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GC-MS analysis of methanolic cold extract of Urochloa distachya (L.) T.Q.Nguyen,whole plant

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ABSTRACT
Urochloa distachya (Poaceae) popularly known as "Signal grass", 20-60 cm long found in tropical Asian origin, Africa, and other countries. It has been used as fodders, animal feeds etc. and least and no therapeutic activities were reported. The present study was performed to identify the phytochemicals by using GC-MS of the U. distachya whole plant part by cold extraction. The whole part of the plant was percolated with methanol by using conical percolator. The GC-MS methanolic cold extract of U. distachya identified 18 phytocomponents; 26-Nor-5-cholesten-3-á-ol-25-one; glycerin; squalene; 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl; Cholesteryl hydrogen phthalate; 1,3-Benzenediol, 5-pentadecyl; 3-Hydroxy-4-methoxybenzaldehyde, acetate; 2-Acetoxyisobutyryl chloride; 5, 6-Dicarbadecaborane(12), 5, 6-dimethyl;Benzenepropanoic acid, 3, 5-bis(1, 1-dimethylethyl)-4-hydroxy, methyl ester; 1-Naphthalenemethanamine; 2-Methyl-4-trimethylsilylbut-1-en-3-yne; Cholestane-3, 5-diol, 5-acetate, (3á, 5à); 2-Methyl-cis-7, 8-epoxynonadecane; Benzeneacetaldehyde; Cholesterol; Cholestan-3-ol;Cyclodeca[b]furan-2(3H)-one, 9-(acetyloxy)-3a, 4, 5, 8, 9, 11a-hexahydro-4-hydroxy-6, 10-dimethyl-3-methylene.The phytoconstituents of methanolic extract of U. distachya has showed remarkable activities like anti-microbial, antioxidant, anti-inflammatory, antipyretic, and anticancer etc.

KEY WORDS: Urochloa distachya, Methanolic cold extraction of whole plant, GC-MS analysis.

I. INTRODUCTION

Allopathic medicines are the backbone of the human beings as well as almost all the living organisms, but mostly all the allopathic medicines produce side effects, adverse effect, and also toxic effect. The herbal medicine shows least or no adverse effect, that's why many people are interested in using herbal medicine. Herbal drugs have been used for many years; with about 90 percent of plants has medicinal properties. Although these plants have medicinal properties but some of them have not scientifically studied [1].

In the last few decades there are several scientific and analytical techniques has been introduced, some of them HPLC, HPTLC, FTIR, and GCMS etc., these techniques provides superior extraction of sample from the plants. High performance liquid chromatography is a type of column chromatography in which a high pressure is generated so that the analyte dissolved in the solvent (solvent is used as mobile phase) through a column. The packing materials are placed inside the column and that portion is known as stationary phase. The retention time depends upon the interaction with stationary phase. The higher interactions with stationary phase shows longer retention time and less interaction with stationary phase causes short retention time [2].High Performance Thin Layer Chromatography (HPTLC) is an advance separation technique of chromatography for qualitative and quantitative analysis [3]. Fourier-Transformed Infrared Spectroscopy (FTIR) is a technique which is used to detect different functional group from the sample [4].Gas chromatography – mass spectroscopy is a combined analytical technique which is used to evaluate the chemical component present in the plant extract. The GC-MS studies are essential for the determination of biological active components from the plant sample.

Urochloa distachya (L.) T.Q. Nguyen (Poaceae) is commonly known as signal grass. It is an annual grass, and the morphology of this plant is 20-60 cm high, lance shaped leaves, 2-3 racemes, bearing spikelet, found in tropical Asian origin, Africa, and other countries. This plant is adapted to the warm tropics, particularly in monsoon environments. This species flowers throughout the

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year. It reproduces vegetative, developing roots from nodes in culms in contact with soil. Stolons develop into new culms and form clones. At 260^0C of soil temperature gets geminate. The plant has been used as fodder, animal feed etc. There is less or no biological activities are reported [5]. Literature review revealed that there was no HPLC, HPTLC, and GC-MS was carried out for the standardization of Urochloa distachya. Therefore the present investigation was focused to analyze the qualitative estimation of phytoconstituents present in the plant by GCMS analysis.

II. MATERIAL AND METHOD

2.1 Collection and preparation of plant materials:

The fresh plants were collected form Hatgaon, Rusuda, Bargarh, Odisha, India and authenticated by Botanical survey of India, Kolkata, India, bearing reference number: CNH/Tech.II/2019/77. The plants were dried under shade. The whole plants were ground into coarse powder by using mechanical grinder. The powder material was stored in an airtight container for further use.

2.2 Preparation of extract

The dried whole plant powder weighing about 400 gm and was placed in the conical percolator. Sufficient amount of solvent (methanol) was introduced into the conical percolator until the crude drugs were immersed. The crude drugs and the solvent were macerated up to 14 days then the extract was collected from the percolator nozzle. The extract was concentrated by the help of rotary evaporator and stored in the desiccator at a temperature 4^0C for further use.

2.3 GC-MS

The methanolic extract of U. distachya was subjected to Thermo Scientific TSQ 8000 Gas Chromatograph - Mass Spectrometer. MS part consists of Triple Quadrupole and the GC part consists of Split/Split less Injectors and multi-mode (including on-column) Programmed Temperature Vaporizing (PTV), the column temperature is 400^0C. The mass spectrometer joined with the TRACE 1300 GC along with Auto-sampler for automated sample handling. About 1 µl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 31.10 minutes. The carrier gas used was Helium gas at a constant flow rate of 1 ml/minute. The ion source was programmed to 350^0C. The name, molecular formula and molecular weight of the components were referred from the library data of National Institute of Standard and Technology (NIST).

