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PHYTOCHEMICAL ANALYSIS OF CHLOROFORM EXTRACT OF ROOTS OF KALANCHOE PINNATA BY HPLC AND GCMS

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ABSTRACT

The plant *Kalanchoe pinnata* is widely used in ayurvedic system of medicine as astringent, analgesic, carminative and also useful in diarrhea and vomiting. Naturalized throughout the hot and moist parts of India. And lots of phytochemical and pharmacological work has done on leaves of plant but the root part is not focused. Hence we have selected roots for phytochemical analysis which will support further evaluation of pharmacological activity. In this first roots are subjected to chloroform for extraction. And preliminary phytochemical test were performed. Chloroform extract was subjected for HPLC and GC-MS analysis which shows various phytoconstituents present in extract.

INTRODUCTION: Various species of KALANCHOE are used medicinally in Indo-China and Philippines Islands, whereas *Kalanchoe pinnata* Pers. (Family Crassulaceae) is naturalized throughout the hot and moist parts of India. The leaves and bark is bitter tonic, astringent to the bowels, analgesic, carminative, useful in diarrhoea and vomiting [1]. Antiulcer [2], anti-inflammatory [3-4] and antimicrobial activity [5] of leaf extract was reported.

Oral treatment with leaf extract significantly delayed onset of disease in BALB/c mice infected with *Leishmania amazonensis* as compared to untreated mice or mice receiving *K. pinnata* by the intravenous or topical routes [6]. Potent cytotoxic compounds bersaldegenin- 1, 3, 5-orthoacetate [7] and bufadienolide-bryophyllin B [8] were isolated. Other chemical constituents from this plant are bryophyllol, bryophollone, bryophollenone, bryophynol and two homologous phenanthrene derivatives 2(9-decenyl)-phenanthrene (I) and 2-(undecenyl)-phenanthrene (II) from leaves [9]. Isolation and structure elucidation of 24-epiclerosterol [24(*R*)-stigmasta-5, 25-dien-3β-ol], 24(*R*)-5α-stigmasta-7, 25-dien-3β-ol, 5α-stigmast-24-en-3β-ol and 25-methyl-5α-ergost-24 (28)-en-3β-ol from aerial parts was done [10].

This species is also included in the plants species, which are used by the tribals of Kerala for treating cancer symptoms [11]. Juice of the fresh leaves is used very effectively for the treatment of jaundice in folk medicines of Bundelkhand region of India, but no systemic study to assess this activity has been carried out.

As the aerial parts of plant have many pharmacological activity but roots of this plant was not focused yet hence the present investigations were carried out to evaluate phytoconstituent of roots of plant with the help of HPLC and GC-MS, which will help for further pharmacological evaluation.

MATERIALS AND METHODS:

Collection of Plant Material: The roots of *Kalanchoe pinnata* was collected from Satpuda hills near Akkalkuwa, Dist: Nandurbar, Maharashtra, India, in June 2010, cleaned and dried at room temperature in shade and away from direct sunlight. The plant authenticated by T. Chakraborthy, Deputy Director, Botanical Survey of India, Koregaon Road Pune, by comparing morphological features and a sample voucher specimen of plant was deposited for future reference (Voucher specimen number QMAKP1).

Preparation of Extract: The root of *Kalanchoe pinnata* was collected and dried in the shade and then pulverized in a grinder. The powdered drug was utilized for extraction. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. A method described in Mukherjee was used for extraction of powdered plant. Extraction was done by Chloroform [12].

Preliminary Phytochemical Screening: The extract was then subjected to preliminary phytochemical screening to detect the presence of various phytoconstituent. The results show presence of Steroids, Saponins, Alkaloids, Glycosides, Flavonoids, Tannins in the chloroform extract [13].

