Table 3.5: Varying parameters of some of the peaks of urine by MEKC buffer at different load times

Peak code	10 seconds				20 seconds			
	M.T	Peak area	Peak height	HHPW	M.T	Peak area	Peak height	HHPW
B	18.23	16.32	6.53	3.90	18.27	230.13	11.25	0.70
C	23.92	62.00	2.79	4.20	23.84	171.98	6.02	1.20
D	30.37	24.79	1.35	1.50	30.83	422.61	24.28	4.50
E	40.10	45.44	1.56	3.50	31.51	27.47		5.00
F	-	-	-	-	33.96	134.66	3.83	-
G	46.85	60.98	2.21	2.50	37.16	334.21	15.69	3.00
H	-	-	-	-	-	-	-	-
I	51.88	358.71	11.62	1.60	42.03	27.80	1.25	-
J	54.76	82.93	2.35	2.30	49.46	164.78	4.29	7.60
Peak code	30 Seconds				40 seconds			
	M.T	Peak area	Peak height	HHPW	M.T	Peak area	Peak height	HHPW
B	18.28	196.53	8.84	3.60	18.37	137.12	8.73	1.20
C	23.76	183.56	9.919*e-1	3.70	22.98	54.36	5.15	2.50
D	30.54	655.26	36.43	2.90	30.20	1083.47	56.48	2.90
E	32.24	62.49	3.33	2.00	32.23	176.76	6.69	2.10
F	33.63	184.41	6.74	4.20	33.36	338.93	8.20	3.40
G	36.78	496.69	22.04	2.90	36.70	844.74	34.91	3.80
H	-	-	-	-	-	-	-	-
I	41.44	92.95	3.60	3.40	41.40	167.57	3.58	3.00
J	49.26	214.72	5.97	4.80	49.18	456.61	9.03	5.20

PC – Peak code, M.T – Migration time (min), PA – Peak area,
P.H – Peak height (cm), HHPW – Half height peak width (cm).

Note – It can be observed that , the migration time of the components is high (better resolution) and the peak area is less compared to the areas observed in other two buffers (SDS and Cholate.

Table 3.6: Varying parameters of some of the peaks of urine by SDS / SCD buffer at different load times

	10 Seconds				20 seconds			
PC	M.T	Peak area	Peak height	HHPW	M.T	Peak area	Peak height	HHPW
B	16.24	84.11	7.76	1.30	16.26	190.63	10.42	1.00
C	21.29	178.12	5.37	2.80	21.48	125.21	5.55	0.60
D	30.35	148.61	2.96	3.40	30.25	250.68	8.11	3.01m
E	37.25	43.24	1.41	2.70	37.10	126.97	4.12	0.40
F	39.67	364.33	12.56	3.50	37.87	623.23	20.50	2.50
G	41.57	337.26	10.83	2.90	40.98	624.50	20.56	3.50
H	42.20	121.27	2.97	1.90	41.71	349.61	13.01	3.10
I	43.87	137.94	2.99	3.60	43.52	197.42	4.78	4.10
J	45.19	227.69	6.01	2.90	45.00	188.63	4.57	4.20
	30 seconds				40 seconds			
PC	M.T	Peak area	Peak height	HHPW	M.T	Peak area	Peak height	HHPW
B	16.41	337.87	9.75	1.00	16.04	215.47	10.35	3.40
C	21.69	209.66	7.22	O.6	21.54	227.79	9.21	1.80
D	30.38	486.91	11.38	3.30	30.16	559.73	13.92	3.30
E	36.68	109.28	3.60	1.40	36.72	105.36	11.22	0.90
F	37.75	990.20	32.01	2.70	37.12	1199.10	46.90	3.00
G	41.22	1001.80	28.97	3.90	40.94	2047.80	58.27	4.20
H	41.58	546.55	22.37	3.20	41.68	76.14	6.44	0.80
I	43.76	371.91	7.67	4.20	43.40	521.42	10.52	3.00
J	45.24	364.29	7.25	3.90	44.83	473.92	9.36	3.30
	50 seconds							
PC	M.T	Peak area	Peak height	HHPW				
B	16.30	137.16	8.96	1.90				
C	21.59	190.82	11.40	0.90				
D	29.98	448.87	13.92	5.00				
E	36.83	1363.40	53.36	1.20				
F	36.83	1326.40	53.36	1.20				
G	40.80	240.30	57.77	1.90				
H	41.88	157.99	3.76					
I	43.53	531.95	11.40	2.80				
J	45.02	561.12	9.78	3.80				

PC – Peak code, M.T – Migration time (min), PA – Peak area, P.H – Peak height (cm), HHPW – Half height peak width (cm).

Note – It can be observed that, the migration time of the components is less than MEKC but still resolution was observed. The peak areas were also higher than MEKC. But the main drawback encountered was blocking of the capillaries.

Table 3.7: Varying parameters of some of the peaks of urine Cholate buffer at different load times

Peak code	10 Seconds				20 seconds				30 seconds			
	M.T	PA	PH	HHPW	M.T	PA	PH	HHPW	M.T	PA	PH	HHPW
B	23.367	55.482	4.9585	1.1	23.475	43.626	3.0248	1.8	23.327	196.55	9.7605	3.6
C	27.819	167.96	2.8997	1.9	28.246	64.502	3.5106	1.8	27.423	137.32	3.2998	3.2
D	33.587	41.963	1.7422	3.8	32.514	26.869	1.6094	4.1	33.569	627.06	10.558	2.7
E	33.587	41.969	1.7422	3.8	34.038	85.786	3.4331	4.6	35.894	398.16	5.6609	2.5
F	42.822	181.25	8.2197	3.5	42.988	395.22	17.072	4.2	41.856	881.59	30.319	3
G	48.936	161.09	5.7245	3.9	48.936	399.21	14.11	3.5	48.03	641.17	21.301	2.8
H	69.214	59.255	1.4307	7	70.841	119.49	2.6097	5.5	69.952	970.58	9.7299	3
I	77.699	362.08	7.154	5.7	79.592	879.14	15.141	7	78.533	2738.6	31.859	4.4
J	81.869	86.108	1.5582	5.5	83.787	192.84	3.269	7.1	82.699	900.75	8.0122	3.7

Peak code	40 seconds				50 seconds			
	M.T	PA	PH	HHPW	M.T	PA	PH	HHPW
B	22.951	399.97	12.944	3.1	22.919	1512.3	19.829	4.6
C	27.529	137.82	7.6008	2.3	28.61	198.79	7.2316	6.5
D	32.383	177.04	7.2953	2.3	32.614	312.63	9.3191	2.9
E					38.827	145.33	3.7061	2
F	39.866	752.76	35.048	2.4	39.613	969.85	45.346	2.5
G	46.12	653.62	24.506	5.1	46.189	978.32	33.033	6.1
H	67.557	210.63	5.1695	5.7	68.134	477.67	7.8858	4.9

PC – Peak code, M.T – Migration time (min), PA – Peak area, P.H – Peak height (cm), HHPW – Half height peak width (cm).

Note – The peak resolution and the areas was quite conviencing compared to MEKC and SDS buffers. The sensitivity was high.

4.3 (I) Preliminary Phytochemical screening

Preparation of Reagents for Phytochemical study

Following reagents and solutions, which are required for the preliminary phytochemical study, were prepared.

Molisch's reagent: 10 g of α-naphthol dissolved in 100 ml 95% ethanol.

Anthrone reagent: 0.2 g of anthrone dissolved in 100 ml of concentrated Sulphuric acid and shaken (the solution should be freshly prepared).

Biuret reagent: 1.5 g of $CuSO_4$, 6 g of sodium potassium tartarate were dissolved in 500 ml of water, 300 ml of freshly prepared NaOH solution was added to this solution and solution made upto the volume of one litre.

Ninhydrin reagent: 0.1 gm of Ninhydrin dissolved in 100 ml of acetone (0.1 %).

Phenolphthalein reagent: 1g of Phenolphthalein dissolved in 100 ml of ethanol.

HCl solution: 50 ml of con HCl dissolved in 50 ml of water.

$CuSO_4$ Solution: 24.7 g of Copper sulphate dissolved in 100 ml of distilled water.

Detection of Alkaloids

The leafy extracts prepared were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were tested carefully with alkaloid reagents.

a) Mayer's Test

1.3 g of mercuric chloride and 5 g of Potassium iodide were dissolved separately in 60 ml and 10 ml of distilled water respectively. Both were mixed thoroughly and diluted up to 100 ml with distilled water. Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow cream precipitate indicated the presence of alkaloids.

b) Wagner's Test

Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed. The formation of brown/reddish brown precipitate indicated the presence of alkaloids.

c) Dragendroff's Test

50 g of tartaric acid was dissolved in 200 ml of distilled water. 4.5 g of basic bismuth nitrate was added and solution was shaken for two hours. 100 ml of 40% Potassium iodide was then added and solution was shaken vigorously and allowed to stand for 24 hours and filtered. Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). The formation of a red precipitate indicated the presence of alkaloids.

d) Hager's Test

Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of a yellow colored precipitate indicated the presence of alkaloids.

Detection of Glycosides

Extracts were hydrolyzed with dilute hydrochloric acid, and the hydrolysate was subjected to glycosides tests.

a) Modified Borntrager's test

The extracts were treated with ferric chloride solution and heated on boiling water bath for about 5 min. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with half its volume of ammonia solution. There was no formation of rose pink or cherry red colour in the ammonical layer, thus indicating the absence of anthranol glycosides.

b) Legal's test

The extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. There was no formation of a pink to blood red colour thus indicating the absence of cardiac glycosides.

Detection of Saponins

a) Froth's test

The extracts were diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam indicated the presence of saponins.

b) Liberrman Buchwald's test

The extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. The formation of a brown ring at the junction indicated the presence of steroidal saponins.