III. RESULTS AND DISCUSSION

The GC-MS analysis of methanolic cold extract of U. distachya (L.) whole plant was depicted in figure 1. The retention time (RT), structure, molecular formula, and percentage peak areas of the identified compounds were given in the table 1. The methanolic extract of U. distachya showed the presence of 18compounds. Out of these compound four compounds were determined as major compound, 26-Nor-5-cholesten-3-á-ol-25-one (56.32) [fig-18], glycerin (11.45) [fig-5], squalene (8.19) [fig-13], 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (3.85) [fig-4], and rest were determined as minor compound Cholesteryl hydrogen phthalate (3.09) [fig-7], 1,3-Benzenediol, 5-pentadecyl (2.35) [fig-10], 3-Hydroxy-4-methoxybenzaldehyde, acetate (1.77) [fig-6], 2-Acetoxyisobutyryl chloride (1.68) [fig-2], 5, 6-Dicarbadecaborane(12), 5, 6-dimethyl (1.68) [fig-14], Benzenepropanoic acid, 3, 5-bis(1, 1-dimethylethyl)-4-hydroxy, methyl ester (1.47) [fig-11], 1-Naphthalenemethanamine (1.37) [fig-7], 2-Methyl-4-trimethylsilylbut-1-en-3-yne (1.20) [fig-9], Cholestane-3, 5-diol, 5-acetate, (3á, 5à) (1.20) [fig-16], 2-Methyl-cis-7, 8-epoxynonadecane (1.15) [fig-12], Benzeneacetaldehyde (1.14) [fig-3], Cholesterol (0.94) [fig-15], Cholestan-3-ol (0.63) [fig-19], Cyclodeca [b]furan-2(3H)-one, 9-(acetyloxy)-3a, 4, 5, 8, 9, 11a-hexahydro-4-hydroxy-6, 10-dimethyl-3-methylene (0.51) [fig-8].

The GC-MS analysis of methanolic cold extract of U. distachya (L.) whole plant showed various biological activities that are represented in the table number 2. 2-Acetoxyisobutyryl chloride showed Antibacterial and antifungal [1]. Benzeneacetaldehyde has been reported as antimicrobial activities [2]. 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl is a flavonoid compound which showed Antimicrobial, anti-inflammatory, antiproliferative, antioxidant, automatic nerve, anticancer, and anti-inflammatory activity [3] [4]. Glycerin which is Simple polyol used in Cough syrups, elixirs and expectorants, toothpaste, mouthwashes, skin care products [2].potent osmotic dehydrating agent with additional effects on brain metabolism, decreases intracranial pressure (Reye's syndrome, stroke, encephalitis, meningitis, pseudotumorcerebri, central nervous system tumor, and space occupying lesions), glaucoma [5]. The Triterpene compound such as squalene has reported as Antioxidant,

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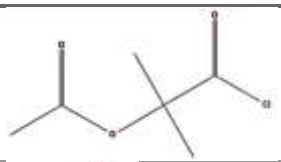
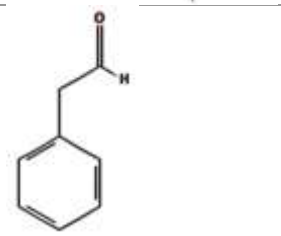
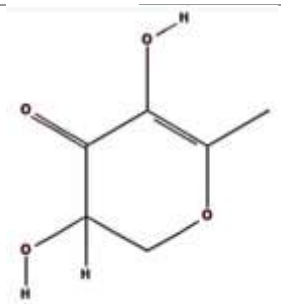
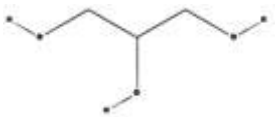
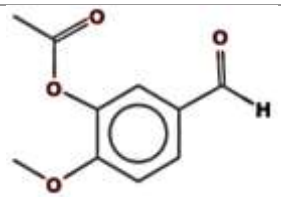
Cardio-protective [6]. Antimicrobial, chemo preventive, anticancer, pesticide, sunscreen, anti-tumor activities [7]. Cholesterol and 26-Nor-5-cholesten-3-á-ol-25-one are steroid cholesterol showed activities like Formation of hormones, vitamin D, and buildup cell membrane [8] and 26-Nor-5-cholesten-3-á-ol-25-one is reported as Antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, and hypoglycemic, antipyretic and estrogenic activities [9].

The GC-MS analysis of methanolic cold extract of U. distachya (L.) whole plant proposed that the biological properties of several components possesses the antioxidant, anti-inflammatory, antimicrobial, antipyretic, and anticancer activities, so further investigation and isolation of the plant can reveled newer molecules which will be helpful for the study of therapeutic and pharmacological activities.

Table 1 GC-MS analysis of methanolic cold extract of U. distachya

Sl. No.	RT	Name of the compound	Chemical structure	Molecular formula	Molecular weight	Peak area %
1	6.33	2-Acetoxyisobutyryl chloride		$C_6H_9ClO_3$	164.59	1.68
2	9.59	Benzene acetaldehyde		C_8H_8O	120.15	1.14
3	11.49	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl		$C_6H_8O_4$	144.12	3.85
4	12.66	Glycerin		$C_3H_8O_3$	92.09	11.45
5	15.11	3-Hydroxy-4-methoxybenzaldehyde, acetate		$C_{10}H_{10}O_4$	194.1840	1.77