HPLC Analysis for Flavonoids [14]:

- Column: Hypersil- ODS Column (250mm × 4.6mm), 5µm particle size
- Mobile phase: Acetonitrile: Phosphate buffer pH 2.4 (25:75)
- Flow rate: 1.2 ml/ min
- Detection: 266 nm

HPLC analysis for steroids [15]:

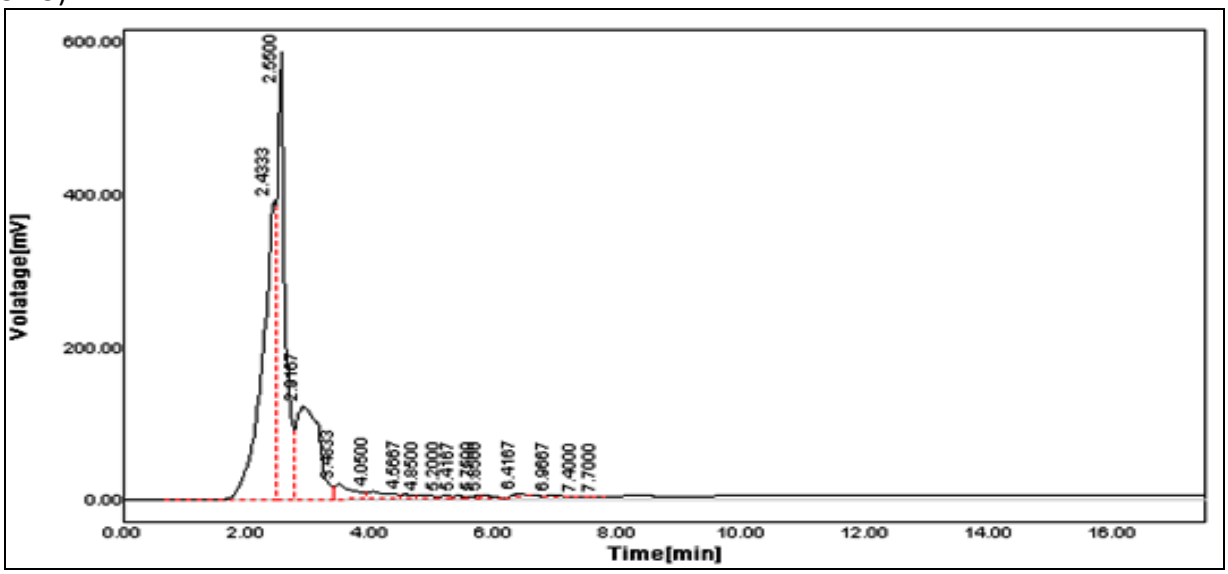
- Column: Hypersil- ODS Column (250mm × 4.6mm), 5µm particle size
- Mobile phase: Methanol: Water (95:5)
- Flow rate: 1ml/ min
- Detection: 210 nm

GC-MS Analysis [16]: The GC-MS analyses were carried out in Perkin Elmer, Auto system XL GC+.

- Carrier gas: helium with a flow rate of 0.7 mL/min;
- Column temperature: 5 min in 180°C, 180-260°C at 3°C/min, 5 min in 260°C, 260-280°C at 0.2°C/min, and finally 5 min in 280°C; injector temperature, 280°C detector temperature, 290°C.
- Volume injected: 1 µL of sample
- Ionization potential: 70 eV
- Ion source temperature: 290°C.

RESULTS AND DISCUSSION:

HPLC Analysis for Flavonoids: Analysis of flavonoids in chloroform extract of plant shows 15 compounds (**Graph 1**) after comparing with standard retention time of various flavonoids, it shows peak at 7.4000 which is nearer to myrcetin [14].