Detection of Phenolics and Tannins

a) Ferric chloride test $FeCl_3$ (5%) solution

5 g of ferric chloride dissolved in 100 ml of ethanol. The extract was treated with a few drops of neutral ferric chloride solution. The formation of a bluish black colour indicated the presence of a phenolic nucleus (tannins).

b) Gelatin test (Gelatin solution containing 10% NaCl)

1 g of gelatin dissolved in 100 ml of 10% NaCl. To the extract, 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicated the presence of tannins.

c) Lead acetate test (lead acetate (10%) solution)

10 g of lead acetate dissolved in 100 ml of ethanol. The extracts were treated with few drops of lead acetate solution. Formation of a yellow precipitate indicated the presence of flavonoids.

d) Alkaline reagent test (NaOH solution 10%)

10 g of NaOH dissolved in 100 ml of distilled water. The extracts were treated with few drops of sodium hydroxide solution. Formation of an intense yellow colour, which became colorless on addition of few drops of dilute acid, indicated the presence of flavonoids.

f) Vanillin hydrochloric test

The extracts were treated with a few drops of vanillin hydrochloride reagent. The formation of pinkish red colour indicated the presence of tannins.

Figure 4.8 (a): 500 MHz LC-NMR spectrum of swertisin, isolated from extracts of *Tetractomia roxburghiana*, in D_2O-CH_3CN with solvent suppression

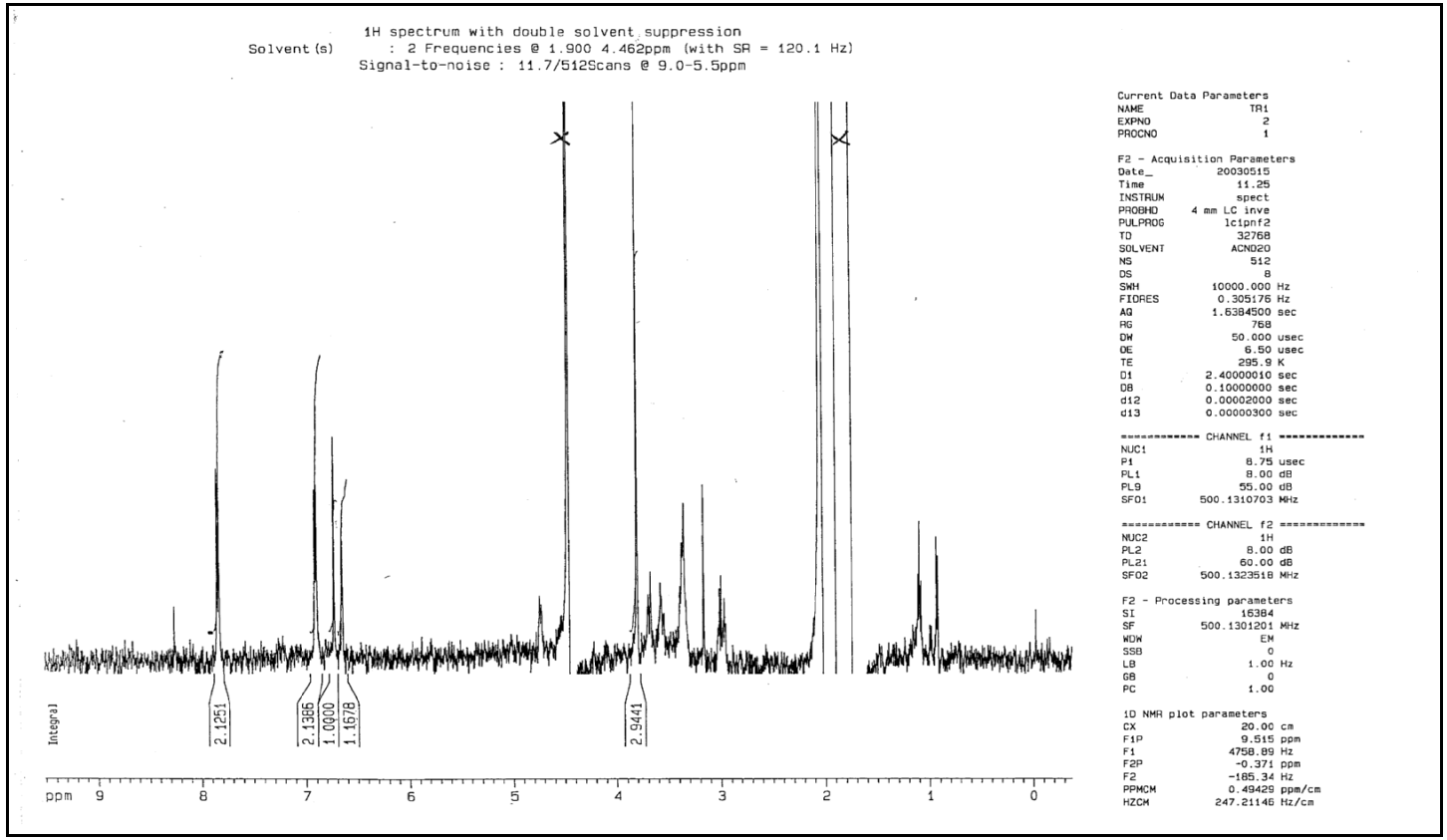

Figure 4.8 (b) 500 MHz LC-NMR spectrum of swertisin, isolated from extracts of *Tetractomia roxburghiana*, in D_2O-CH_3CN with solvent suppression

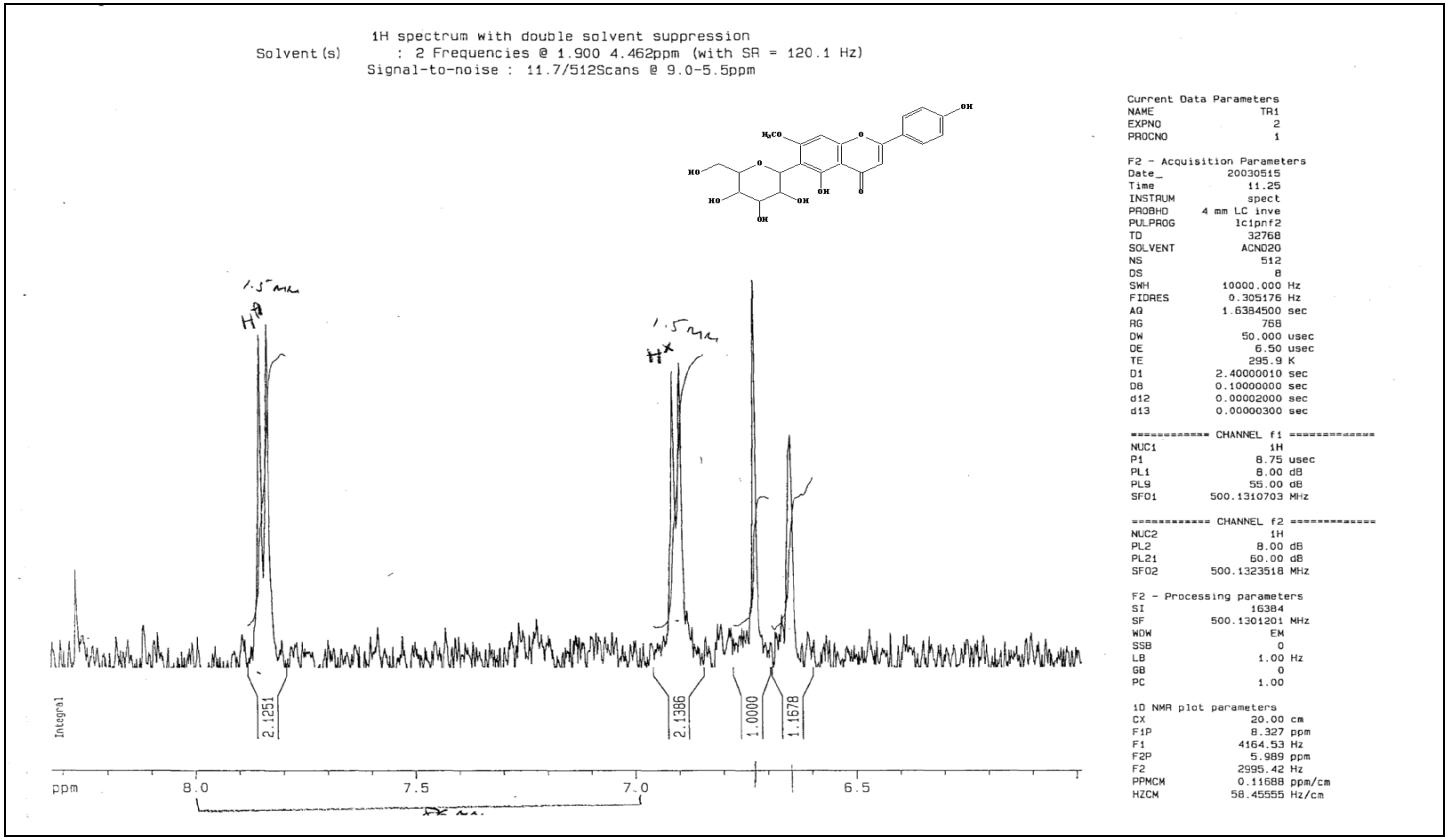

Figure 4.9 (a) Mass spectrum of TRF1(Gallic acid) isolated from methanolicextracts of *Tetractomia roxburghiana*

