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ELUCIDATION OF BIOACTIVE COMPOUNDS FROM DIFFERENT POLARITY EXTRACTS OF BENINCASA HISPIDA (THUNB.) COGN. , FRESH FRUIT PULP USING GC-MS ANALYSIS.

Vedha Balakumar, M.Gopalakrishnan and T. Sekar *

Post Graduate and Research Department of Botany, Pachaiyappa's College, Chennai - 600 030, Tamil Nadu, India

*Corresponding author E-mail***: *vedhabalakumar@yahoo.co.in***

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Key Words

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ABSTRACT

The present study was to trace out the variations in the profile of bioactive compounds from chloroform, methanol and aqueous extracts of Benincasa hispida fresh fruit pulp using Gas chromatography Mass spectrometry analysis (GC-MS). The identity of the bioactive components of the extracts was established on the basis of GC retention indices, by comparing their mass spectra with National Institute of Standards and Technology (NIST) data center. According to GC-MS spectral data, in methanolic extract 22 bioactive compounds were identified and characterized in which the major components are α1-Sitosterol (97.24%), Lupan-3-ol (56..83 %), CucurbitacinB, dihydro- (25.29 %). and cis-13-Octadecenoic acid (20.56 %) , whereas in chloroform extract 16 compounds were identified , in which the major components are 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (100%), Methanecarboxylic acid (34.49 %), In aqueous extract 13 compounds were identified and the major components are Methanecarboxylic acid (100%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy -6-methyl-(21.64%) . The identified compounds may have significant therapeutic value.

INTRODUCTION:

A large trailing gourd, Benincasa hispida (Thunb.) Cogn. of family Cucurbitaceae, commonly known as ash gourd ,winter melon, and wax gourd. It is cultivated throughout the plains of India and on hills up to 1200 meter altitude as a vegetable. The fruit is large, cylindrical and completely covered with waxy bloom. In Ayurvedic system of medicine, ash gourd pulp is employed as a main ingredient in Kushmanda leyham or rasayana which potential application for neurodegenerative disorders and to improve immunity and strength. The perusal of literature studies also reveals its anti-inflammatory (Gover JK et al.,1994)[1], anti-diuretic (Dong MY et al.,1995)[2], anti diarrheal (vrushabendra Swamy Bhyrapur Mathad et al.,2005)[3], antioxidant, anti-diabetic (Abhishek mahatma et al., 2014)[4], antiulcer (Gover JK et al.,2001)[5], antihistamine (Anilkumar D et al.,2002) [6], anti-obesity, anticancer (Kumar A et al., (2002)[7], anti compulsive (Shika Girdhar 2010)[8], anti-Alzheimer's, (Chandra Roy et al.,)[9], gastroprotective effect (Manish A et al., 2008)[10]. anti convulsant (S

K Nimbal et al., 2011)[11] and its potential usefulness in the management of morphine withdrawal (Gover JK et al.,2000)[12]. The seeds are rich in protein and play active role in antifungal, antiviral, anti-proliferative, antitumor and immune dilatory activities. The ash gourd rind extract possesses significant diuretic activity with a potassium sparing effect.

The major phytoconstituents of the fruits are triterpenoids, flavonoids, glycosides,carotenes, vitamins, and uronic acid. Phyto chemical screening on the extracts of B. hispida fruit pulp revealed that triterpenoids and saponins were the major constituents of the sample. Certain literature studies carried out for checking the bioactive components in the fruit of Benincasa hispida reveals the presence of beta-sitosterol, lupeol, iso-vitexin, iso-multiflorenol, cucurbitacin-B, and different amino acids by Thin Layer Chromatograhy study.(Manish A et al.,2008)[10]. The present study was to compare and determine the profile of bioactive compounds of Benincasa hispida fresh fruit pulp extracts prepared with solvents of different polarity using GC-MS technique.

MATERIALS AND METHODS

Plant Materials

The fresh fruit of Benincasa hispida was purchased from local vegetable market. The identification and authentification of the material was done in Postgraduate and Research Department of Botany, Pachaiyappa's college, Chennai 600 030, Tamil Nadu.

