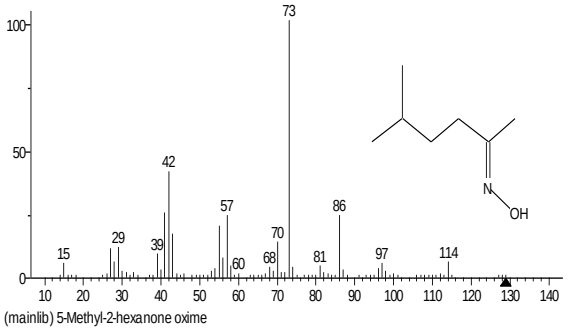

Fig 4 Chromatogram

Name: Cyclohexanol,2-amino-,trans-
Formula: $C_6H_{13}NO$
MW: 115

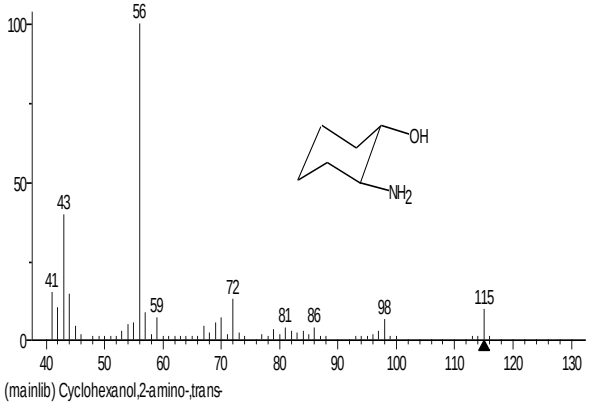

Fig 5 Chromatogram

Name: Azocine, octahydro-
Formula: $C_7H_{15}N$
MW: 113

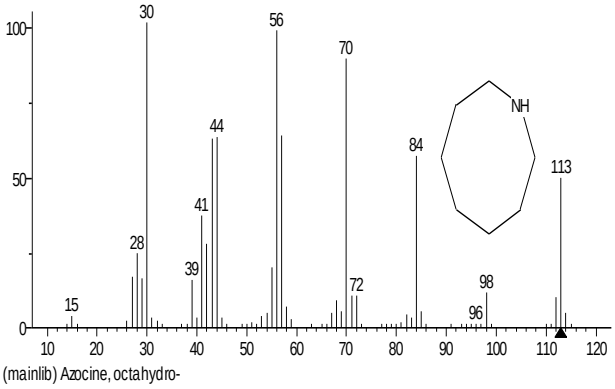

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Fig 6 Chromatogram

Name: Butanal, O-methyloxime
Formula: $C_5H_{11}NO$
MW: 101

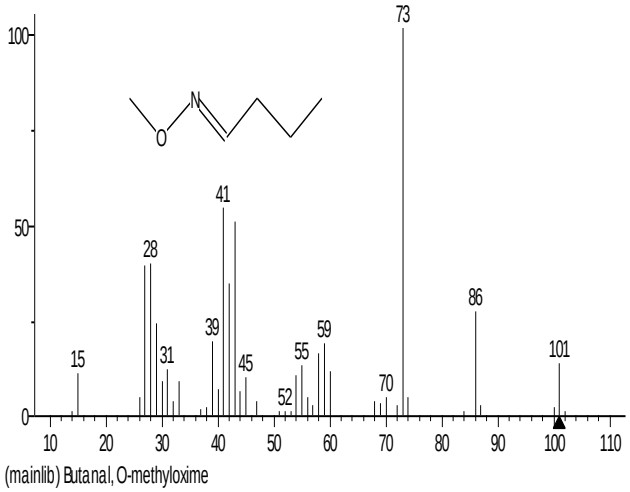

Fig 7 Chromatogram

Name: L-Homocitrulline
Formula: $C_7H_{15}N_3O_3$
MW: 189