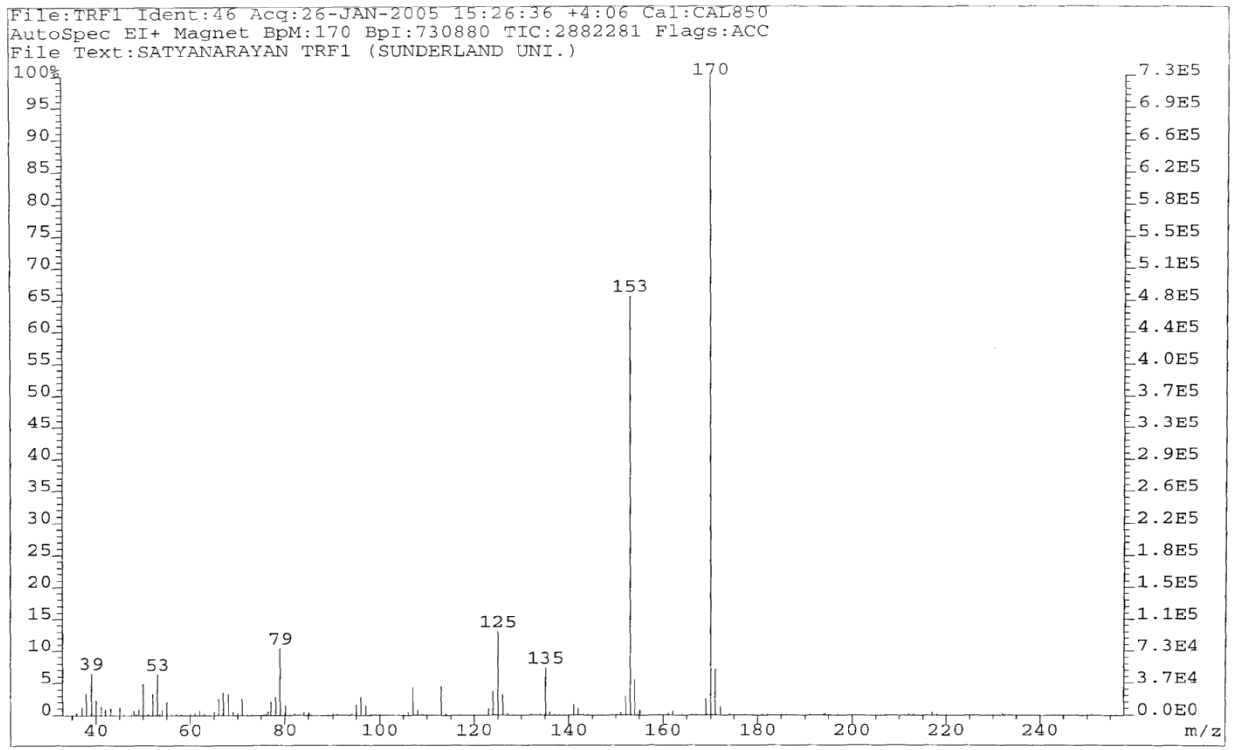

Figure 4.9 (b) : Mass of TRF1, library match of fragmentation pattern (Gallic acid) isolated from methanolicextracts of *Tetractomia roxburghiana,*

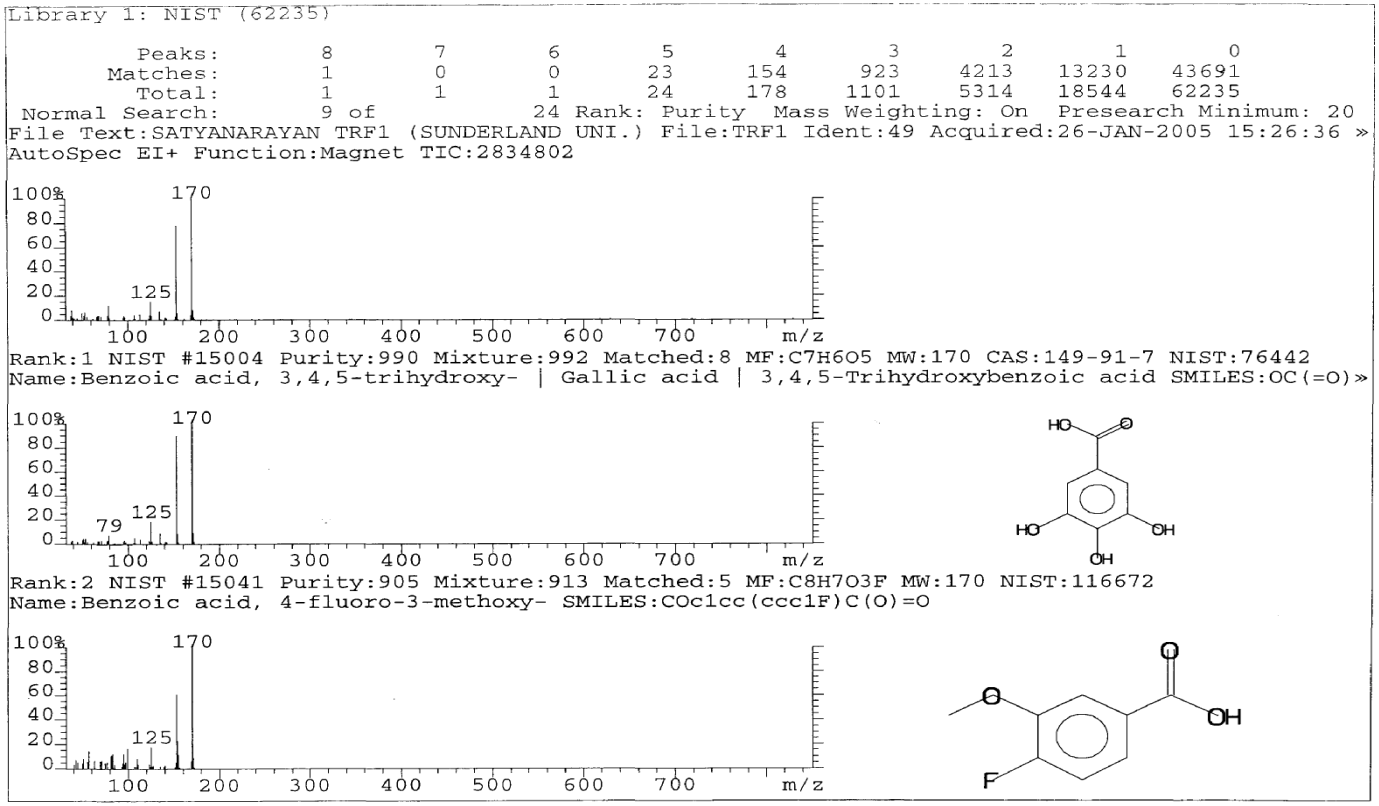

Figure 4.9 (c) ^{1}HNMR (300MHz, CDCl3) spectrum of TRF1(Gallic acid) isolated from methanolicextracts of *Tetractomia roxburghiana*

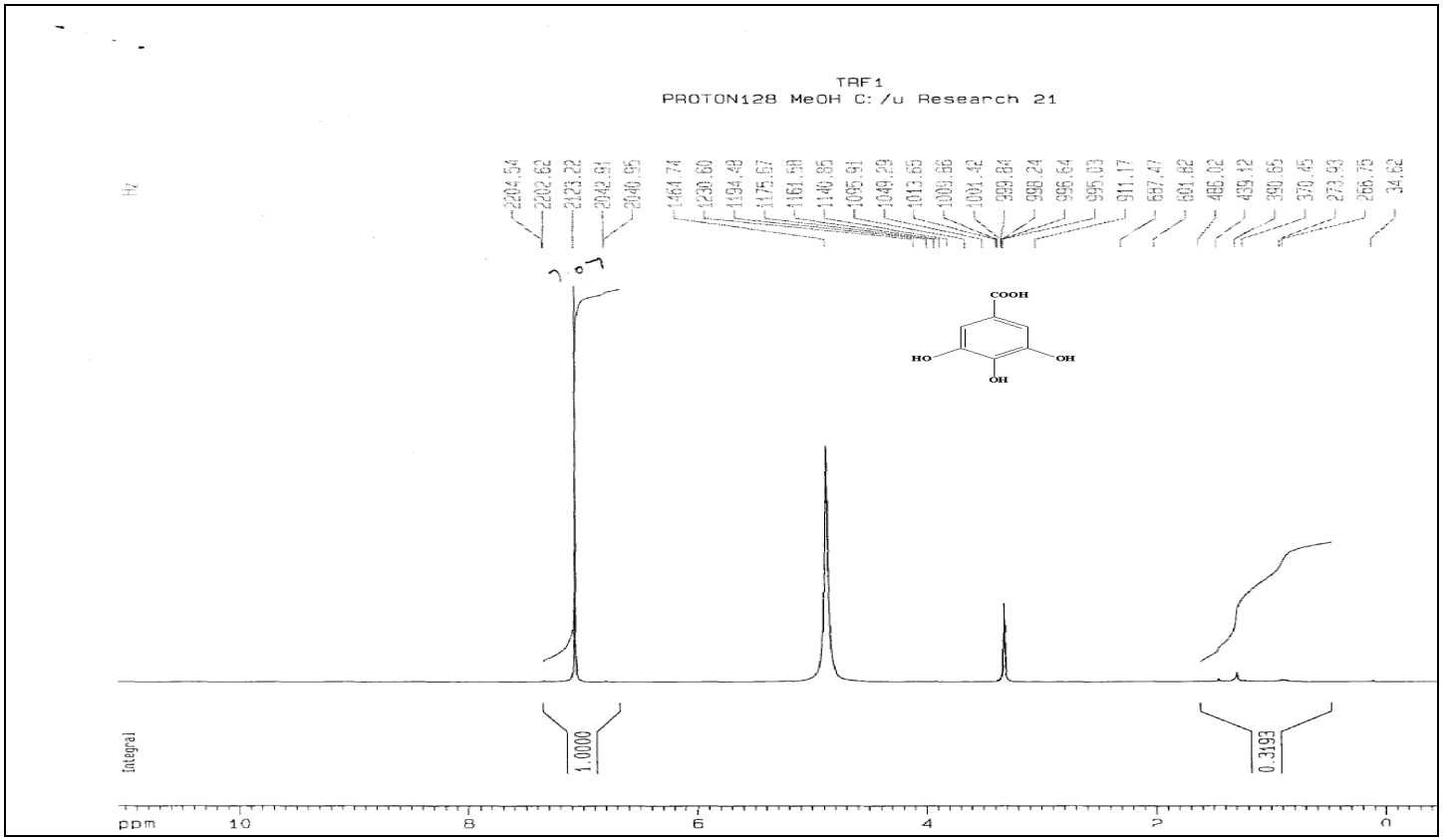

Figure 4.10 (a) : Mass of TRF2, α-asarone (library match of fragmentation pattern) isolated from methanolicextracts of *Tetractomia roxburghiana*