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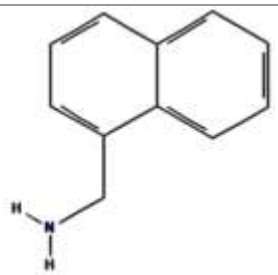
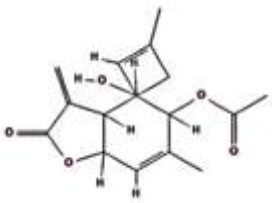
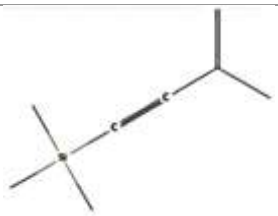
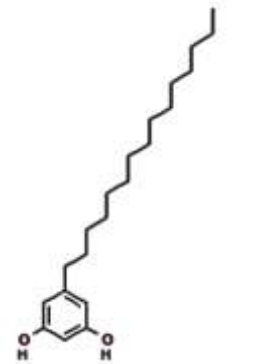
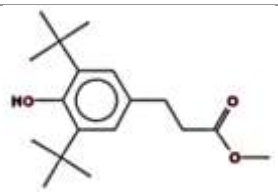
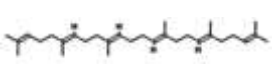
6	17.27	1-Naphthalene methanamine		$C_{11}H_{11}N$	157.21	1.37
7	18.06	Cyclodeca[b]furan-2(3H)-one, 9-(acetyloxy)-3a, 4, 5, 8, 9, 11a-hexahydro-4-hydroxy-6, 10-dimethyl-3-methylene		$C_{17}H_{22}O_5$	306.4	0.51
8	18.51	2-Methyl-4-trimethylsilyl but-1-en-3-yne		$C_8H_{14}Si$	138.28	1.20
9	19.80	1,3-Benzenediol, 5-pentadecyl		$C_{21}H_{36}O_2$	320.5	2.35
10	21.00	Benzenepropanoic acid, 3, 5-bis(1, 1-dimethylethyl)-4-hydroxy, methyl ester		$C_{18}H_{28}O_3$	292.4131	1.47
11	22.11	2-Methyl-cis-7, 8-epoxynonadecane		$C_{20}H_{40}O$	296.5	1.15
12	22.43	Squalene		$C_{30}H_{50}$	410.7	8.19

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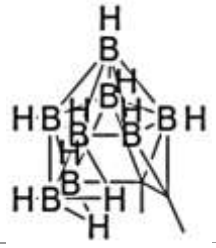
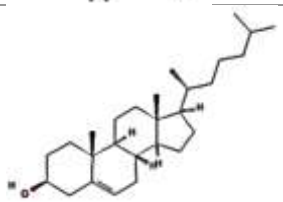
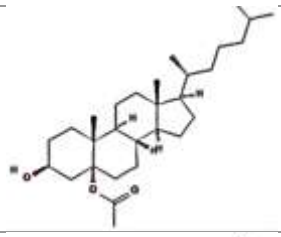
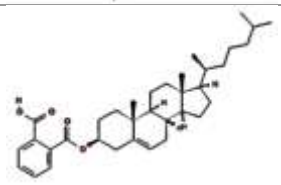
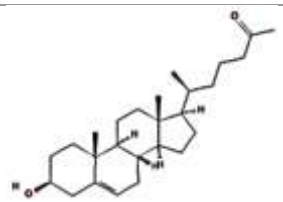
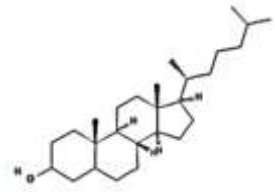
13	23.14	5, 6-Dicarbadecaborane(12), 5, 6-dimethyl		$C_4H_{16}B_8$	152.199643	1.68
14	25.98	Cholesterol		$C_{27}H_{46}O$	386.7	0.94
15	26.20	Cholestane-3, 5-diol, 5-acetate, (3á, 5à)		$C_{29}H_{50}O_3$	446.7	1.20
16	26.44	Cholesteryl hydrogen phthalate		$C_{35}H_{50}O_4$	534.8	3.09
17	27.00	26-Nor-5-cholesten-3-á-ol-25-one		$C_{26}H_{42}O_2$	386.6	56.32
18	27.28	Cholestan-3-ol		$C_{27}H_{48}O$	388.7	0.63

Table 2 Biological properties of the phytocompounds

Sl. No.	**Name of the compound**	**Nature of the compound**	**Biological activity**
1	2-Acetoxyisobutyryl chloride	-	Antibacterial and antifungal [6]
2	Benzeneacetaldehyde	Benzene	Antimicrobial [7]
3	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl	Flavonoid	Antimicrobial, anti-inflammatory, anti-proliferative, antioxidant, automatic nerve activity, anticancer, anti-inflammatory[8] [9]

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4	Glycerin	Simple polyol	Cough syrups, elixirs and expectorants, toothpaste, mouthwashes, skin care products [7].Potent osmotic dehydrating agent with additional effects on brain metabolism, decreases intracranial pressure (Reye's syndrome, stroke, encephalitis, meningitis, pseudotumorcerebri, central nervous system tumor, and space occupying lesions), glaucoma[10].
5	Squalene	Triterpene	Antioxidant, Cardio-protective activity [11]. Antimicrobial, chemo preventive, anticancer, pesticide, sunscreen, anti-tumor [12].
6	Cholesterol	Steroid	Formation of hormones, vitamin D, and buildup cell membrane etc. [13].
7	26-Nor-5-cholesten-3-á-ol-25-one	Steroid	Antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, hypoglycemic, antipyretic and estrogenic activities [14].

IV. CONCLUSION

The present investigation concluded that the methanolic extract of whole plant of U. distachya has several bio-active phytocomponents which are responsible for various biological activities. The local tribals and Vaidyas use this plant in some diseases and disorders. So, further investigation and isolation of newer compounds will be helpful for the study of the pharmacological activities.

V. ACKNOWLEDGEMENT

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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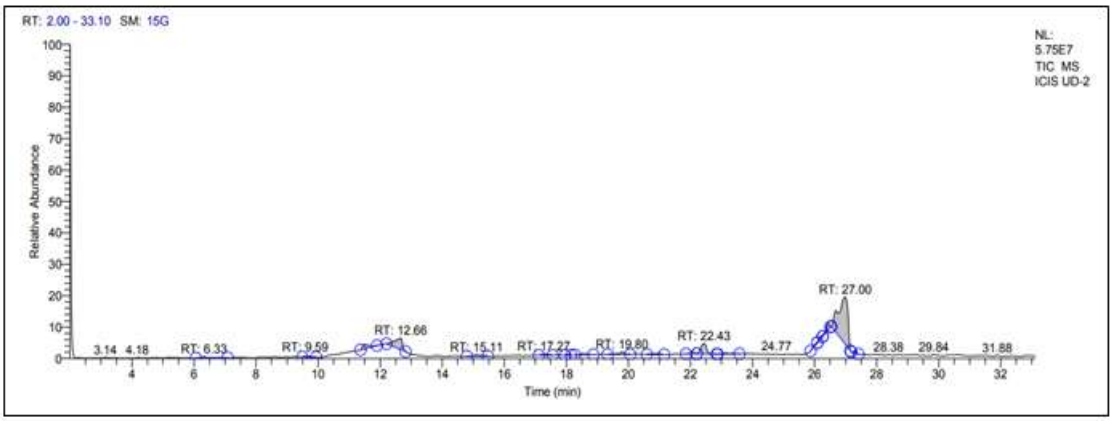