Preparation of extract

The fruit was initially washed under running tap water, then again washed with distilled water and wiped dry. The rind was peeled off and seeds were removed. The fresh fruit pulp cut into pieces then meshed to fine juice in an air blender. The fresh juice was serially extracted with chloroform, methanol and aqueous based on their polarity. For chloroform extraction 500ml fruit juice was mixed with 1000ml of chloroform and kept covered for 36h at room temperature, the slurry was stirred intermittently for 2h and left overnight (J.K. Grover et al., 2001)[4]. The mixture was then filtered using what man no. 1 filter paper and the filtrate was concentrated on a vacuum rotatory evaporator, the concentrated final extract was thick brown liquid and the yield of extract was 2.4ml/100ml fresh juice. The residue was used for methanolic extraction with same procedure. For aqueous extract hot distilled water is used. The crude extract is stored in refrigerator throughout the study.

GC-MS analysis

GC-MS analysis of these extracts was carried out by Agilent 7890B,Gas chromatography connected to 5977A Mass Spectrometer. Column name HP_5MS, column capillary composed of 5% Phenyl Methyl siloxane. GC working temperature starts from -60^0 C to 325^0 C. Column capillary film thickness 30m x 250µm x0.25µm. An electron ionization system with ionizing energy of 70eV was used for GC-MS detection. Oven temperature programmed at 50^0 C for 2min then ramp up to 280^0 C, increased by 5^{0}C/min; at 280^0 C maintained for 15min; total run time 63min. In MS the solvent delay 2min, scan range 50-700m/z, carrier gas was helium; gas flow rate 1ml/min; GC injector port temperature 250^0 C, and the detector temperature 290^0 C. 1µl of extract injected through auto sampler by split less injection mode.

Identification of components

The spectral output of GC-MS analysis was interpreted using the database of National

Institute of Standard and Technology (NIST) database center. The mass spectrum of the unknown components was compared with the spectrum of the known components data stored in the NIST library. Name of the components, molecular weight and the molecular structure of the components of test sample were illustrated.

RESULTS AND DISCUSSION

The GC-MS analysis was utilized successfully for the investigation and characterization of bioactive compound profile of Benincasa hispida fruit sample prepared by successive extraction with different polarity solvents. The method applied provided good separation for each compounds and their abundance of compounds detected, retention time (RT), peak percentage varies with different polarity solvents. GC-MS chromatogram of total ion concentration (TIC) of extracts are shown in figure1,2 and3, while detected compounds are displayed in table 1, 2 and 3.and fig 4,5,6,7,and 8 illustrates the mass spectrum of major components. According to the result(table 1- 3) the mass spectra of the constituents compared with NIST library, in methanolic extract 22 peaks of components were identified and characterized in which the major components are α1-Sitosterol (97.24%), Lupan-3-ol (56..83 %), Cucurbitacin B, dihydro-(25.29 %)., cis-13-Octadecenoic acid (20.56 %) , 3-Hydroxy-3-methyl-2-butanone oxime (5.16%),1,2-Cyclopentanedione (4.16%), whereas in chloroform extract 16 compounds were identified , the major components were 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (100 %), Methanecarboxylic acid (34.49 %), α-Pyrrolidone, 5-[3-hydroxybutyl]-(10.01%), Ethanoic acid. (6.88%). In aqueous extract (Table 3) 13 compounds were identified and the major components are Methanecarboxylic acid (100%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (21.64%).Cyclotrisiloxane, hexamethyl (11.36%), 2-Hydroxy-gamma-butyrolactone(8.00%.). Certain literature studies reveals the medicinal applications of bioactive compound obtained in the present analysis , n-hexadeconic acid the structural basis of the anti-inflammatory property and the kinetic parameters of inhibition by n-hexadeconic acid have been determined (Vasudevan aparna et al,.2012)[13], furan and 2- furanmethanol are decomposition products of thymine(Adriano A.S. et al., 2003)[14], cis-Vaccenic acid, cis-13-Octadecenoic acid are the phosphofatty acids play role in brain responses(M. G. Murphy et al., 2006)[15], 3-methyl-1,2-cyclopentanedione (Young Choi et al., 2007)[16] suggest that this agent may have potential to minimize inflammation, cucurbiticin –b which are implicated in apoptosis and the cell cycle rich source of potential anticancer compounds(L. Rios, J. et al., 2012)[17], α1-Sitosterol and Lupan-3-ol are triterpenoids, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- have anti inflammatory and antimicrobial activity,(Ramalakhmi S. et al.,2012)[18] , Butanoic acid, 4-hydroxy- and hexonic acid contribute quantitative aroma composition to the fruit (M Larsen et al.,1992)[19]

CONCLUSION

GC-MS analysis applied to investigate the bioactive compound profile of Benincasa hispida fruit extracts prepared with solvents of different polarity was compared and determined. Significant difference between methanol, chloroform and aqueous were observed in which methanolic and chloroform extracts have distinct classes of bioactive compounds. The present analysis was useful for drawing profile of detected bioactive compounds which may proceed to do further studies.