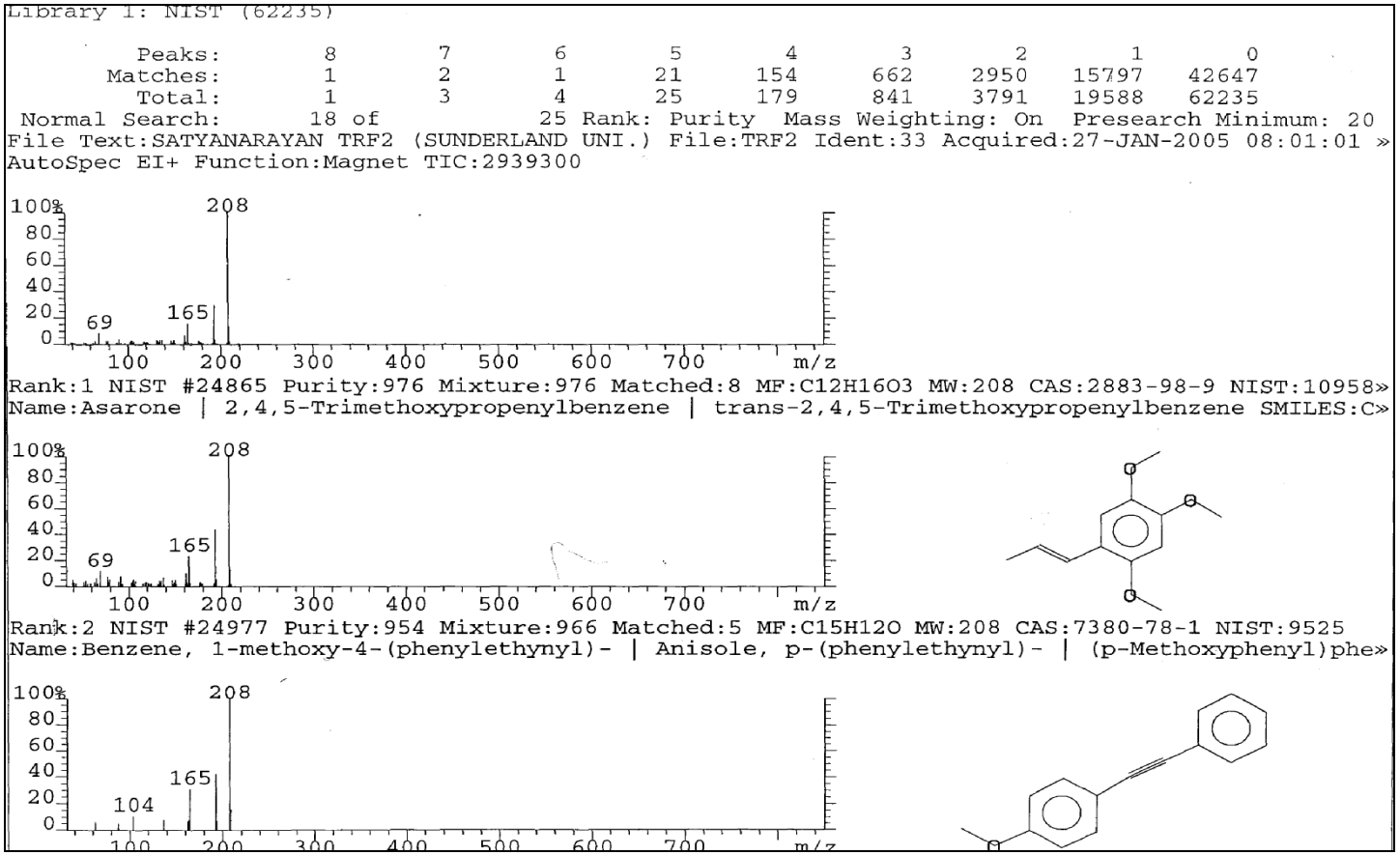

Figure 4.10 (b): Mass spectrum of TRF2 (α-asarone) isolated from methanolic extract of *Tetractomia roxburghiana*

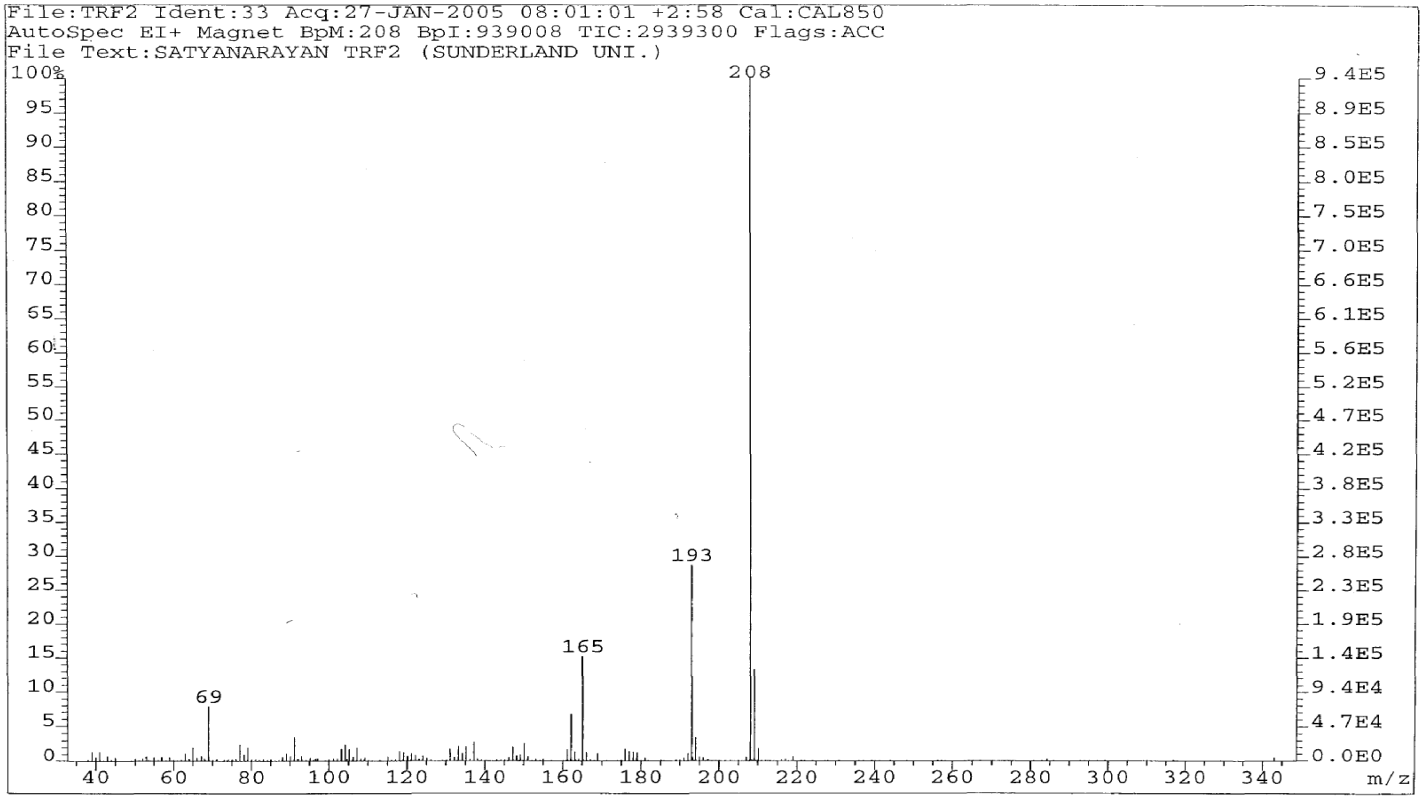

Figure 4.10 (c) : ^{1}HNMR (300MHz, CDCl3) spectrum of TRF2 (α-asarone) isolated from methanolicextracts of *Tetractomia roxburghiana*