Fig-01: GC-MS chromatogram of the methanolic cold extract of *U. distachya*

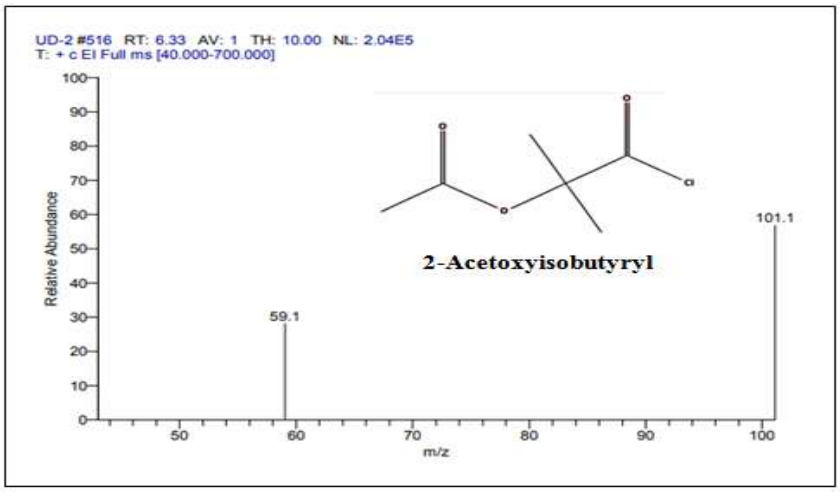

Fig-02: GC-MS chromatogram of 2-Acetoxyisobutyryl

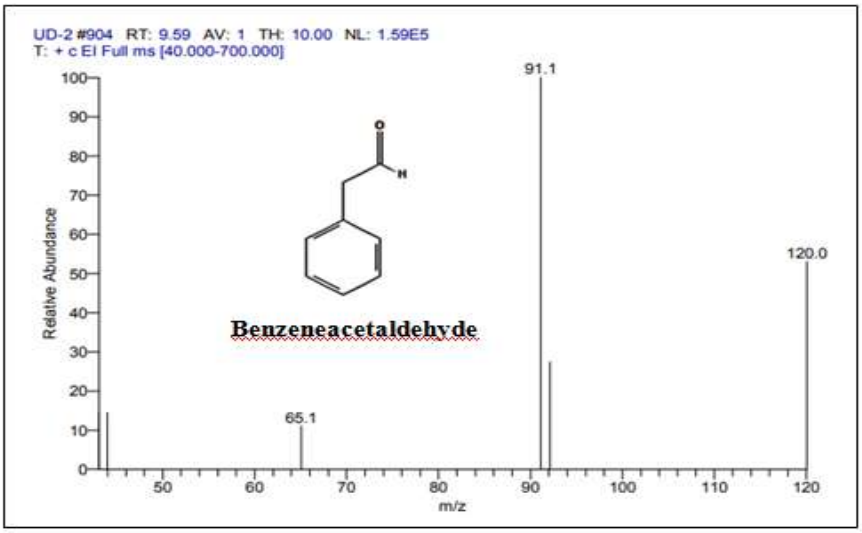

Fig-03: GC-MS chromatogram of Benzeneacetaldehyde

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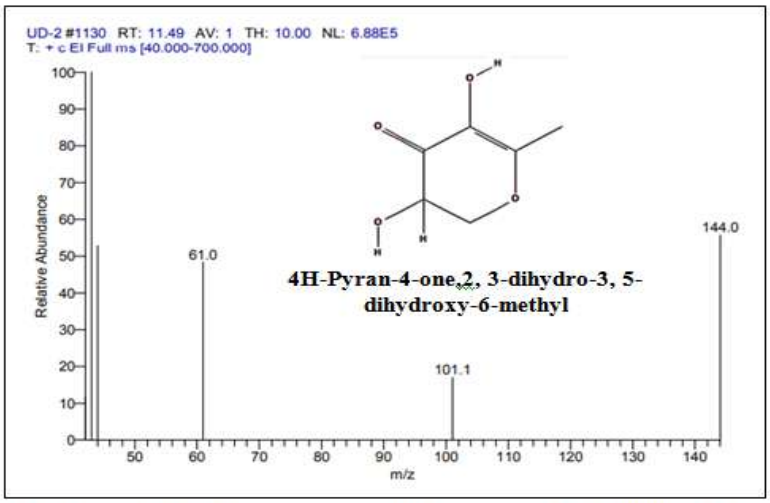

Fig-04: GC-MS chromatogram of 4H-Pyran-4-one,2, 3-dihydro-3, 5-dihydroxy-6-methyl