Table 1. Bioactive components identified in the methanolic extract of Benincasa hispida fruit pulp by GC-MS.

Peak No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	2.137	Furfural	$C_5 H_4 O_2$	96	0.71
2	2.793	2-Furanmethanol	$C_5 H_6$ O2	98	0.86
3	3.091	3-Hydroxy-3-methyl-2-butanone oxime	$C_5 H_{11} NO_2$	117	5.16
4	3.499	Dihydroxyacetone	C_3H6 O3	90	1.36
5	3.735	1,2-Cyclopentanedione	$C_5 H_6 O_2$	98	4.16
6	4.168	2-Hydroxy-gamma-butyrolactone	$C_4 H_6 O_3$	102	2.08
7	4.701	Methyl acetoxyacetate	$C_5 H_8 O_4$	132	0.23
8	4.912	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6 H_8 O_3$	128	0.28
9	5.085	D-Alanine, N-propargyloxycarbonyl-, isohexyl ester	$C_{13} H_{21} NO_4$	255	0.30
10	5.222	Pentanal	$C_5 H_{10} O$	86	0.53
11	5.668	l-Alanine, N-methoxycarbonyl-, butyl ester	$C_9 H_{17} NO_4$	203	1.10
12	6.795	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6 H_8 O_4$	144	0.38
13	6.956	5-Hydroxymethylfurfural	$C_6 H_6 O_3$	126	1.03
14	7.303	Ketone, methyl 2-methyl-1,3-oxothiolan-2-yl	$C_6 H_{10} O_2 S$	146	0.88
15	7.910	d-Mannose	$C_6 H_{12} O_6$	180	3.01
16	8.307	Methyl β-d-galactopyranoside	$C_7 H_{14} O_6$	194	1.27
17	9.335	n-Hexadecanoic acid	$C_{16} H_{32} O_2$	256	0.21
18	9.620	cis-Vaccenic acid	$C_{18} H_{34} O_2$	282	0.63
19	9.781	cis-13-Octadecenoic acid	$C_{18} H_{34}$ O2	282	20.56
20	10.376	Cucurbitacin B, dihydro-	$C_{32} H_{48} O_8$	560	25.29
21	42.019	α1-Sitosterol	$C_{30} H_{50} O$	426	97.24
22	42.106	Lupan-3-ol	$C_{30} H_{52} O$	428	56.83

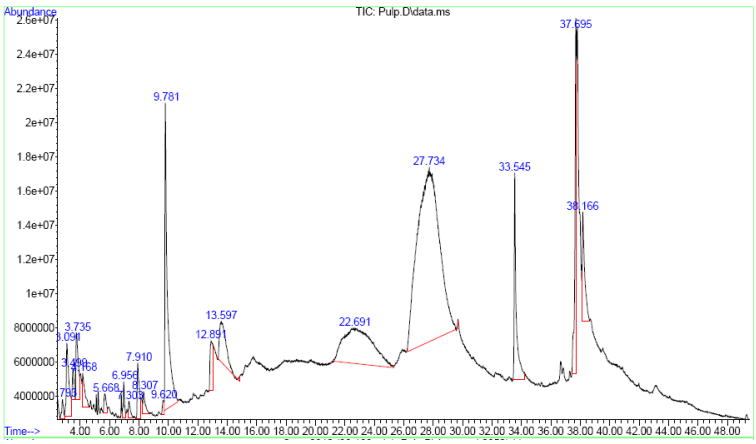

Fig 1: GC-MS chromatographic peak condition of methanolic extract of Benincasa hispida fruit pulp.

Table 2: Bioactive components identified in the chloroform extract of Benincasa hispida fruit pulp by GC-MS analysis.