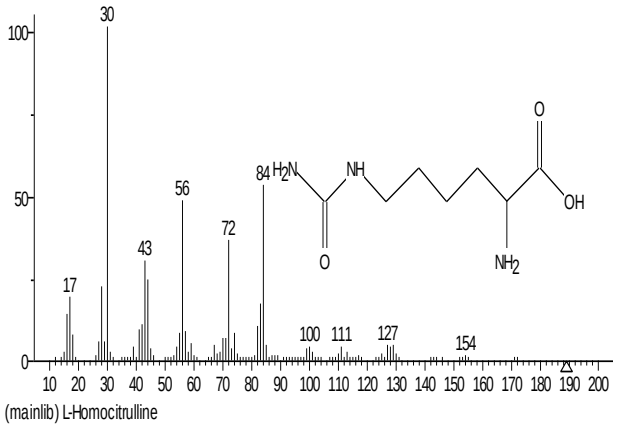

Fig 8 Chromatogram

Name: Pentanal, oxime
Formula: $C_5H_{11}NO$
MW: 101

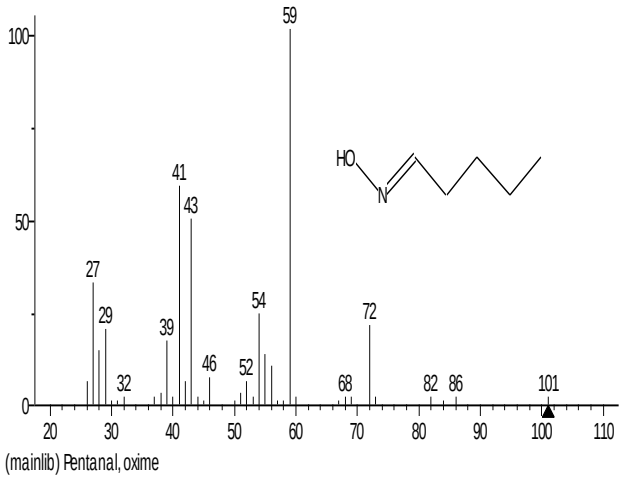

Fig 9 Chromatogram

Name: E-2-Tetradecen-1-ol
Formula: $C_{14}H_{28}O$
MW: 212

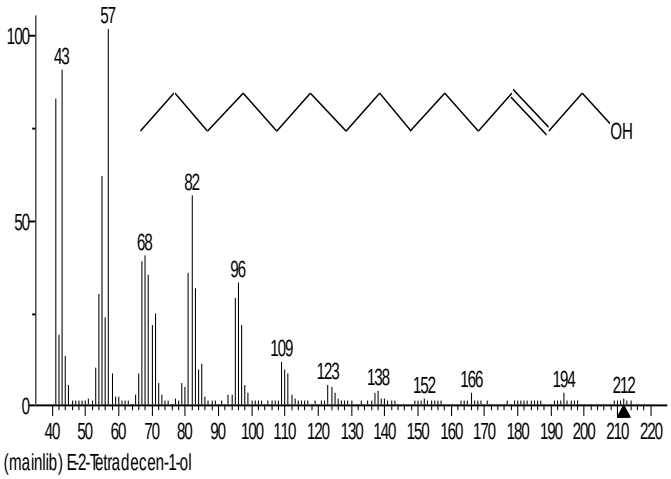

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Fig 10 Chromatogram

Name: Z-10-Pentadecen-1-ol
Formula: $C_{15}H_{30}O$
MW: 226

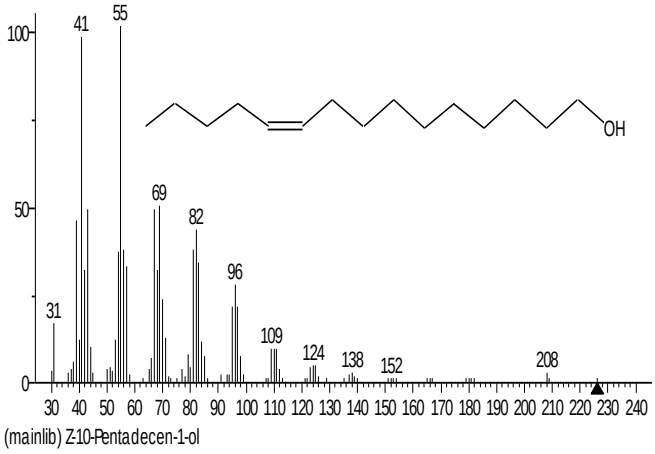

Fig 11 Chromatogram

Name: Lysine
Formula: $C_6H_{14}N_2O_2$
MW: 146

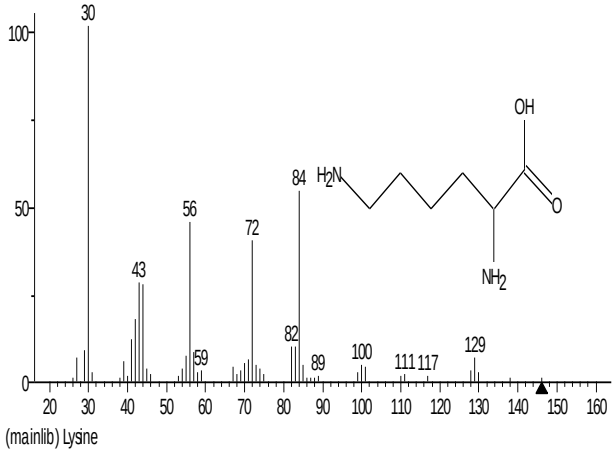