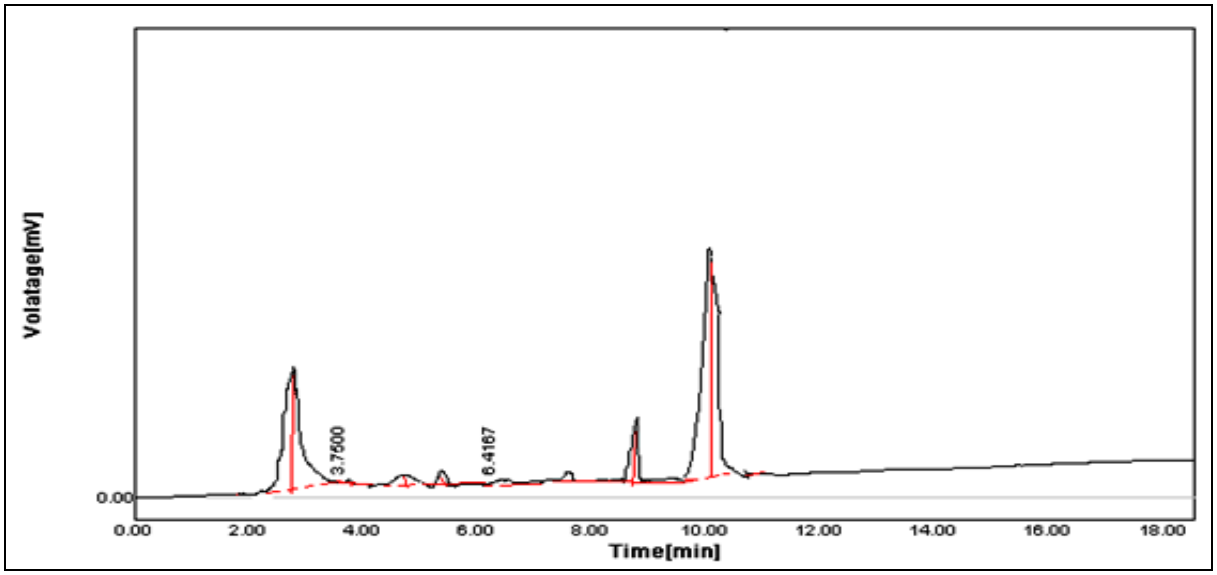


GRAPH 1: CHROMATOGRAM OF HPLC FOR CHLOROFORM EXTRACT FOR FLAVONOIDS

TABLE 1: RESULTS OF HPLC FOR CHLOROFORM EXTRACT FOR FLAVONOIDS

S. No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1		2.4333	5665.9653	37.51	14.9	0.7500	0.0000
2		2.5500	5225.7778	34.60	2649.1	1.0000	0.1120
3		2.9167	3129.7407	20.72	866.4	1.6111	1.0476
4		3.4833	414.4306	2.74	747.6	1.2000	1.0625
5		4.0500	234.2879	1.55	300.7	1.0000	0.6667
6		4.5667	84.4501	0.56	14.1	1.1667	0.1512
7		4.8500	86.1647	0.57	2.2	1.4286	0.0269
8		5.2000	45.6691	0.30	863.7	1.0833	0.0432
9		5.4167	44.1830	0.29	208.5	1.0833	0.1667
10		5.7500	42.2993	0.28	4858.2	0.6000	1.4286
11		5.8500	48.9324	0.32	23.6	2.8750	0.3243
12		6.4167	65.7003	0.43	3653.1	0.8571	0.1838
13		6.9667	9.1030	0.06	2422.2	1.4000	0.9429
14		7.4000	4.7364	0.03	9034.4	1.0000	0.8387
15		7.7000	2.2466	0.01	11835.9	0.9286	0.8571
Sum			**15103.6875**				

HPLC Analysis for Steroids: Analysis of flavonoids in chloroform extract of plant shows 8 compounds (**Graph 2**) after comparing with standard retention time of various flavonoids, it shows peak at 7.8920 and 10.1563 which is nearer to cholesterol and beta sitosterol respectively [15].