Peak No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	2.986	Ethanoic acid	$C_2H_4O_2$	60	15.38
2	3.290	Methanecarboxylic acid	$C_2H_4O_2$	60	34.49
3	3.394	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	74	6.88
4	4.093	Pyridine	$C5H_5N$	79	0.78
5	4.761	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222	1.94
6	5.616	2-Furanmethanol	$C_5H_6O_2$	98	1.67
7	6.782	Butanoic acid, 4-hydroxy-	C_4H_8O3	104	2.08
8	7.109	1,2-Cyclopentanedione	$C_5H_6O_2$	98	1.16
9	8.268	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	1.86
10	11.209	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	C_6H_8O3	128	3.05
11	13.550	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C6H_8O_4$	144	100.00
12	14.864	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	$C_6H_6O_4$	142	5.62
13	20.146	`α-Pyrrolidone, 5-[3-hydroxybutyl]-	$C_8H_{15}NO_2$	157	10.01
14	29.566	Acetic acid, 2-oxo-2-[2-(1-oxopropylamino)phenyl]-	$C_{11}H_{11}NO_4$	221	0.66
15	32.344	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.30
16	32.344	4H-Pyran-3-carboxylic acid, 6-amino-5-cyano-2-methyl-4-phenyl-, benzyl ester	$C_{21}H_{18}N_2O_3$	346	1.00

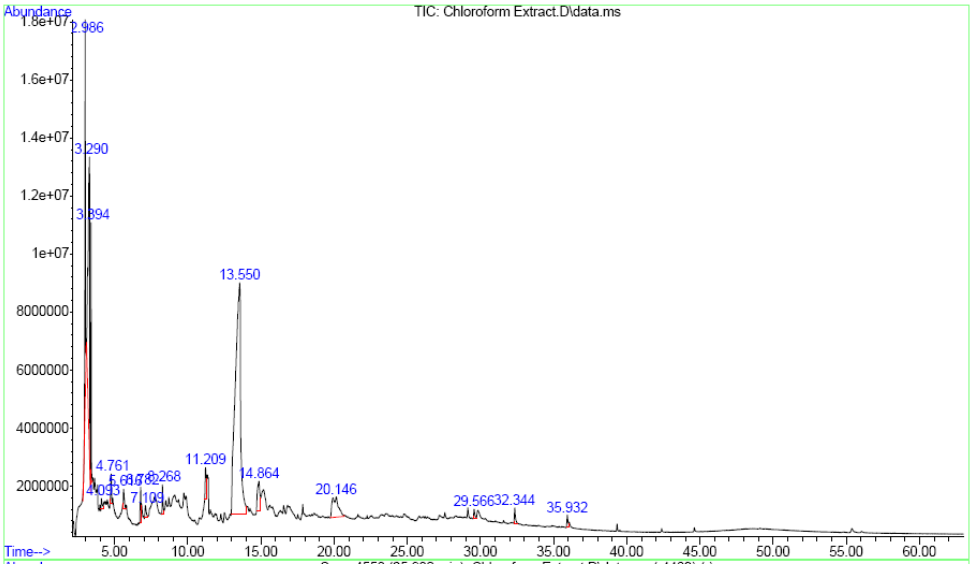

Fig 2: GC-MS chromatographic peak condition of chloroform extract of Benincasa hispida fruit pulp.

Table 3: Bioactive components identified in the aqueous extract of Benincasa hispida fruit pulp by GC-MS.

Peak No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	3.335	Methnecarboxylic acid	$C_2H_4O_2$	60	100.00
2	3.439	Pyridine	C_5H_5N	79	0.31
3	3.498	Propanoic acid, 2-oxo-, methyl ester	$C_4H_6O_3$	102	1.08
4	4.739	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222	11.36
5	7.985	Silane, dimethyl(dimethylisobutoxysilyloxy) isobutoxy-	$C_{12}H_{30}O_3Si_2$	278	1.04
6	8.282	L-Lactic acid	: $C_3H_6O_3$	90	7.26
7	8.684	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296	1.67
8	9.055	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102	8.00
9	10.949	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	128	6.47
10	11.521	1,2,4-Triazino[5,6-E][1,2,4]-triazine-3,6-dione, hexahydro-	$C_4H_8N_6O_2$	172	2.23
11	13.029	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	21.64
12	15.347	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296	0.87
13	17.843	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444	1.23

