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GC – MS Determination of Bioactive Components of *Naringi crenulata* (Roxb) Nicolson

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Abstract: The investigation was carried out to determine the possible bioactive components of leaves and bark of *Naringi crenulata* using GC-MS. The chemical compositions of the ethanolic extract of leaves and bark of *Naringi crenulata* were investigated using Perkin-Elmer Gas chromatography – Mass spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. 17 components from leaves and 23 components from bark of the above said plant were identified.
Key words: *Naringi crenulata*, GC-MS analysis, bioactive components.

Introduction

Naringi crenulata (Roxb) Nicolson belongs to Rutaceae family (sub family – Aurantioideae) is known as Kattuelumichai. It is commonly known as '*Kattunarakam*', *Malanarakam, Narinarakam, Cheriyakuttunaragam* in Malayalam, '*Kukka velaga*' in Telugu. It is a wide spread species of the genus (*Naringi*) growing as understorey trees in evergreen forests upto 1200 m. Leaves are compound, imparipinnate, 15cm long, alternate, spiral; rachis with oblanceolate wings, glabrous, leaflets 5-7, opposite, sessile 2-4.5 x 1-1.5cm, elliptic to obovate, apex emarginated or abtuse, base acute, margin crenulata or irregularly serrulate, glandular punctuate, glabrous; secondary nerves 7-10 pairs, looped near margin tertiary nerves ademedially ramified. Young branchlets terete, glabrous, thorny. It has been used as folk medicine [(1)]. The root extract of this plant is used for curing vomiting, dysentery and colic disorders.[(2,3)] Fruit decoction is used as antidote to insect poison[(3)]. Pectic polysaccharides [(4)] have been isolated from the fruits of *Naringi crenulata* by extraction with water. The bark juice [(5)] is applied externally for getting speedy relief in sprain. A preliminary evaluation of anthelmintic activity [(6)] of leaves is reported. Taking into consideration of the medicinal importance of this plant, the ethanolic extract of leaves and bark of *Naringi crenulata* were analyzed for the first time using GC MS. This work will help to identify the compounds of therapeutic value. GC-MS is one of the best techniques to identify the bioactive constituents of long chain, branched chain hydrocarbons, alcohols, acids, ester etc.

Materials and Methods

Leaves and bark of *Naringi crenulata* were collected in the month of February and March, 2010 from the Agasthiarmalai Biosphere Reserve, Western Ghats, Tamilnadu. Leaves were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept aside and again shaken after 24 hrs. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS analysis

GC/MS analysis of these extracts were performed using a Perkin-Elmer GC clauses 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column(30m x 0.25 mm ID x 1 µ df), composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2 µl was employed split ratio of 10:1) injector temperature 250^0C; ion-source temperature 280^0C. The oven temperature was programmed from 110^0C (isothermal for 2 min) with an increase of 10^0C / min to 2000^0C, then 5^0C/min to 280^0C, ending with a 9 min isothermal at 280^0C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass.

Table 1 : Phytocomponents in the ethanolic extract of leaves of *Naringi crenulata*

S.No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	2.13	Butane, 1,1-diethoxy-2-methyl-	$C_9H_{20}O_2$	160	11.76
2	2.40	Vitamin d3	$C_{27}H_{44}O$	384	4.52
3	2.50	Octane, 3,5-dimethyl-	$C_{10}H_{22}$	142	10.86
4	2.94	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	13.12
5	3.02	4-Trifluoroacetoxytridecane	$C_{15}H_{27}F_3O_2$	296	3.17
6	3.06	Acetic acid, trifluoro-, 3,7-dimethyloctyl ester	$C_{12}H_{21}F_3O_2$	254	4.52
7	3.22	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324	0.45
8	3.37	1-Butanol, 3-methyl-, formate	$C_6H_{12}O_2$	116	8.14
9	5.54	Tetratetracontane	$C_{44}H_{90}$	618	3.62
10	5.65	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	$C_{27}H_{54}$	378	4.98
11	5.76	Tetratetracontane	$C_{44}H_{90}$	618	3.17
12	7.23	Caryophyllene	$C_{15}H_{24}$	204	12.22
13	10.00	Cholesta-8,24-dien-3-ol, 4-methyl-, (3á,4à)-	$C_{28}H_{46}O$	398	4.98
14	11.59	Sumatriptan	$C_{14}H_{21}N_3O_2S$	295	3.17
15	13.17	Docosanoic acid, 1,2,3-propanetriyl ester	$C_{69}H_{134}O_6$	1058	0.45
16	14.18	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	502	2.26
17	15.28	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	4.07