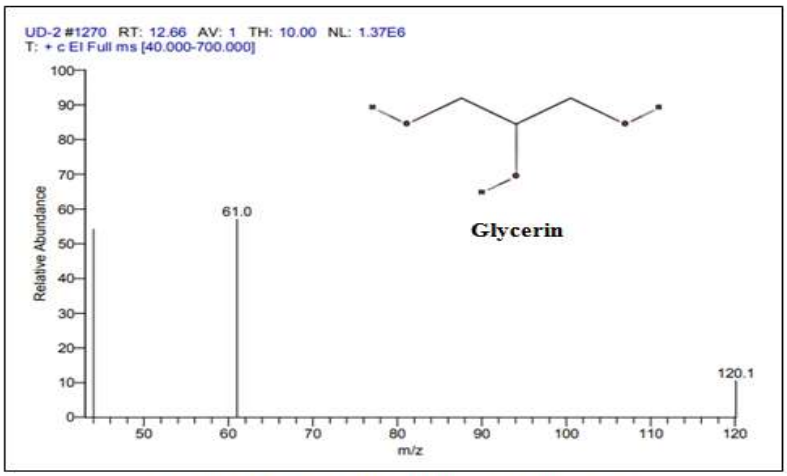

Fig-05: GC-MS chromatogram of Glycerin

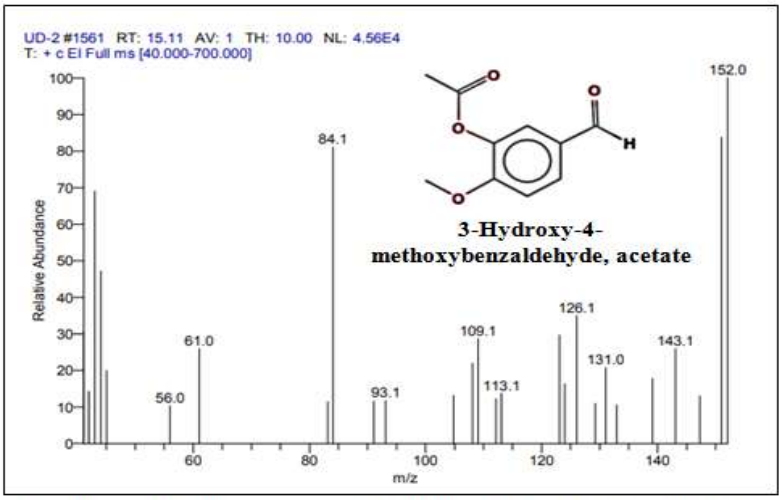

Fig-06: GC-MS chromatogram of 3-Hydroxy-4-methoxybenzaldehyde, acetate

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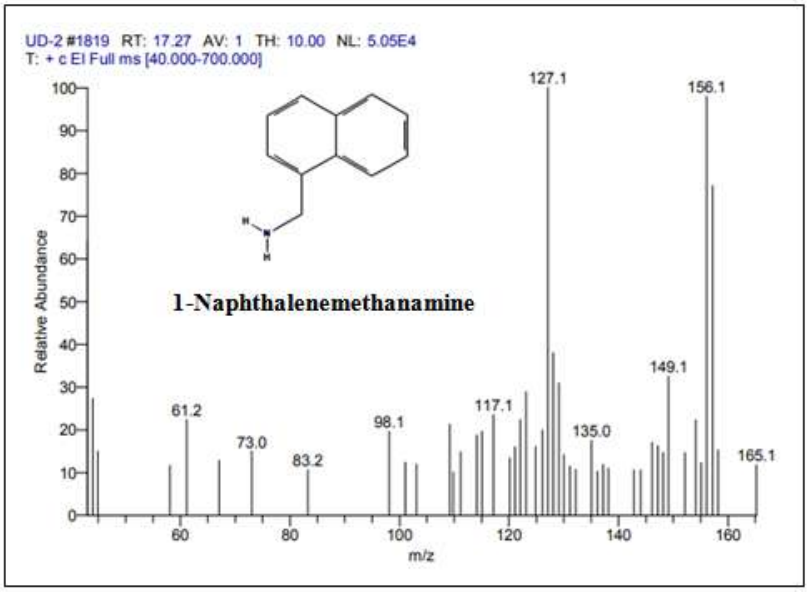

Fig-07: GC-MS chromatogram of 1-Naphthalenemethanamine

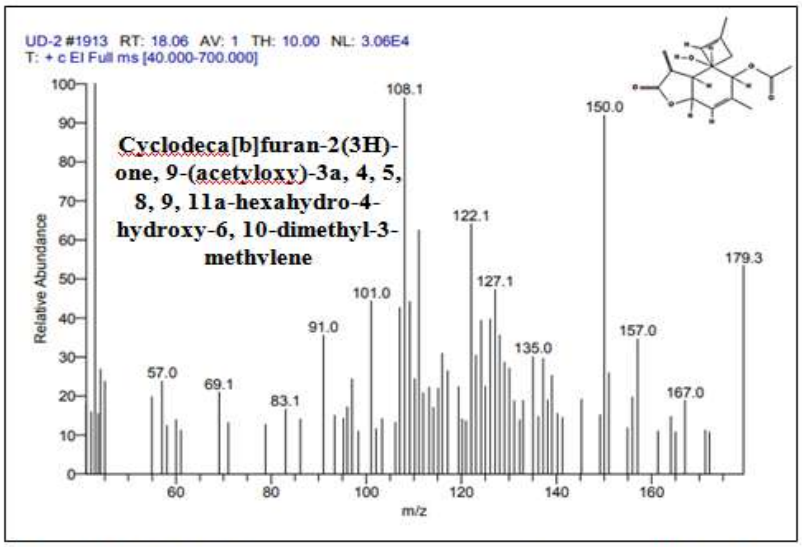

Fig-08: GC-MS chromatogram of Cyclodeca[b]furan-2(3H)-one, 9-(acetyloxy)-3a, 4, 5, 8, 9, 11a-hexahydro-4-hydroxy-6, 10-dimethyl-3-methylene