Fig 12 Chromatogram

Name: Trimethylamine, compd. with borane (1:1)
Formula: $C_3H_{12}BN$
MW: 73

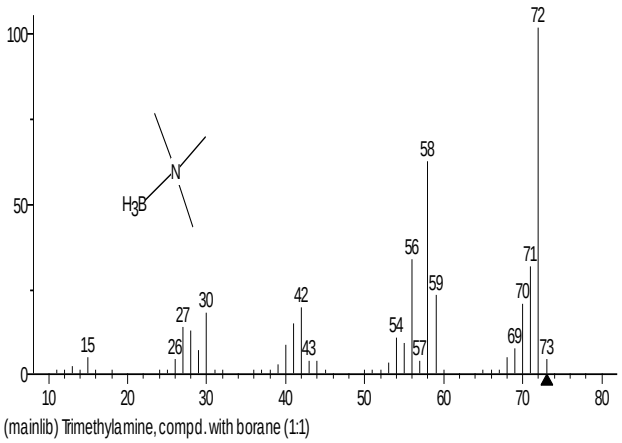

Fig 13 Chromatogram

Name: 1,1-Cyclopropanedicarbonitrile, 2,2-dimethyl-
Formula: $C_7H_8N_2$
MW: 120

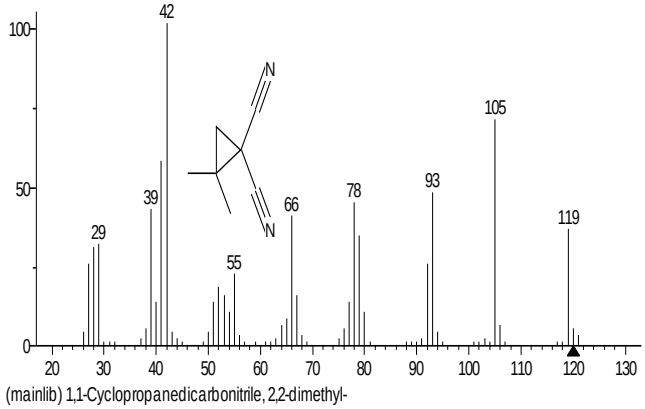

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Fig 14 Chromatogram

Name: Cyclopentanol, 2-(aminomethyl)-, cis-
Formula: $C_6H_{13}NO$
MW: 115

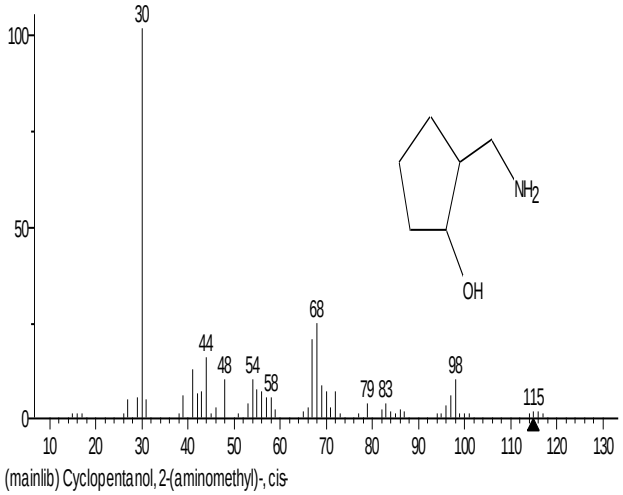

Fig 15 Chromatogram

Name: Tricyclo[4.2.1.1(2,5)]decan-3-ol
Formula: $C_{10}H_{16}O$
MW: 152