Table 2 : Phytocomponents in the ethanolic extract of bark of *Naringi crenulata*

S.No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	2.12	2-Dimethylsilyloxytridecane	$C_{15}H_{34}OSi$	258	11.54
2	2.50	Octane, 3,5-dimethyl-	$C_{10}H_{22}$	142	24.96
3	2.83	Undecane	$C_{11}H_{24}$	156	2.34
4	2.94	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	4.68
5	3.06	1-Octanol, 3,7-dimethyl-	$C_{10}H_{22}O$	158	10.14
6	3.23	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	2.50
7	4.30	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324	1.56
8	4.52	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	252	4.05
10	5.55	4-Trifluoroacetoxyhexadecane	$C_{18}H_{33}F_3O_2$	338	3.12
11	5.66	1-Undecene, 5-methyl-	$C_{12}H_{24}$	168	4.68
12	5.77	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	3.28
13	8.35	2-Nonadecanone 2,4-dinitrophenylhydrazine	$C_{25}H_{42}N_4O_4$	462	0.47
14	8.58	17-Pentatriacontene	$C_{35}H_{70}$	490	1.09
16	13.25	Isoquinolin-6-ol, 7-methoxy-1-methyl-	$C_{11}H_{11}NO_2$	189	5.15
17	13.35	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	2.34
18	16.01	4H-Pyrazolo[3,4-b]pyran-5-carbonitrile, 6-amino-4-(4-hydroxy-3-metoxyphenyl)-3-methyl-	$C_{15}H_{14}N_4O_3$	298	2.34
19	18.33	2H-Furo[2,3-h]-1-benzopyran-2-one, 8,9-dihydro-8-(1-hydroxy-1-methylethyl)-, (S)-	$C_{14}H_{14}O_4$	246	10.14
20	20.69	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	$C_{27}H_{54}$	378	1.40
21	21.25	Hexa-t-butylselenatrisiletane	$C_{24}H_{54}SeSi_3$	506	0.78
22	25.14	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	1.72
23	31.89	psi.,.psi.-Carotene,1,1',2,2'-tetrahydro-1,1'-dimethoxy-	$C_{42}H_{64}O_2$	600	1.72