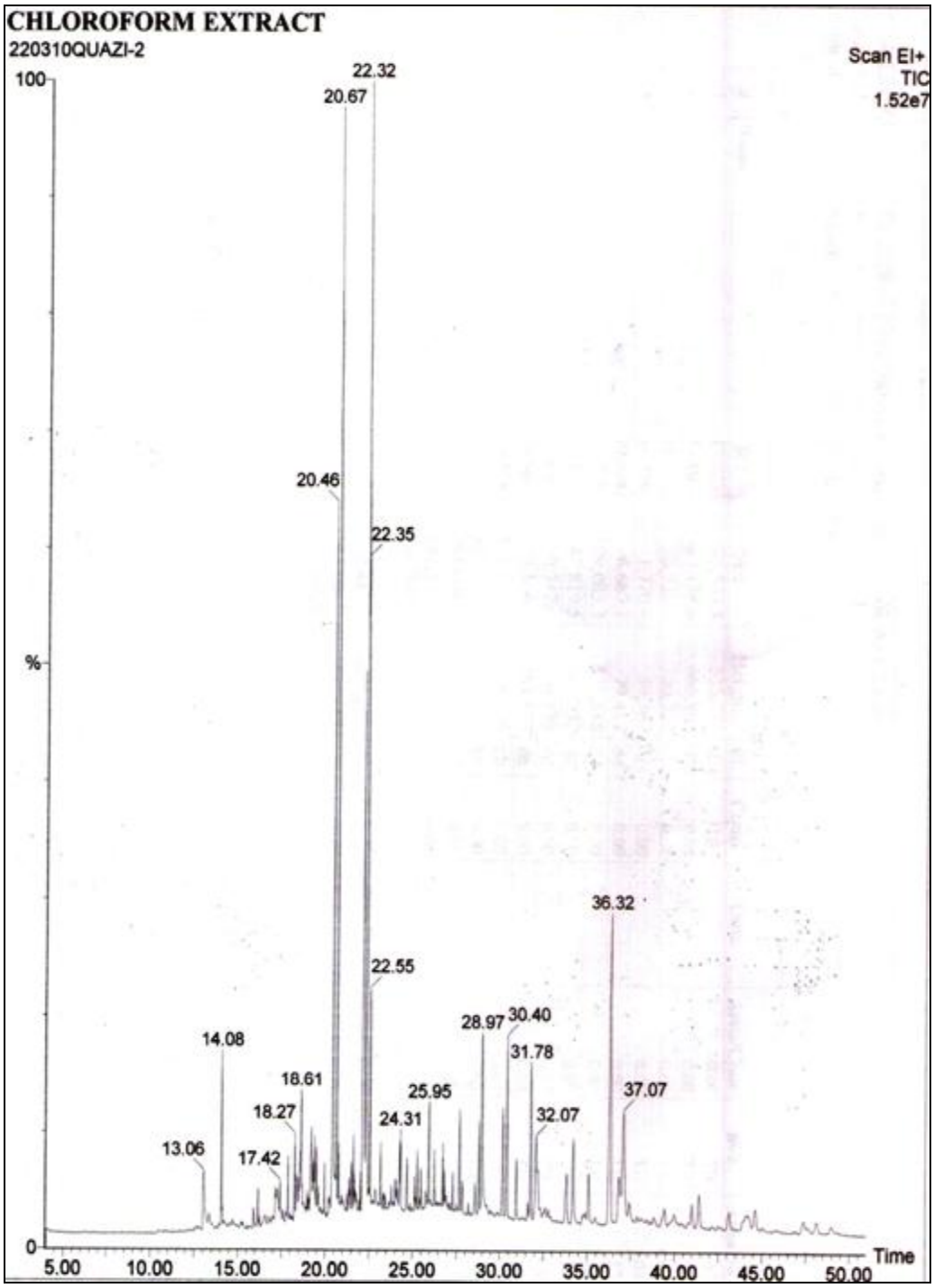


GRAPH 2: CHROMATOGRAM OF HPLC FOR CHLOROFORM EXTRACT FOR STEROIDS

TABLE 2: RESULTS OF HPLC FOR CHLOROFORM EXTRACT FOR STEROIDS

S. No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1		2.8167	285.2793	24.96	1955.3	8.6667	0.0000
2		3.7500	9.0759	0.79	5729.1	1.1429	0.1622
3		4.8500	38.1647	3.34	198.4	1.4286	0.0269
4		5.4167	44.1830	3.86	208.5	1.0833	0.1667
5		6.4167	8.7238	0.76	4193.6	1.0385	0.9375
6		7.8920	10.2466	0.89	11835.9	0.9286	0.8571
7		8.7418	167.2904	14.64	135.0	0.7143	1.5775
8		10.1563	579.5357	50.72	2683.9	1.3333	0.6667
Sum			**1142.49**				