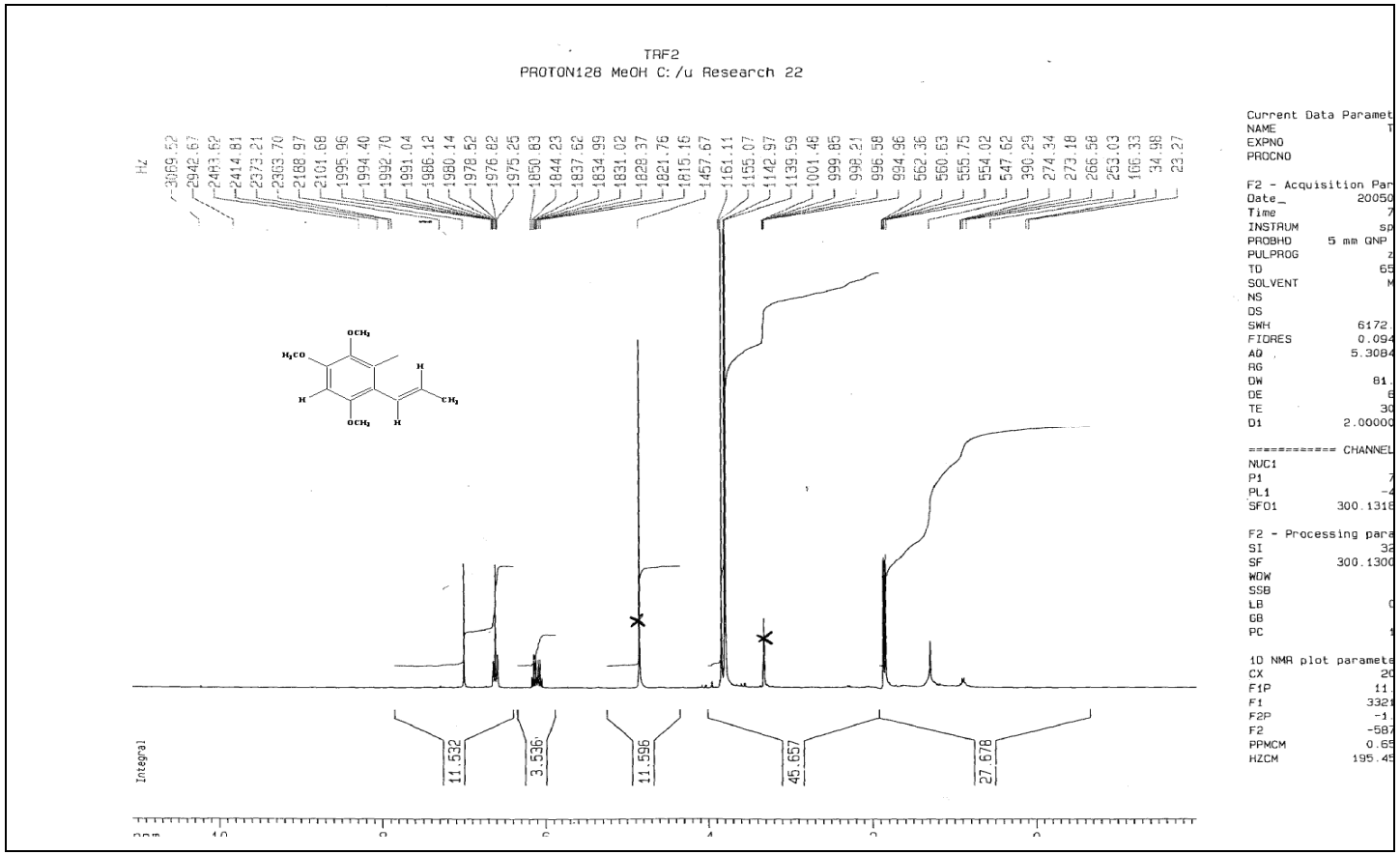

Figure 4.11 (a) Mass of TRF3, angelicin (library match of fragmentation pattern) isolated from methanolicextracts of *Tetractomia roxburghiana*

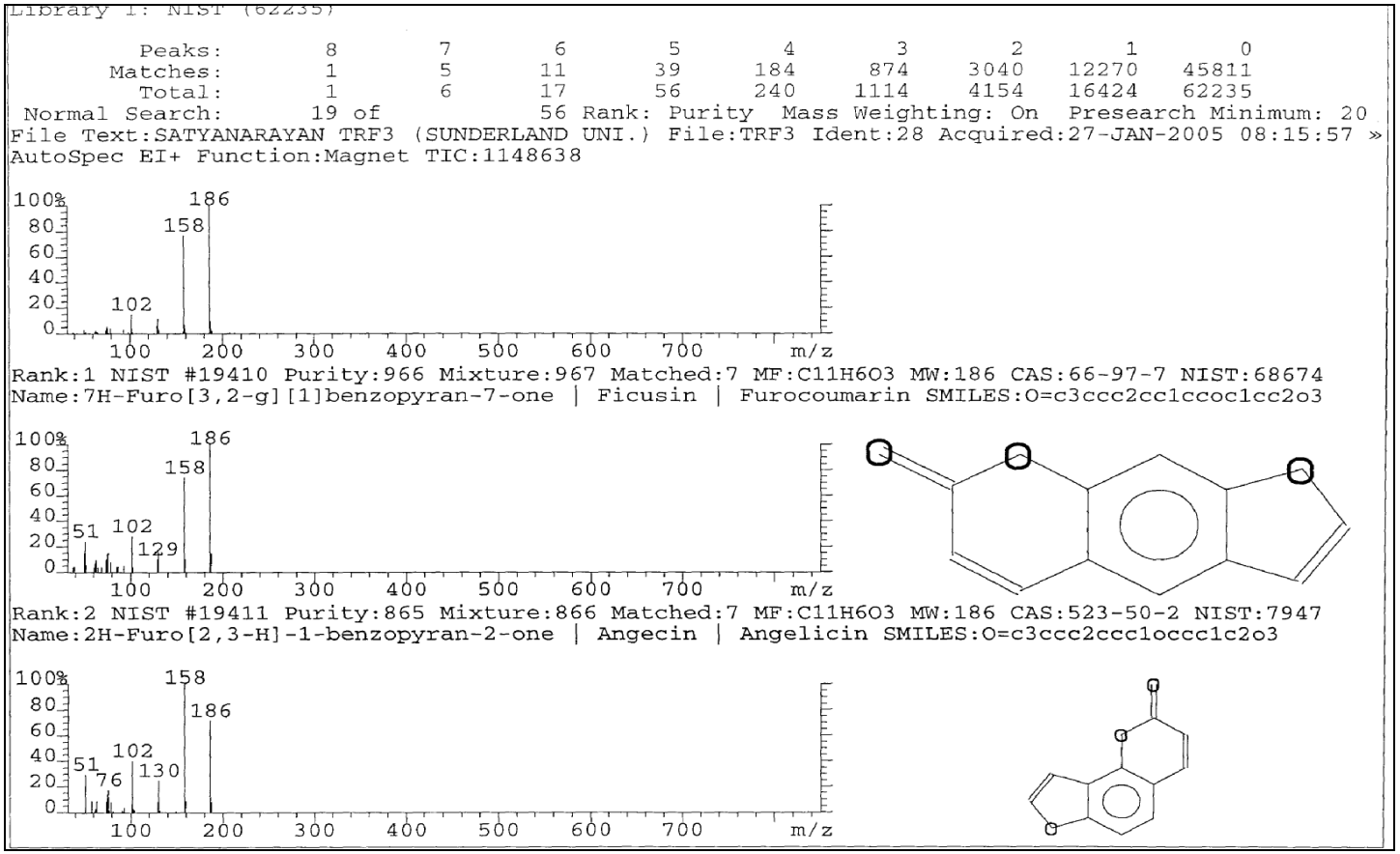

Figure 4.11 (b) ^{1}HNMR (300MHz, CDCl3) spectrum of TRF3 (angelicin) isolated from methanolicextracts of *Tetractomia roxburghiana*

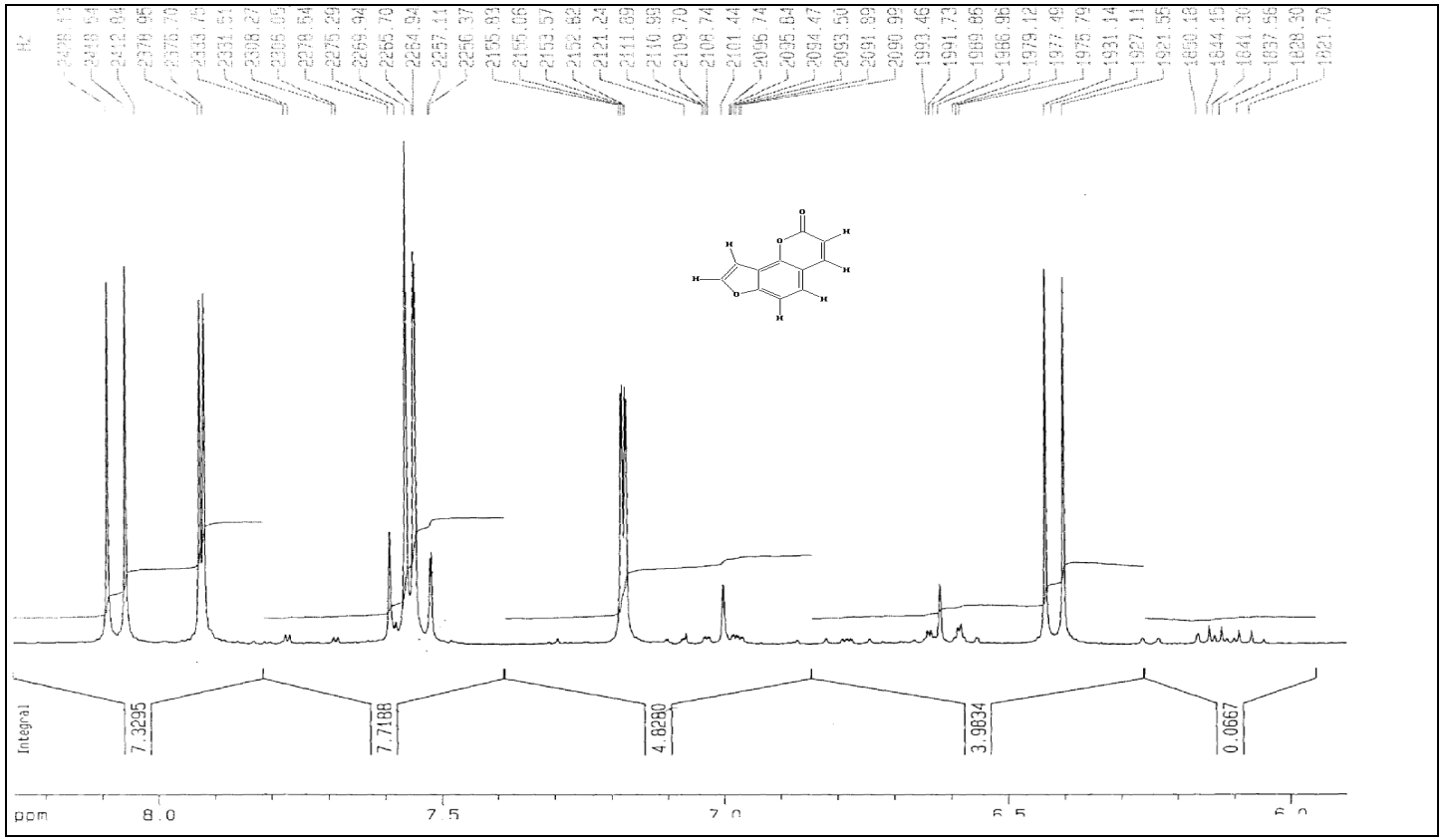

Figure 4.11 (c) ^{1}HNMR (300MHz, CDCl3) spectrum of TRF3 (angelicin) isolated from methanolicextracts of *Tetractomia roxburghiana*