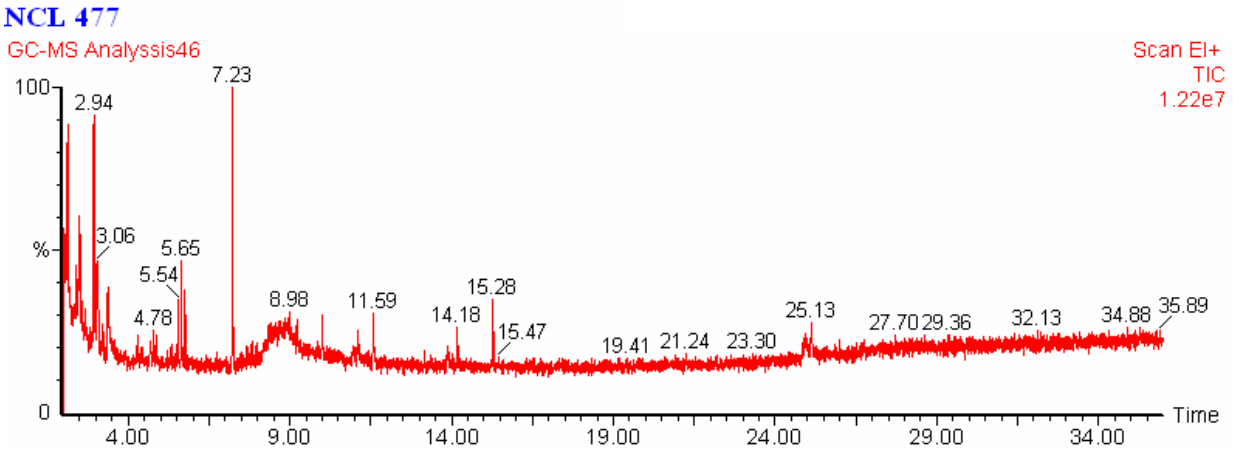

Fig 1. GC-MS Chromatogram of ethanolic extract of leaves of *Naringi crenulata*

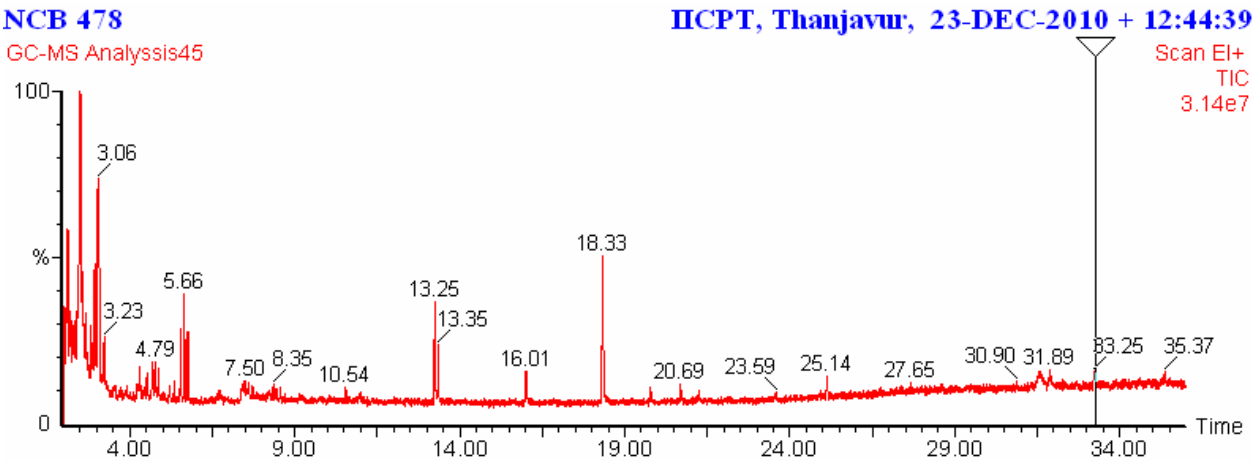

Fig 2. GC-MS Chromatogram of ethanolic extract of bark of *Naringi crenulata*

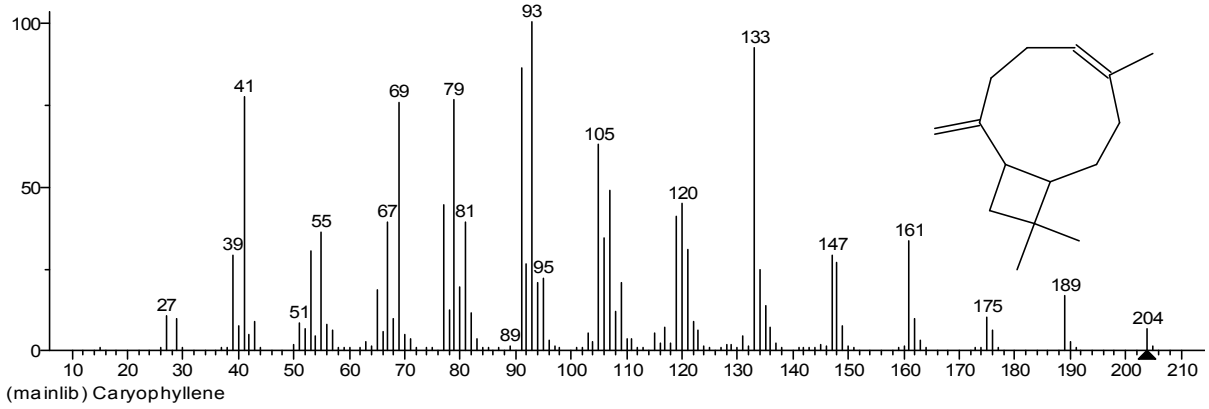

Fig. 3. Mass spectrum of Caryophyllene (RT. 7.23)