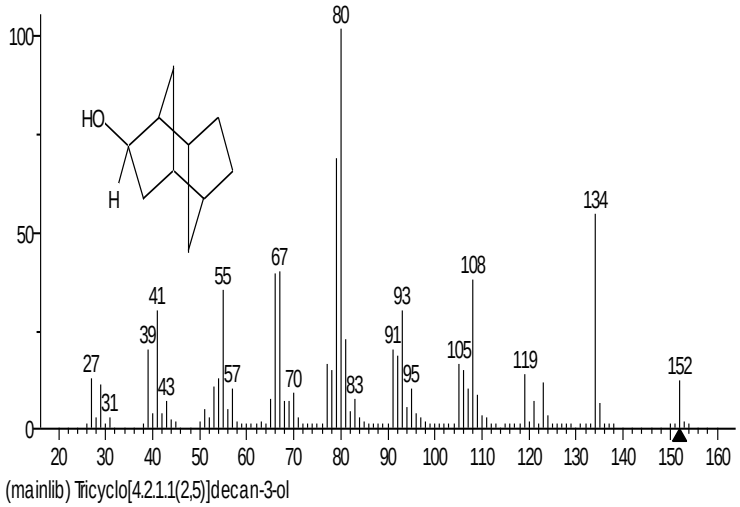

Fig 16 Chromatogram

Name: n-Nonanoylmorpholine
Formula: $C_{13}H_{25}NO_2$
MW: 227

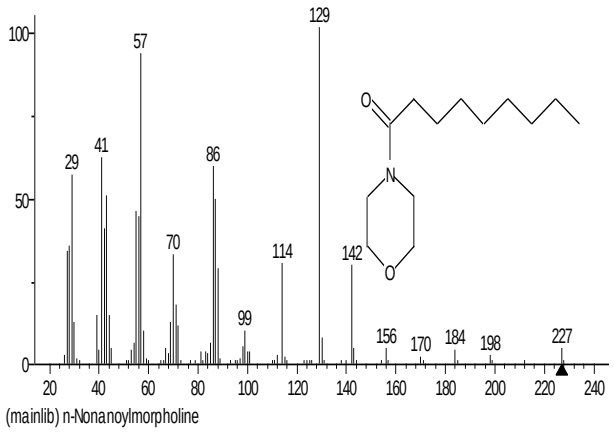

Fig 17 Chromatogram

Name: Thiomorpholine
Formula: C_4H_9NS
MW: 103

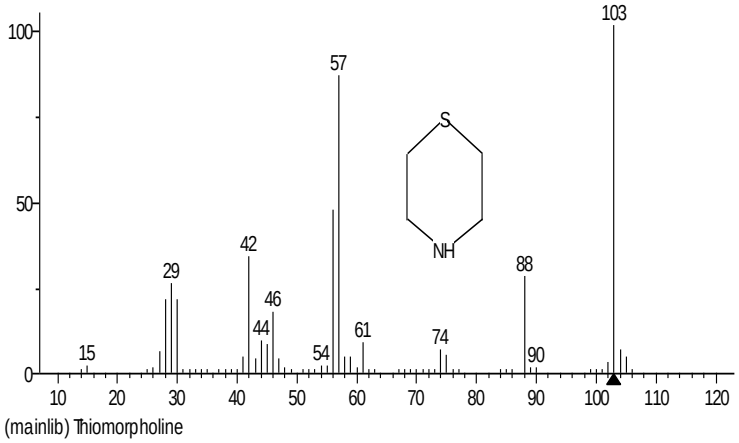

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Fig 18 Chromatogram

Name: Semioxamazide

Formula: $C_2H_5N_3O_2$

MW: 103

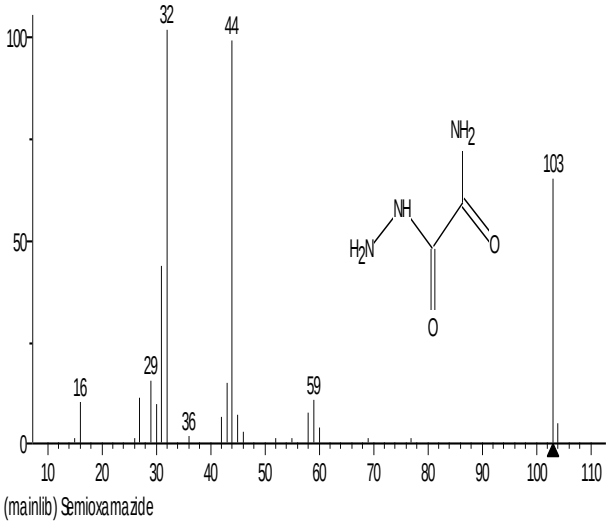

Fig 19 Chromatogram

Name: Cholan-24-oic acid, 3-oxo-, methylester, (5à)-

Formula: $C_{25}H_{40}O_3$

MW: 388