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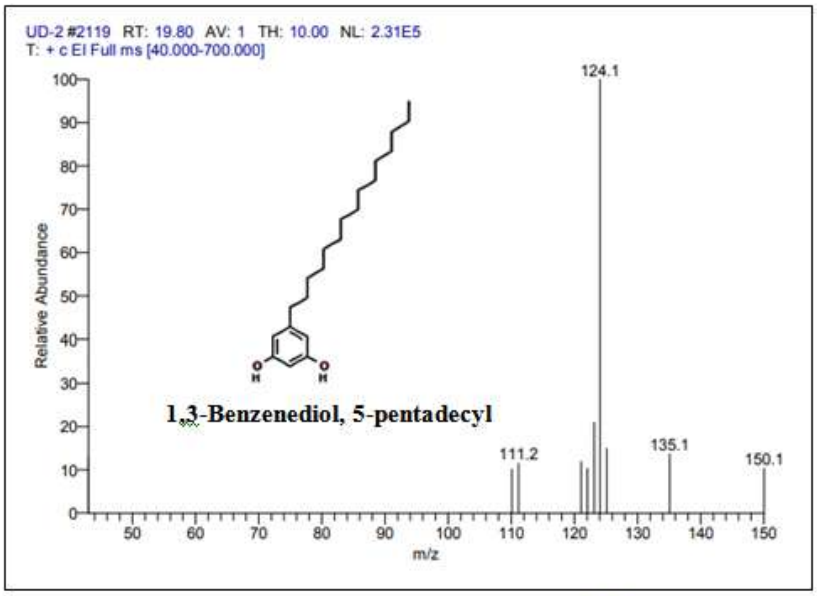

Fig-10: GC-MS chromatogram of 1, 3-Benzenediol, 5-pentadecyl

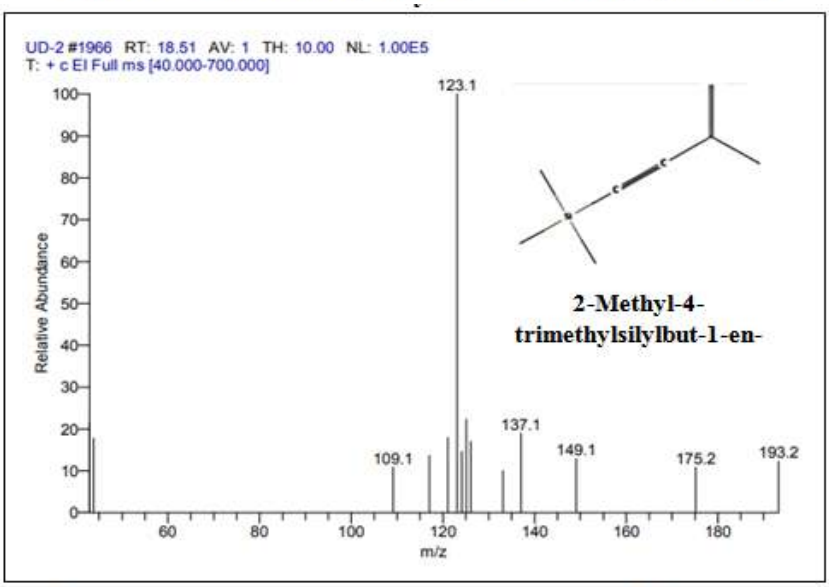

Fig-09: GC-MS chromatogram of 2-Methyl-4-trimethylsilylbut-1-en-3-yne

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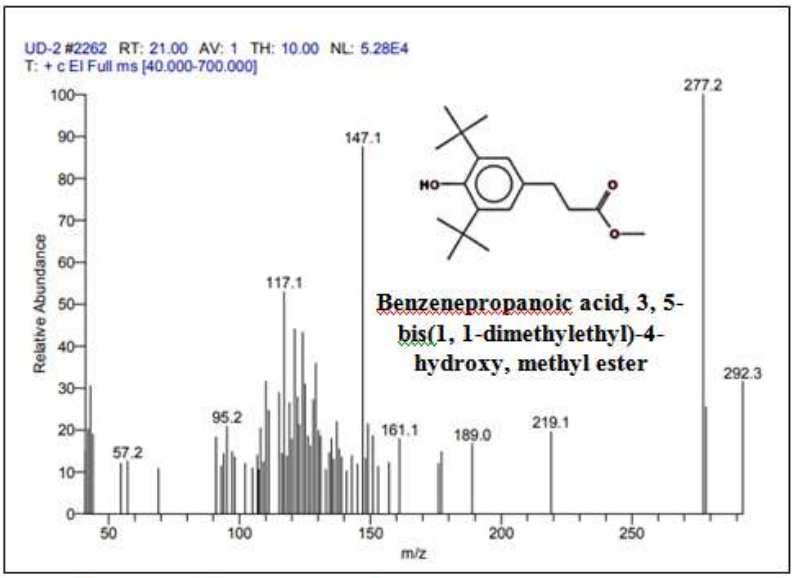

Fig-11: GC-MS chromatogram of Benzenepropanoic acid, 3, 5-bis (1, 1-dimethylethyl)-4-hydroxy, methyl ester