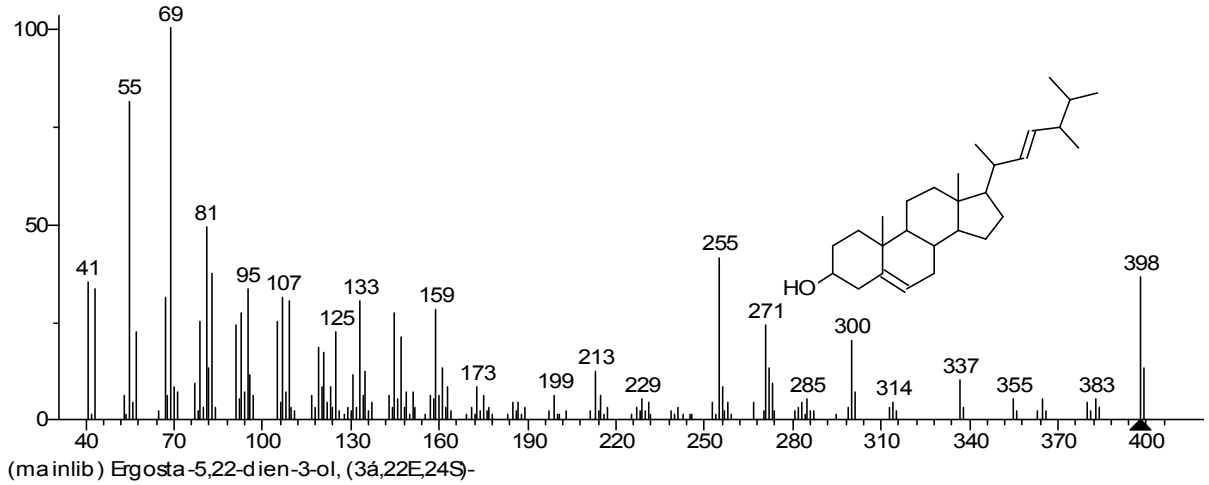

Fig.4 Mass spectrum of Cholesta (RT : 10)

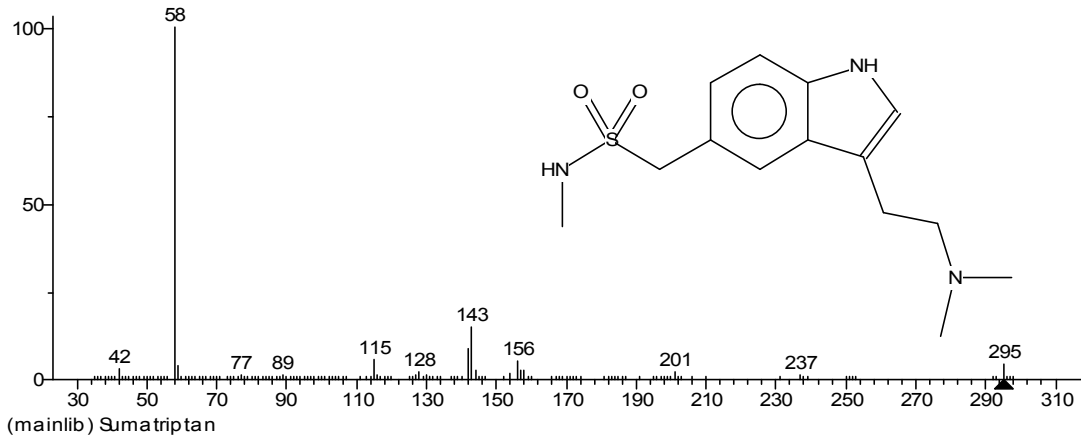

Fig.5 : Mass spectrum of sumatriptan (RT : 11.54)

Table 3.: Activity of phyto components identified in the ethanolic extract of leaves of *Naringi crenulata*