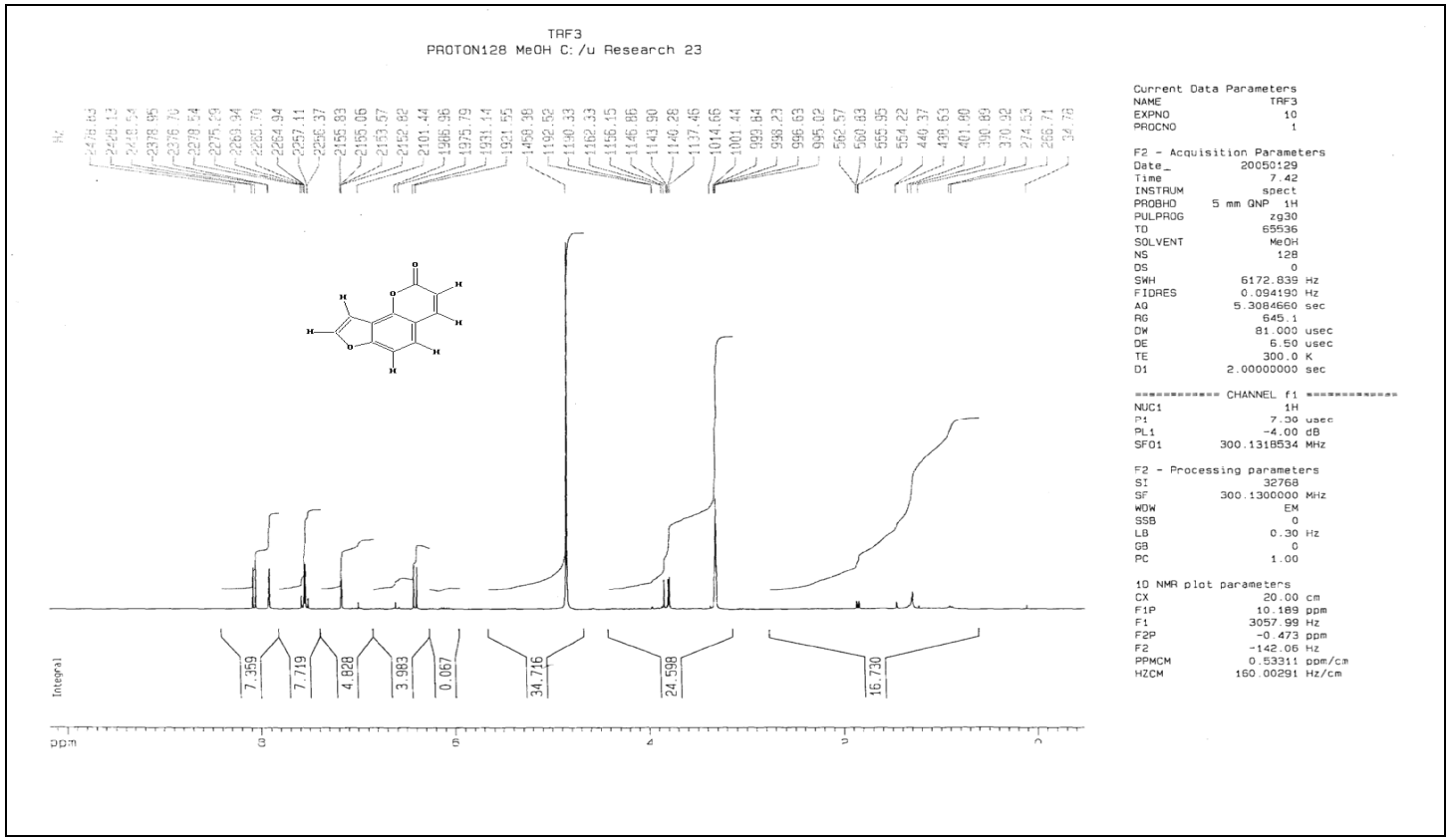

Figure 5.3 (a) ^{1}HNMR (300MHz, CDCl3) spectrum of GCZE 4(angelicin) isolated from methanolicextracts of *Glycosmis calcicola*

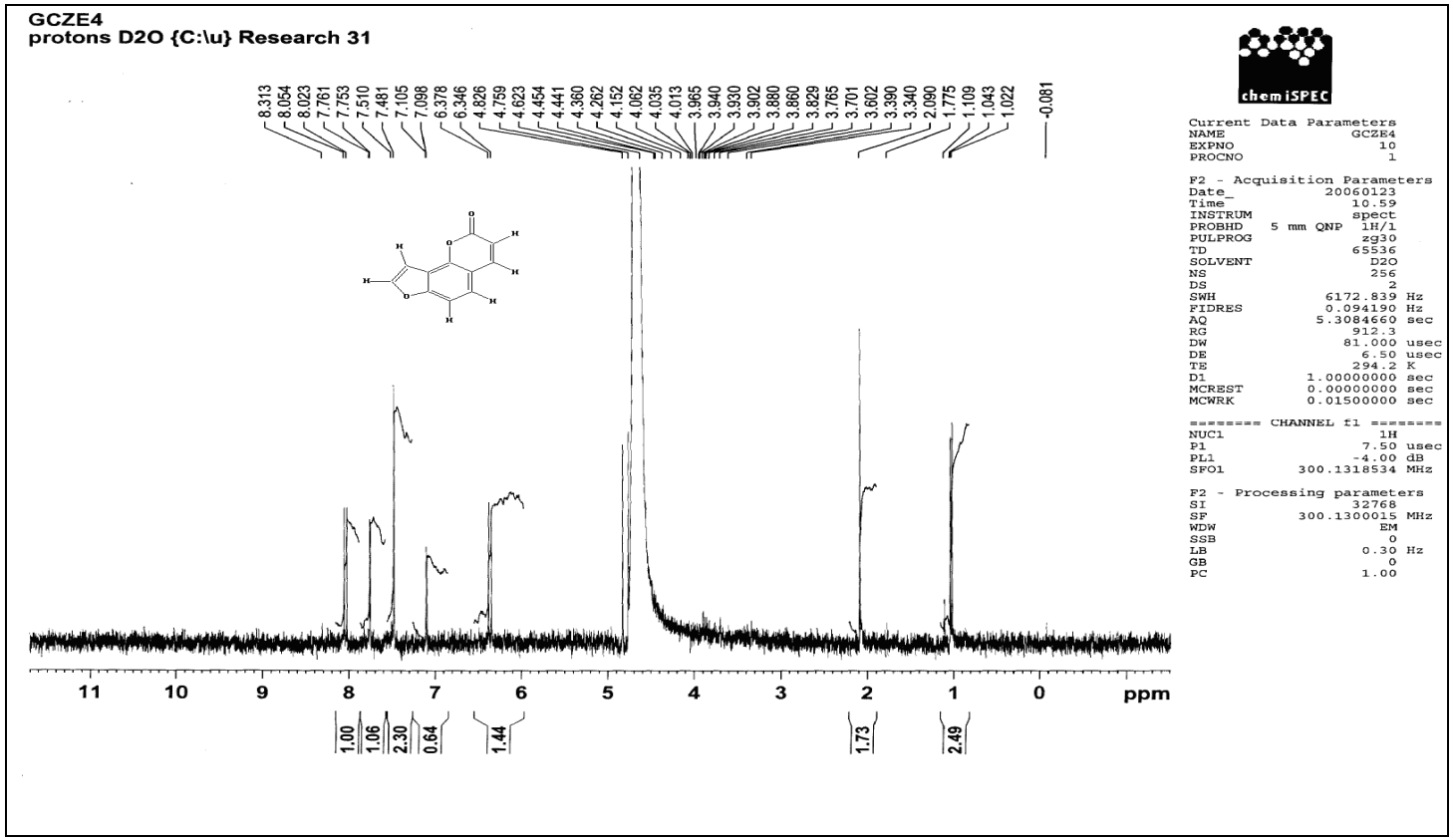

Figure 5.3 (b) ^{1}HNMR (300MHz, CDCl3) spectrum of GCZE 4(angelicin) isolated from methanolicextracts of *Glycosmis calcicola*