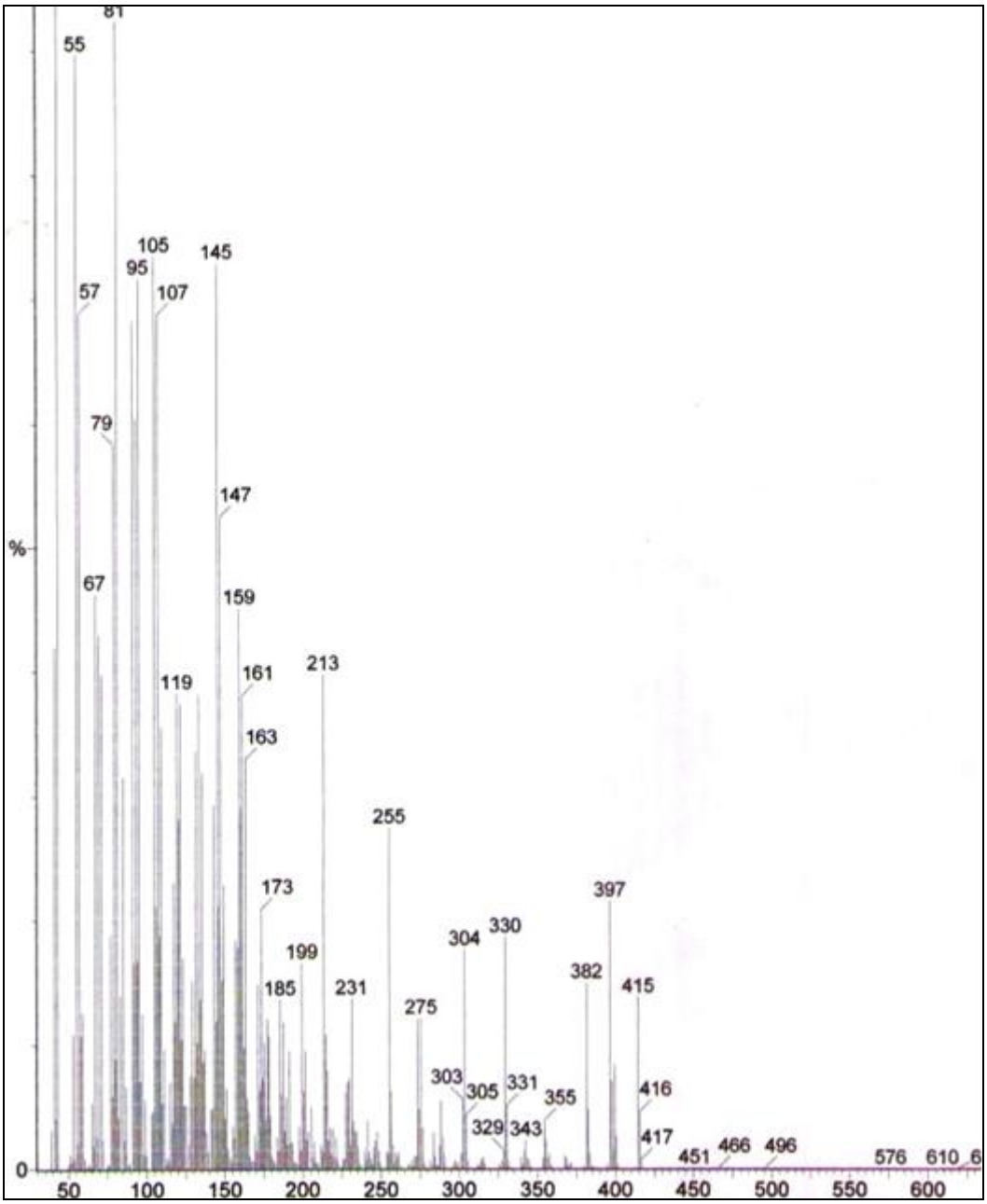
GC-MS Analysis: After preliminary phytochemical investigation and HPLC analysis shows the presence of various phytoconstituent in chloroform extract (**Graph 3**). Further investigation done by GCMS. Gaschromatograph of chloroform extract shows the presence of 40 phtoconstituent (**Table 3**) which are subjected to mass spectroscopy. The mass spectroscopy of these peaks does not give any prominence result it shows only presence of steroids and various sugar molecule (**Graph 4**), here ms data of chloroform extract for peak at 37.075 RT (**Table 4**). The list of possible phytoconstituent is given by checking compound in NIST and NBS library.