S.No	Name of the compound	Nature of compound	*Activity
1	Vitamin d3	Vitamin compound	Nutrient
2	4-Trifluoroacetoxytridecane	Fluro compound	Antimicrobial
3	Acetic acid, trifluoro-, 3,7-dimethyloctyl ester	Ester compound	Antimicrobial
4	1-Butanol, 3-methyl-, formate	Alcoholic compound	Antimicrobial
5	Caryophyllene	Sesquiterpene	Antimicrobial Anticancer
6	Cholesta-8,24-dien-3-ol,4-methyl-, (3á,4à)-	Steroid	Antimicrobial Diuretic Anti-inflammatory Antiasthma
7	Sumatriptan	Sulfur compound	Antimicrobial
8	7,8-Epoxylanostan-11-ol, 3-acetoxy-	Alcoholic compound	Antimicrobial

Results and Discussion

The components present in the ethanolic extracts of leaves and bark of *Naringi crenulata* were identified by GC-MS analysis (Figures 1 and 2). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanolic extracts of leaves and bark of *Naringi crenulata* are presented in Tables 1 and 2. Seventeen compounds were identified in ethanolic extracts of leaves of *Naringi crenulata*. The prevailing compounds were caryophyllene (12.22%), propane, 1,1,3-triethoxy-(13.12%), butane, 1,1-diethoxy-2-methyl- (11.76%), octane, 3,5-dimethyl-(10.86%), cyclohexane, 1,3,5-trimethyl-2-octadecyl-(4.98%), cholesta -8,24-dien-3-ol, 4-methyl-(3a, 4a) (4.98%), sumatriptan (3.17%) and 4-triflouroacetoxytridecane (3.17%) Figures 3, 4 and 5 show the mass spectrum and structures of caryophyllene, cholesta-8,24-dien-3-ol, 4-methyl-,(3a, 4a) and sumatriptan respectively. Table 3 listed the major phyto components and its biological activities obtained through the GC/MS study of leaves of *Naringi crenulata.*

Twenty three compounds were identified in the ethanolic extract of bark of *Naringi crenulata*. The results revealed that octane, 3,5-dimethyl-(24.96%) was formed as major component followed by 2-Dimethylsilyloxytridecane(11.54%), l-Octanol, 3,7-dimethyl-(10.14%), Isoquinolin-6-ol, 7-methoxy-1-methyl-(5.15%), 1-Hexadecanol, 2-methyl-(3.28%) and Dibutyl phthalate (2.34%) were found as the major components in the ethanolic extracts of bark of *Naringi crenulata*. Figure 6,7,8 and 9 shows the mass spectrum and structures of 2-Nonadecanone 2,4-dinitrophenyl hydrazine, Isoquinolin-6-ol, 7methoxy-1-methyl-, 4H-pyrazolo(3,4,-b)pyran-5-carbonitrile, 6-amino-4-(4-hydroxy-3-metoxyphenyl)3-methyl and

Ethyl iso-allocholate respectively. Major phytocompounds and its biological activities obtained through the GC/MS study of bark of *Naringi crenulata* has been tabulated (Table 4).

In the present study, 17 and 23 phyto -components have been identified from ethanolic extracts of leaves and bark of *Naringi crenulata* respectively by Gas chromatography and Mass spectrometry analysis. So it is recommended as a plant of phyto pharmaceutical importance. However, further studies will need to be undertaken to ascertain fully its pharmacological activity.

Table 4. : Activity of Components identified in the Plant Sample: NCB 478

S.No	Name of the compound	Nature of compound	*Activity
1	1-Octanol, 3,7-dimethyl-	Alcoholic compound	Antimicrobial
2	13-Heptadecyn-1-ol	Alcoholic compound	Antimicrobial
3	4-Trifluoroacetoxyhexadecane	Fluro compound	Anti microbial
4	1-Hexadecanol, 2-methyl-	Alcoholic compound	Antimicrobial
5	2-Nonadecanone 2,4-dinitrophenylhydrazine	Ketone compound	Antimicrobial
6	Isoquinolin-6-ol, 7-methoxy-1-methyl-	Alkaloid	Antimicrobial Antioxidant Anti-inflammatory
7	Dibutyl phthalate	Plasticizer compound	Antimicrobial Anti fouling
8	4H-Pyrazolo[3,4-b]pyran-5-carbonitrile, 6-amino-4-(4-hydroxy-3-metoxyphenyl)-3-methyl-	Alkaloid	Antimicrobial Antioxidant Antiinflammatory
9	Ethyl iso-allocholate	Steroid	Antimicrobial Diuretic Anti-inflammatory Antiasthma
10	psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	Carotene compound	Nutrient