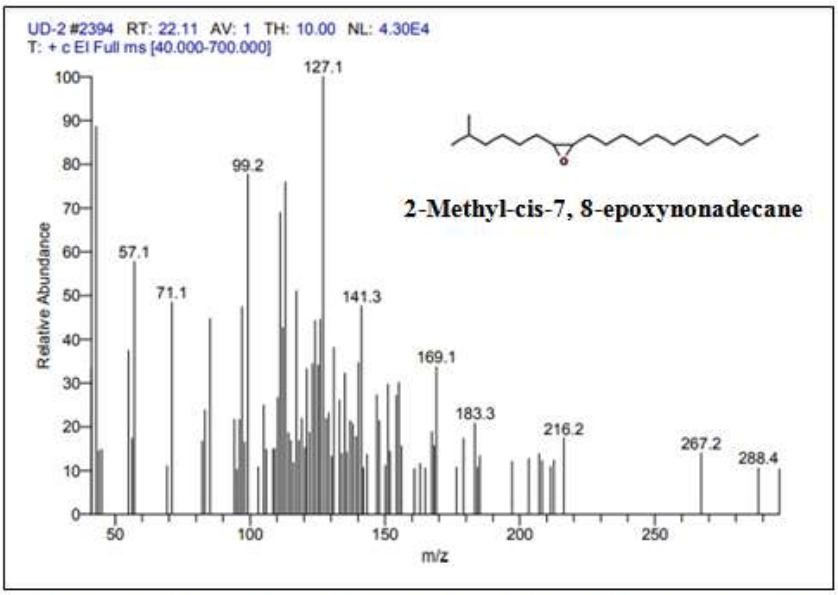

Fig-12: GC-MS chromatogram of 2-Methyl-cis-7, 8-epoxynonadecane

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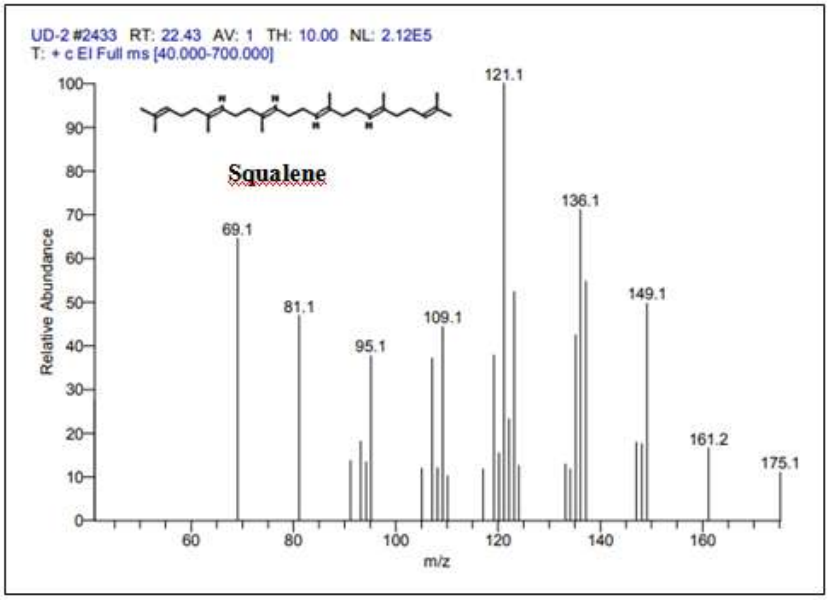

Fig-13: GC-MS chromatogram of Squalene

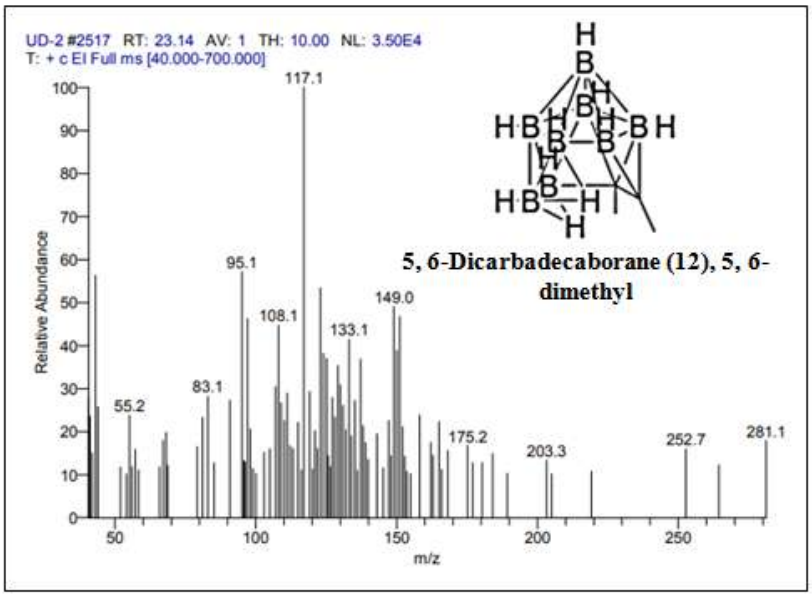

Fig-14: GC-MS chromatogram of 5, 6-Dicarbadecaborane (12), 5, 6-dimethyl

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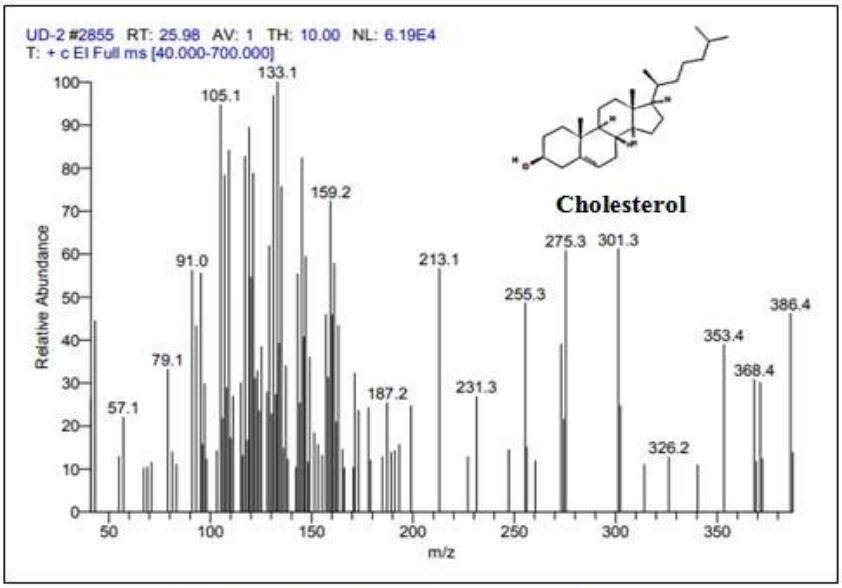