GRAPH 3: GC CHROMATOGRAM OF CHLOROFORM EXTRACT

TABLE 3: AREA PERCENT REPORT OF CHLOROFORM EXTRACT

S. No.	Name	RT	Area (A)	Height	BL	Conc	Units	A/conc	m/z	Area%
1		13.075	85,564.8	535,711	MM	0.00		0.00	TIC	1.67
2		14.102	87,138.6	666,805	MM	0.00		0.00	TIC	1.70
3		18.612	98,726.1	7,000,724	MM	0.00		0.00	TIC	1.93
4		18.924	1,770.4	39,347	MM	0.00		0.00	TIC	0.03
5		19.034	6,687.1	79,412	dd	0.00		0.00	TIC	0.13
6		19.217	109,085.3	675,518	dd	0.00		0.00	TIC	2.13
7		19.364	47,819.5	491,052	dd	0.00		0.00	TIC	0.93
8		19.456	30,747.4	376,129	dd	0.00		0.00	TIC	0.60
9		19.566	20,306.2	253,570	db	0.00		0.00	TIC	0.40
10		20.446	511,244.9	3,955,516	bb	0.00		0.00	TIC	9.99
11		20.629	395,723.8	3,111,630	bb	0.00		0.00	TIC	7.73
12		22.169	491,098.3	3,958,713	dd	0.00		0.00	TIC	9.60
13		22.352	937,544.6	6,627,489	db	0.00		0.00	TIC	18.32
14		22.536	95,700.8	750,374	bb	0.00		0.00	TIC	1.87
15		23.178	22,920.9	183,161	bd	0.00		0.00	TIC	0.45
16		24.296	62,441.9	442,625	db	0.00		0.00	TIC	1.22
17		25.176	12,894.2	121,168	bd	0.00		0.00	TIC	0.25
18		25.249	21,468.0	182,992	db	0.00		0.00	TIC	0.42
19		25.469	20,694.0	166,177	bb	0.00		0.00	TIC	0.40
20		25.946	73,754.4	431,432	bb	0.00		0.00	TIC	1.44
21		26.277	26,776.4	201,552	bd	0.00		0.00	TIC	0.52
22		26.789	41,042.9	356,064	bd	0.00		0.00	TIC	0.80
23		26.863	15,781.6	173,783	dd	0.00		0.00	TIC	0.31
24		27.284	16,823.0	133,426	MM	0.00		0.00	TIC	0.33
25		27.688	57,702.4	415,391	bd	0.00		0.00	TIC	1.13
26		27.834	19,976.5	157,620	db	0.00		0.00	TIC	0.39
27		28.586	22,695.0	174,179	MM	0.00		0.00	TIC	0.44
28		28.824	61,601.7	478,529	bd	0.00		0.00	TIC	1.20
29		28.989	203,248.0	1,262,146	dd	0.00		0.00	TIC	3.97
30		30.145	88,541.1	685,055	bb	0.00		0.00	TIC	1.73
31		30.401	127,118.6	984,709	bb	0.00		0.00	TIC	2.48
32		30..951	46,526.9	354,349	bb	0.00		0.00	TIC	0.91
33		31.776	133,637.9	981,008	MM	0.00		0.00	TIC	2.61
34		32.106	145,933.9	747,893	MM	0.00		0.00	TIC	2.85
35		33.811	77,236.9	491,155	MM	0.00		0.00	TIC	1.51
36		34.196	84,839.0	631,156	MM	0.00		0.00	TIC	1.66
37		35.076	43,705.1	343,011	MM	0.00		0.00	TIC	0.85
38		36.305	449,108.7	2,951,895	MM	0.00		0.00	TIC	8.78
39		36.836	82,568.8	504,613	bd	0.00		0.00	TIC	1.61
40		37.075	239,332.1	1,167,576	dd	0.00		0.00	TIC	4.68