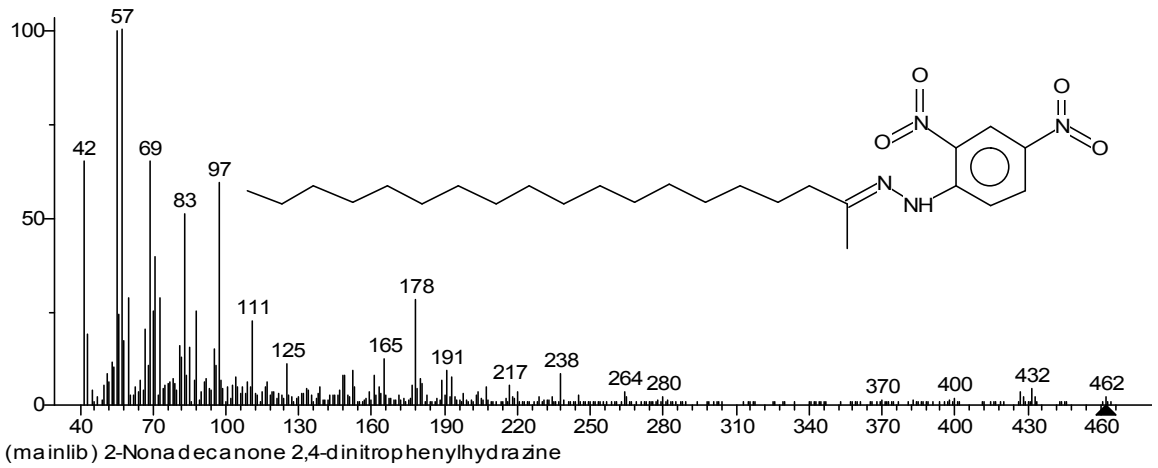

Fig.6 : 2-Nonadecanone 2,4-dinitrophenylhydrazine

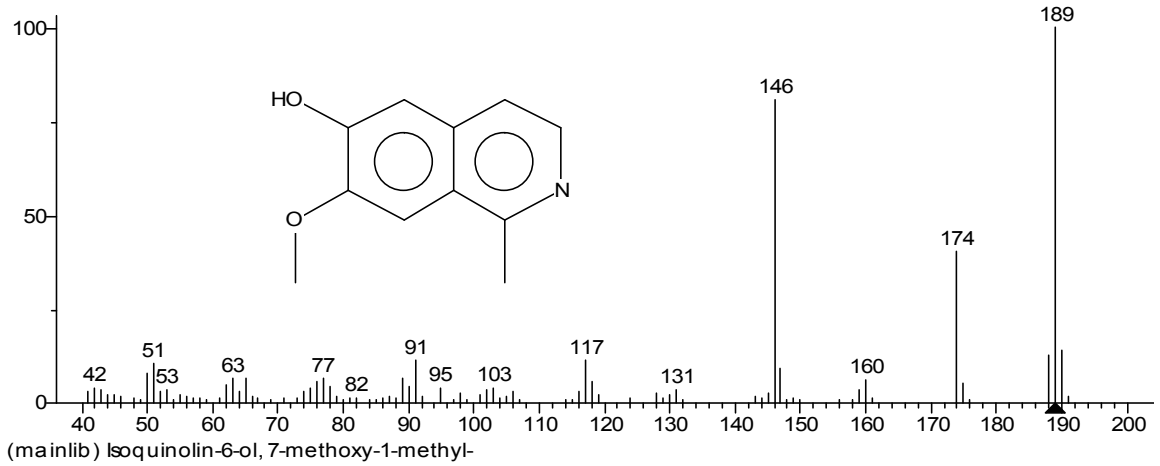

Fig.7 : Isoquinolin-6-ol, 7-methoxy-1-methyl-

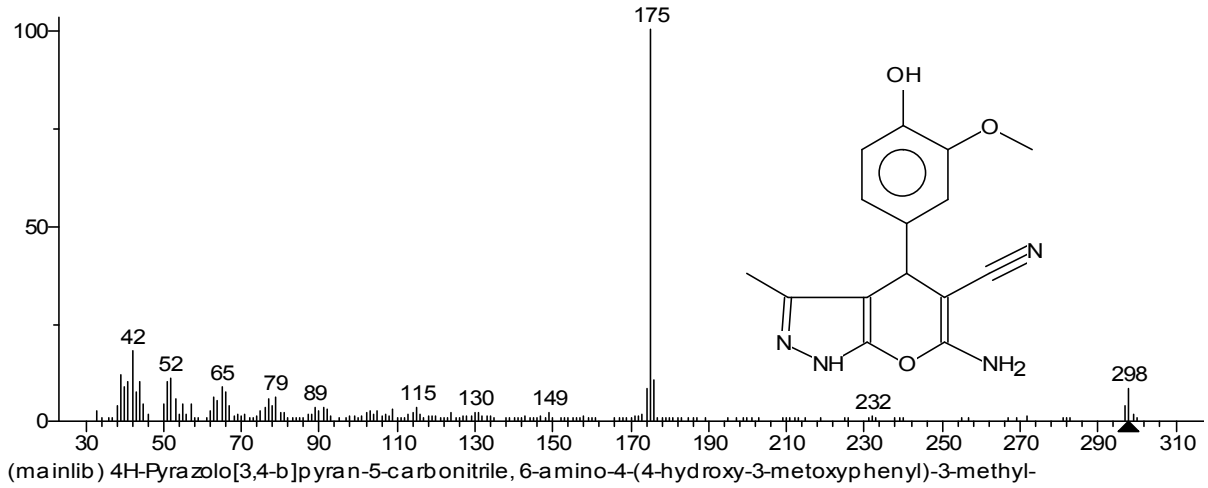