Fig-15: GC-MS chromatogram of Cholesterol

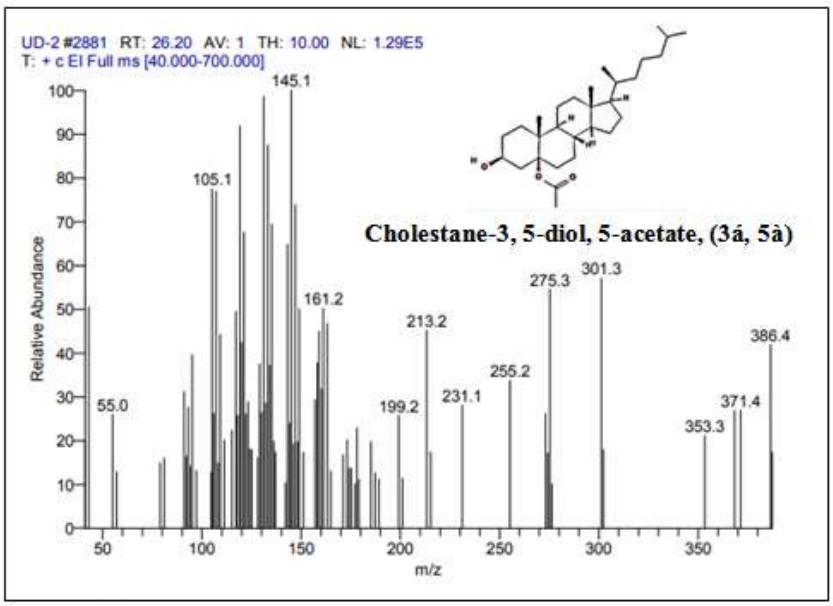

Fig-16: GC-MS chromatogram of Cholestane-3, 5-diol, 5-acetate, (3á, 5à)

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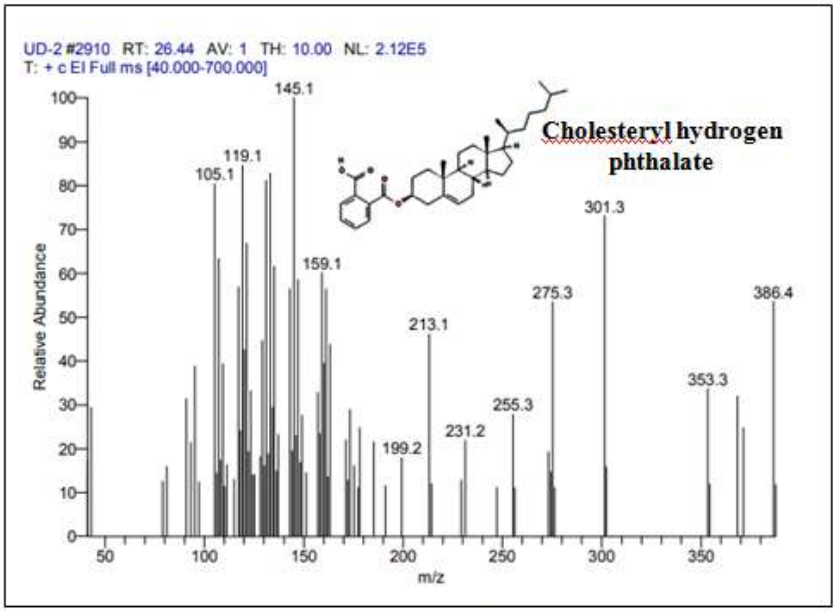

Fig-17: GC-MS chromatogram of Cholesteryl hydrogen phthalate

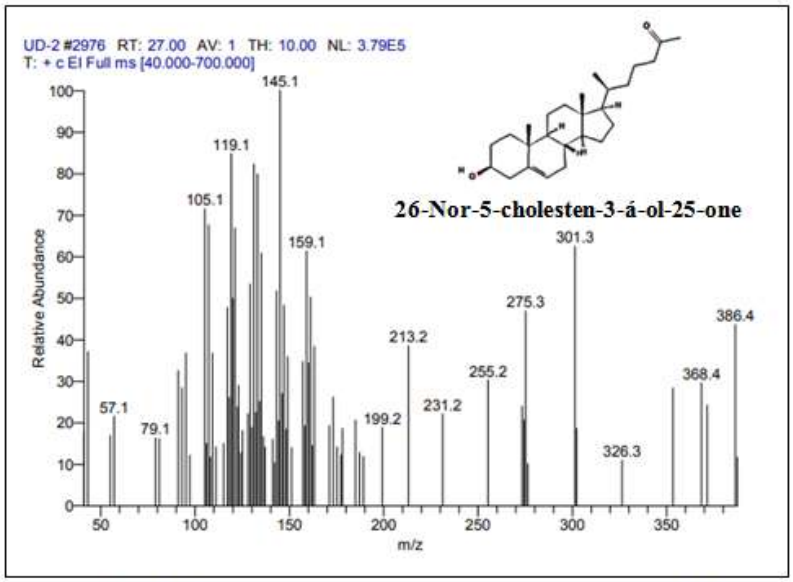

Fig-18: GC-MS chromatogram of 26-Nor-5-cholesten-3-á-ol-25-one

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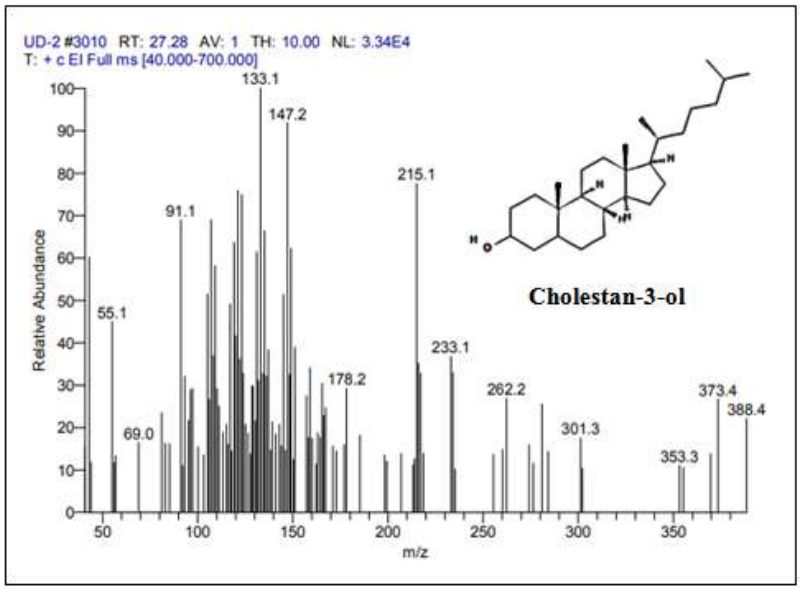

Fig-19: GC-MS chromatogram of Cholestan-3-ol

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