GRAPH 4: MS DATA FOR CHLOROFORM EXTRACT FOR PEAK AT 37.075 RT

TABLE 4: LIST OF PROBABLE COMPOUND OF CHLOROFORM EXTRACT FOR PEAK AT 37.075 RT

Hit	REV	for	Compound name	M.W.	Formula	CAS	Library
1	851	658	Campesterol	400	C28H48O	474-62-4	Nbs
2	828	728	Cholesta-5- ene, 3-ol, (3 beta)-carbanochloridate	448	C28H45O2Cl	7144-08-3	Nist
3	818	669	Cholesterol	386	C27H46O	57-88-5	Nbs
4	817	675	Beta-sitosterol acetate	456	C31H52O2	915-05-9	Nbs
5	811	687	Cholest-5- ene, 3bromo-, (3 beta)-	448	C27H44Br	516-91-6	Nist
6	789	696	Gamma-sitosterol	414	C29H50O	83-47-6	Nbs
7	779	684	Cholest-3- en-3-ol (3 beta)-propanoate	442	C30H50O2	633-61-8	Nist
8	772	582	Cholesterol	386	C27H46O	57-88-5	Nbs
9	767	608	Methyl(25RS)-3beta acetoxy-5-cholestin-26-oate	472	C30H48O4	103160-13-0	Nist
10	764	671	Cholesta-3, 5-diene	368	C27H44	747-90-0	Nbs
11	757	665	Cholesta-3, 5-diene	368	C27H44	747-90-0	Nbs

12	756	635	Cholest-5- en-3-ol (3 beta)-tetradecanoate	596	C41H72O2	1989-52-2	Nist
13	752	570	Cholest-5- ene, 3methoxy-, (3 beta)-	384	C47H82O2	1174-92-1	Nbs
14	740	460	3-B-phenpxy-24-nor-cholan-5,20(22)-diene	404	C29H40O	1000148-77-6	
15	735	645	Cholesta-3,5-diene	368	C27H44	747-90-0	Nbs
16	724	508	Cholane-5,20(22)-diene-3b-phenoxy	418	C30H42O	10014-88-4	Nist
17	719	534	5-(7a-isopropenyl-4,5-dimethyl-octahydroinden-4-yl)	288	C20H32O	1000193-54-2	Nist
18	719	586	Cholest-8- en- (3 beta)-ol, acetate	428	C29H48O2	17137-74-5	Nist
19	715	605	21-acetoxypregnenolone	374	C23H34O4	566-78-9	Nist
20	701	371	Pregna-3, 5-dien-20-one	298	C21H30O	1093-87-4	Nbs

CONCLUSION: Preliminary phytochemical screening of chloroform extracts of *K.Pinnata* shows the presence of Steroids, Saponins, Alkaloids, Glycosides, Flavonoids and Tannins. HPLC analysis shows the presence of myrcetin, cholesterol and beta sitosterol. While but GCMS data show only steroids and sugar moieties, no molecule from flavonoids. The possible reason should be due lack of derivetisation technique which degrade the flavonids on heating into glycon and aglycon moiety. From these above phytochemical investigation it was concluded that chloform extract of Kalanchoe pinnata root contain various flavonoids and steroids. Which will leads further pharmacological investigation of this plant.

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