Fig.8 : 4H-Pyrzolo[3,4-b] pyran-5-carbonitrile, 6-amino 4-(4-hydroxy-4metoxyphyenyl)-3-methyl-

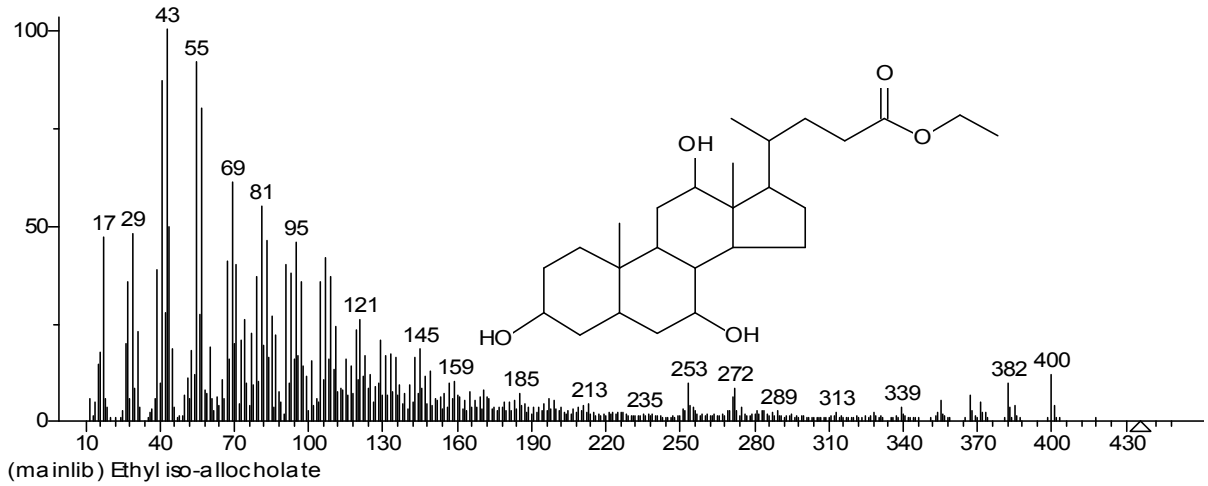

Fig.9 : Ehtyl Iso-allocholate

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