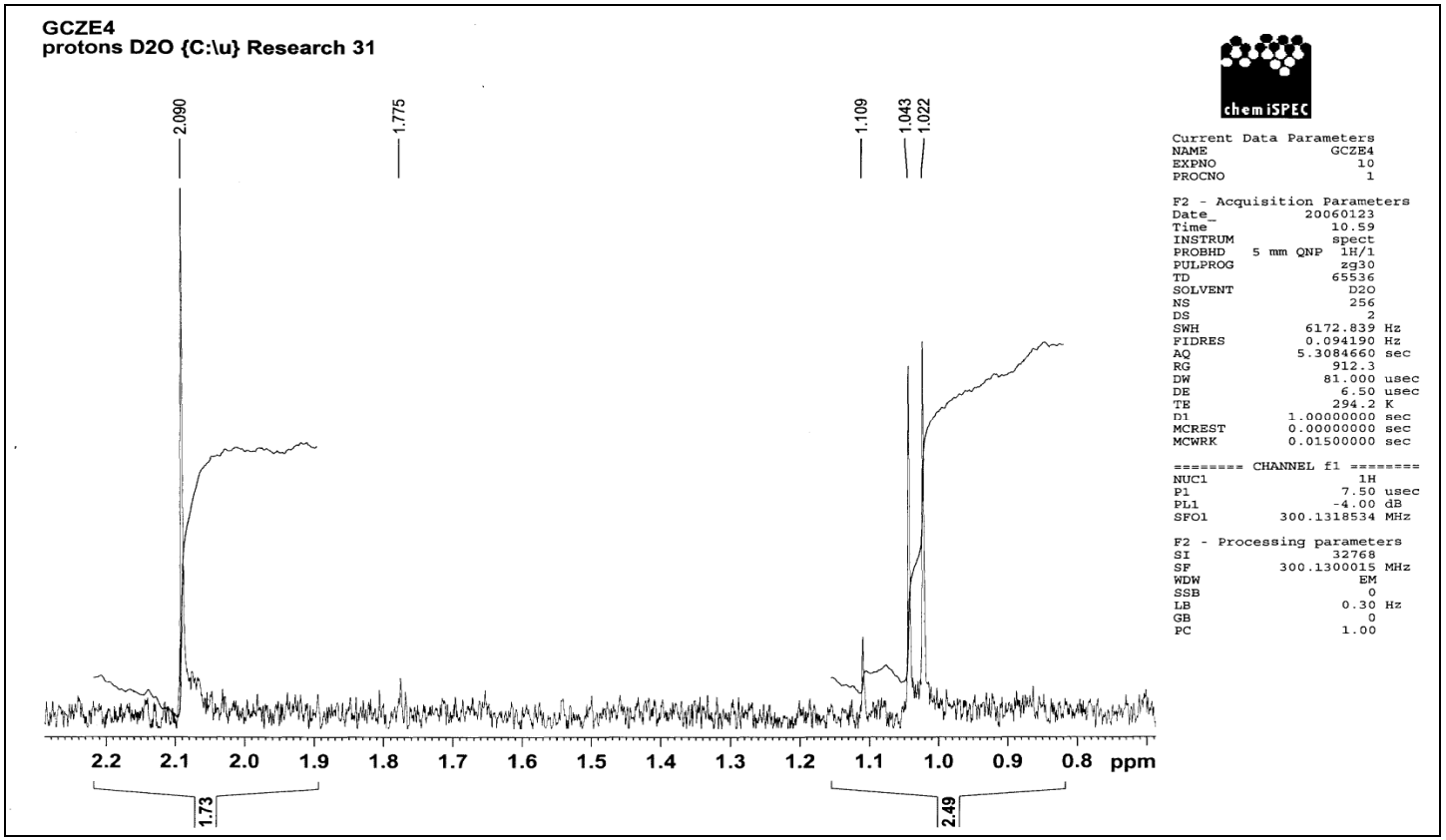

Figure 6. 3 (a): Mass spectrum of Peak 7, [2-(3, 4-dimthoxy-phenyl) 3, 7-dihydroxy-5-methoxy-chromen-4-one] isolated from (CE 3) fraction of methanolic extract of *Clausena excavata*

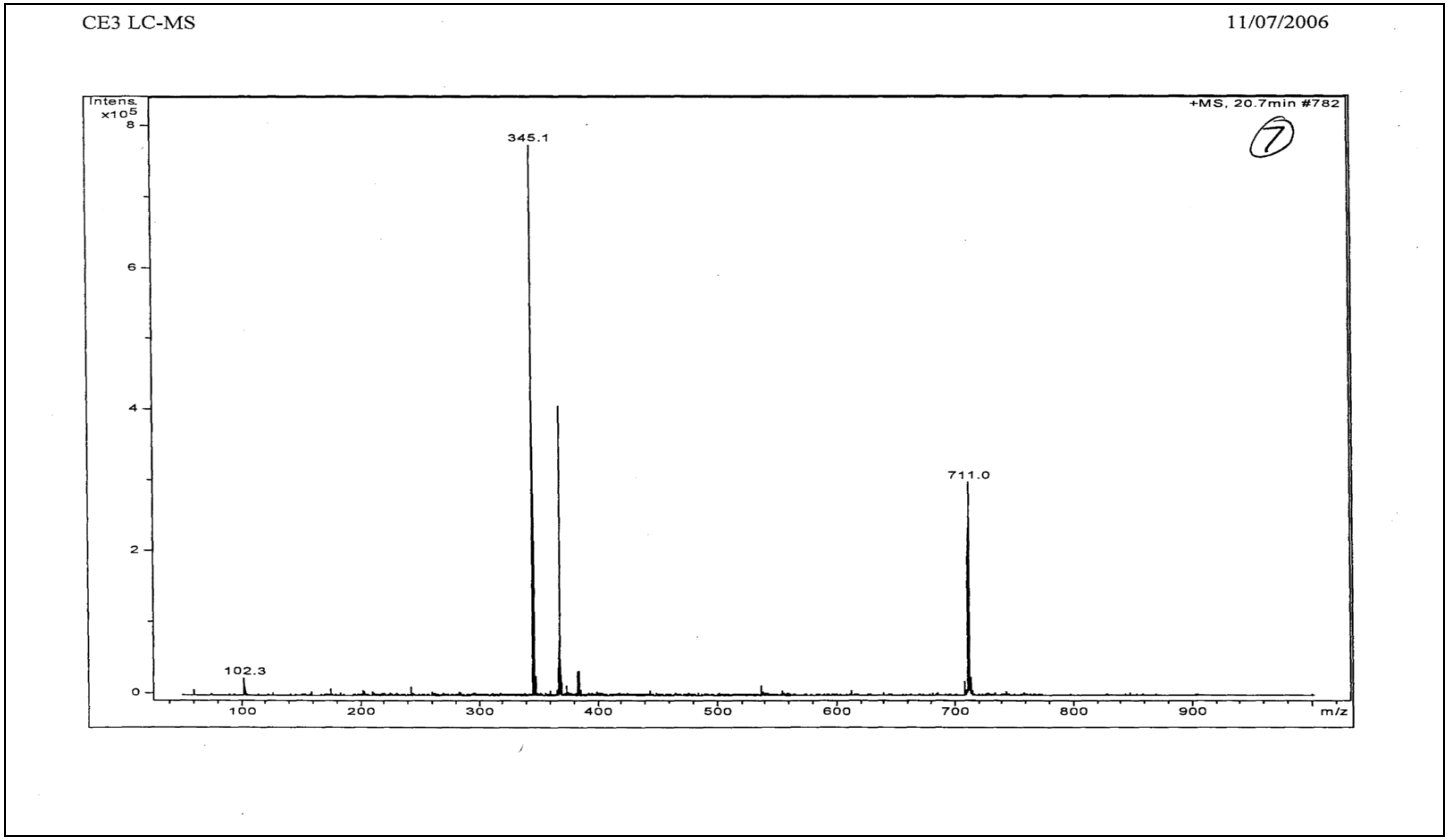

Figure 6.3 (b): ^{1}HNMR: (500 MHz, CD_3OD): of Peak 7 , [2-(3, 4-dimthoxy-phenyl) 3, 7-dihydroxy-5-methoxy-chromen-4-one] isolated from (CE 3) fraction of methanolic extract of *Clausena excavata*