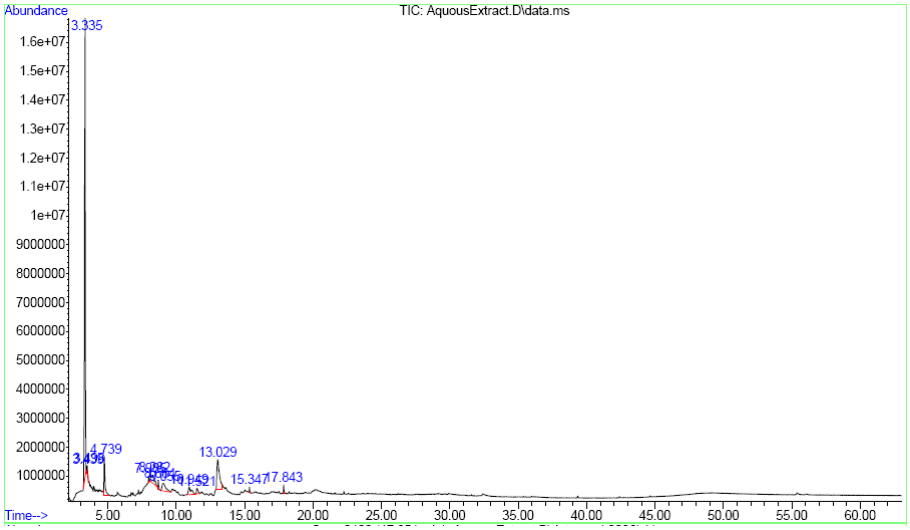

Fig 3: GC-MS chromatographic peak condition of aqueous extract of Benincasa hispida fruit pulp.

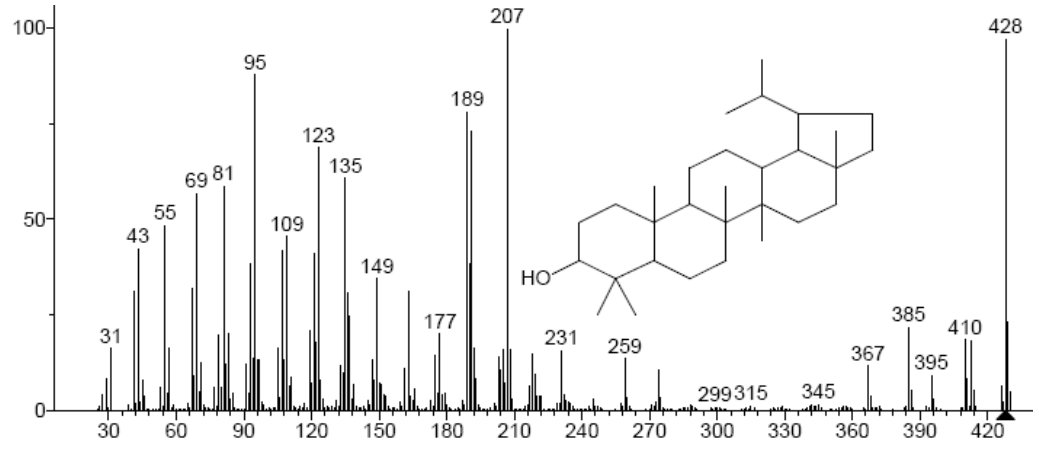

Fig 4: Mass spectrum of Lupan-3-ol

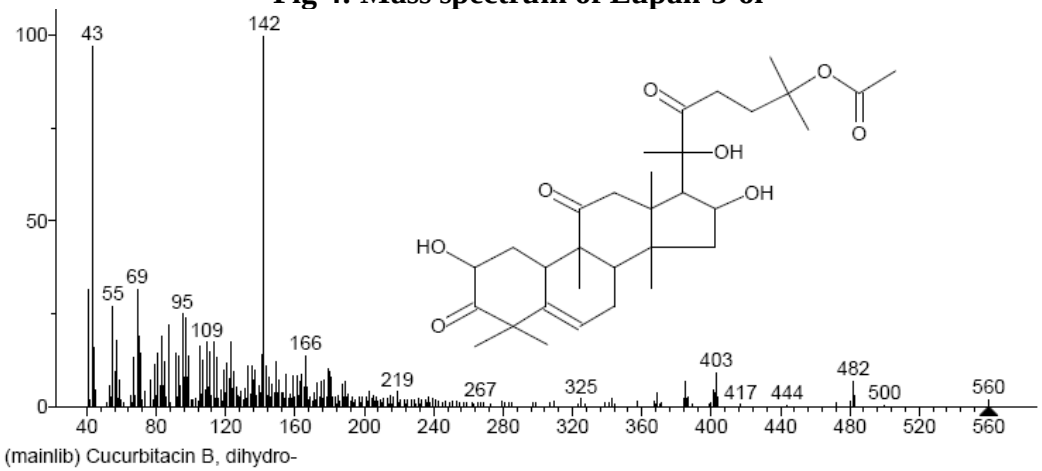

Fig 5: Mass spectrum of Cucurbitacin B

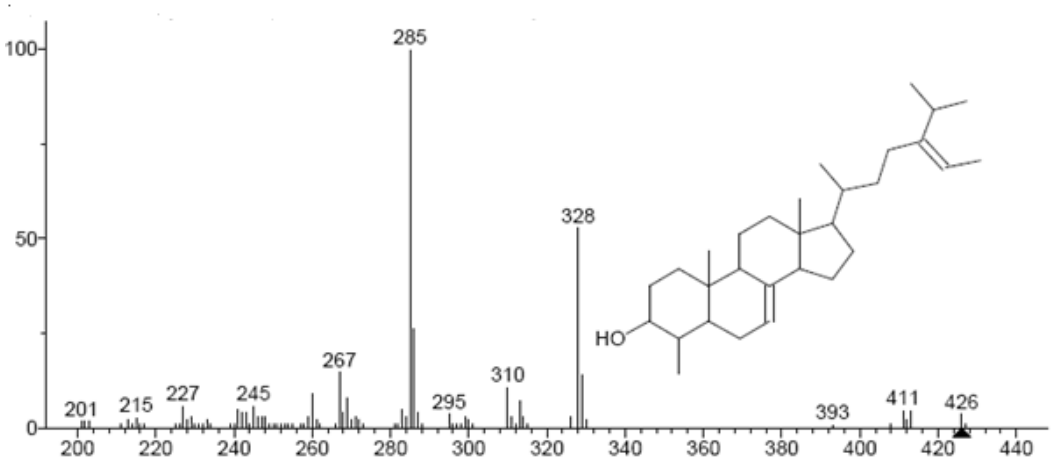

Fig 6: Mass spectrum of Lupan-3-ol

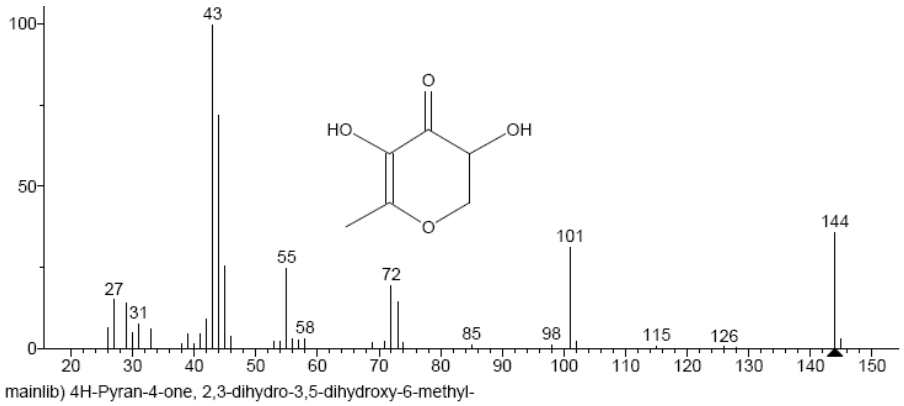

Fig 7: Mass spectrum of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

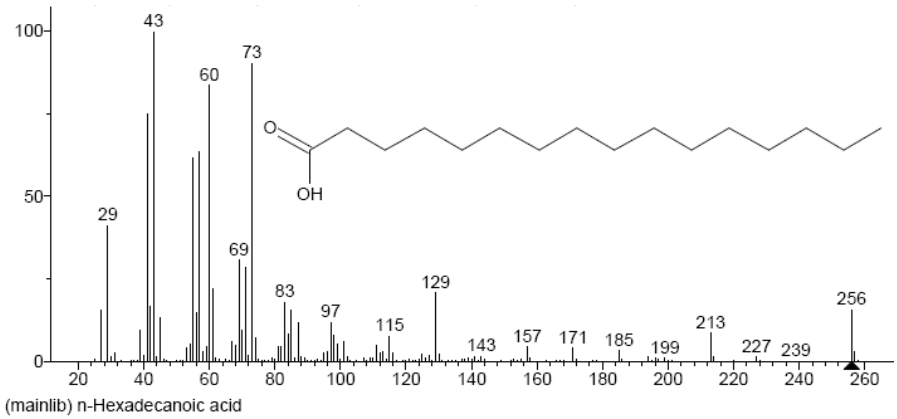

Fig 8: Mass spectrum of n-Hexadecanoic acid

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