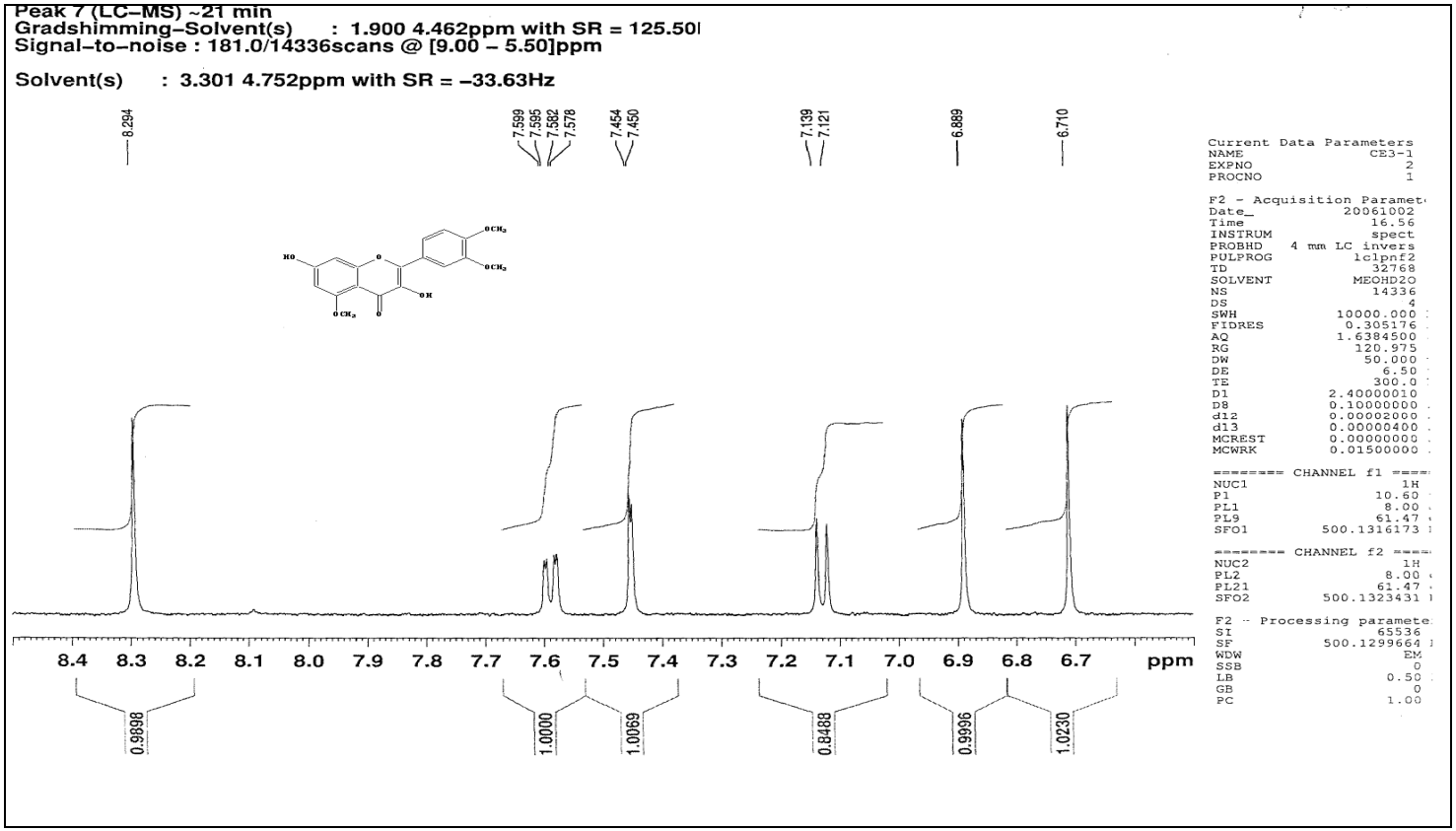

Figure 6.4 (a): Mass spectrum of Peak 8 [2-(3, 4-dimethoxy-phenyl)-5, 7-dimethoxy-chromen-4-one] identified from (CE 3) fraction of methanolic extract of *Clausena excavata*

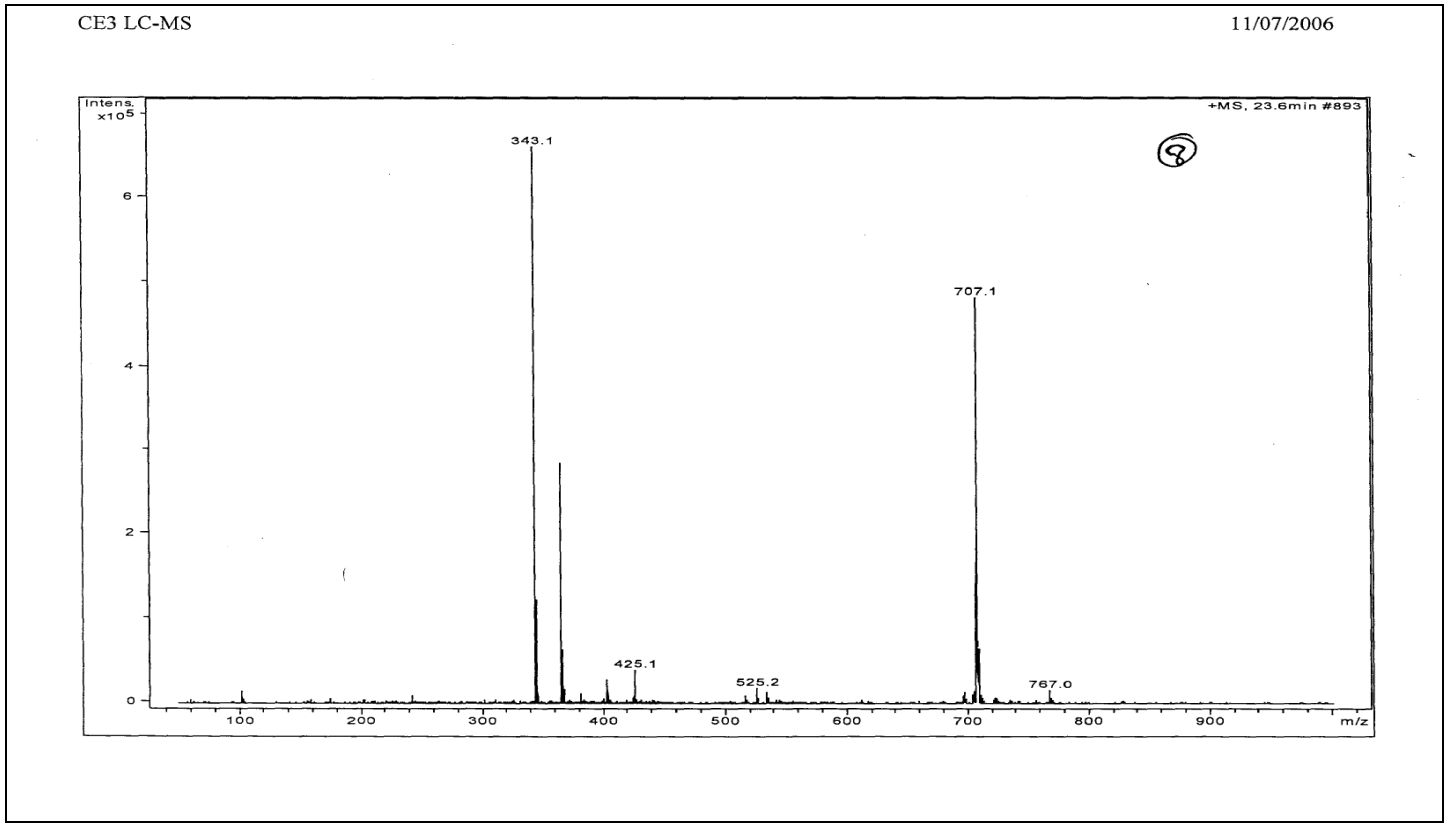

Figure 6.4 (b) ^{1}HNMR: (500 MHz, CD_3OD): of Peak 8 [2-(3, 4-dimethoxy-phenyl)-5, 7-dimethoxy-chromen-4-one] identified from (CE 3) fraction of methanolic extract of *Clausena excavate*

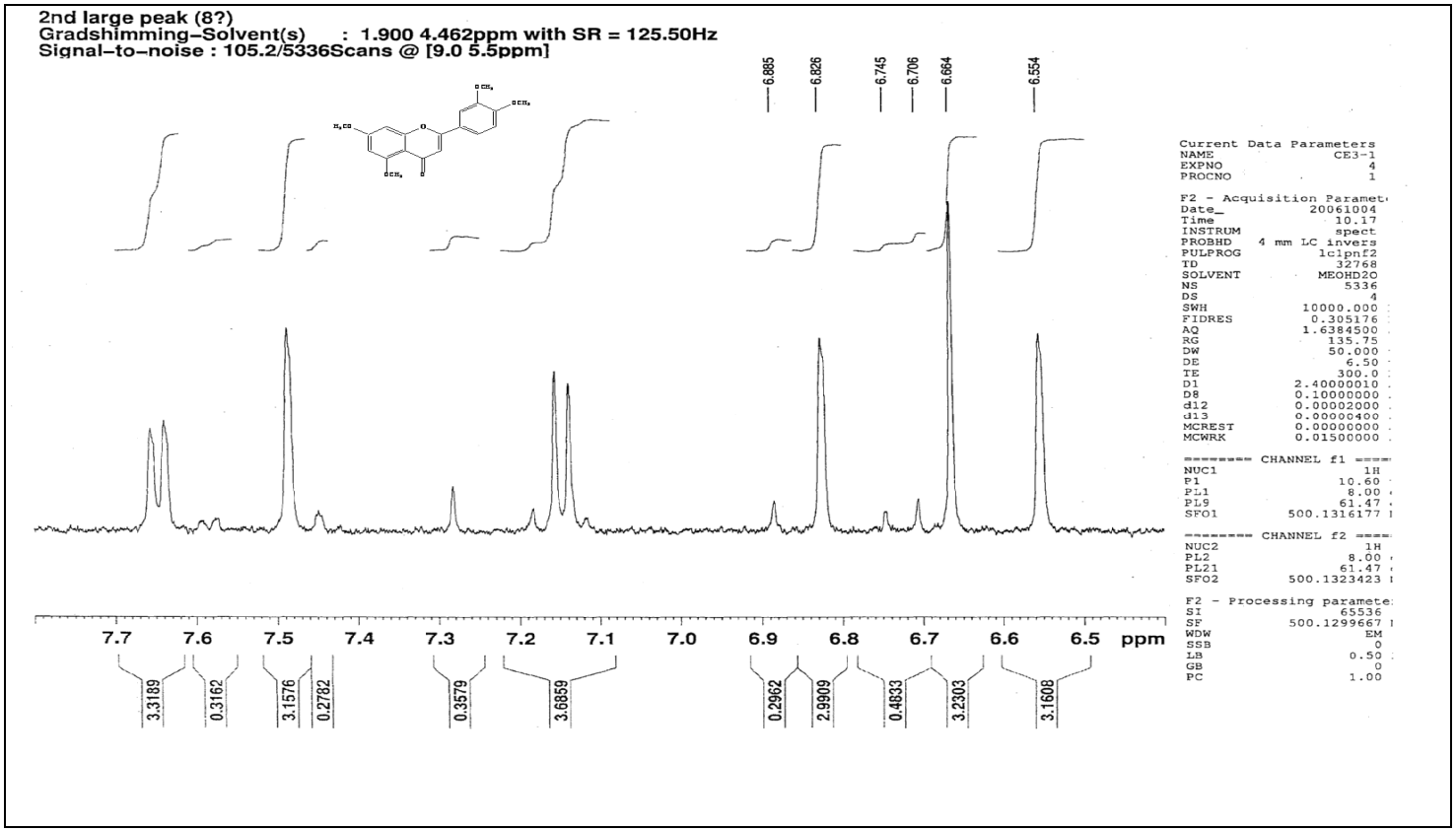

Figure 6.5 (a): ^{1}HNMR: (500 MHz, CD_3OD): of CE 7 identified from methanolic extract of *Clausena excavate*

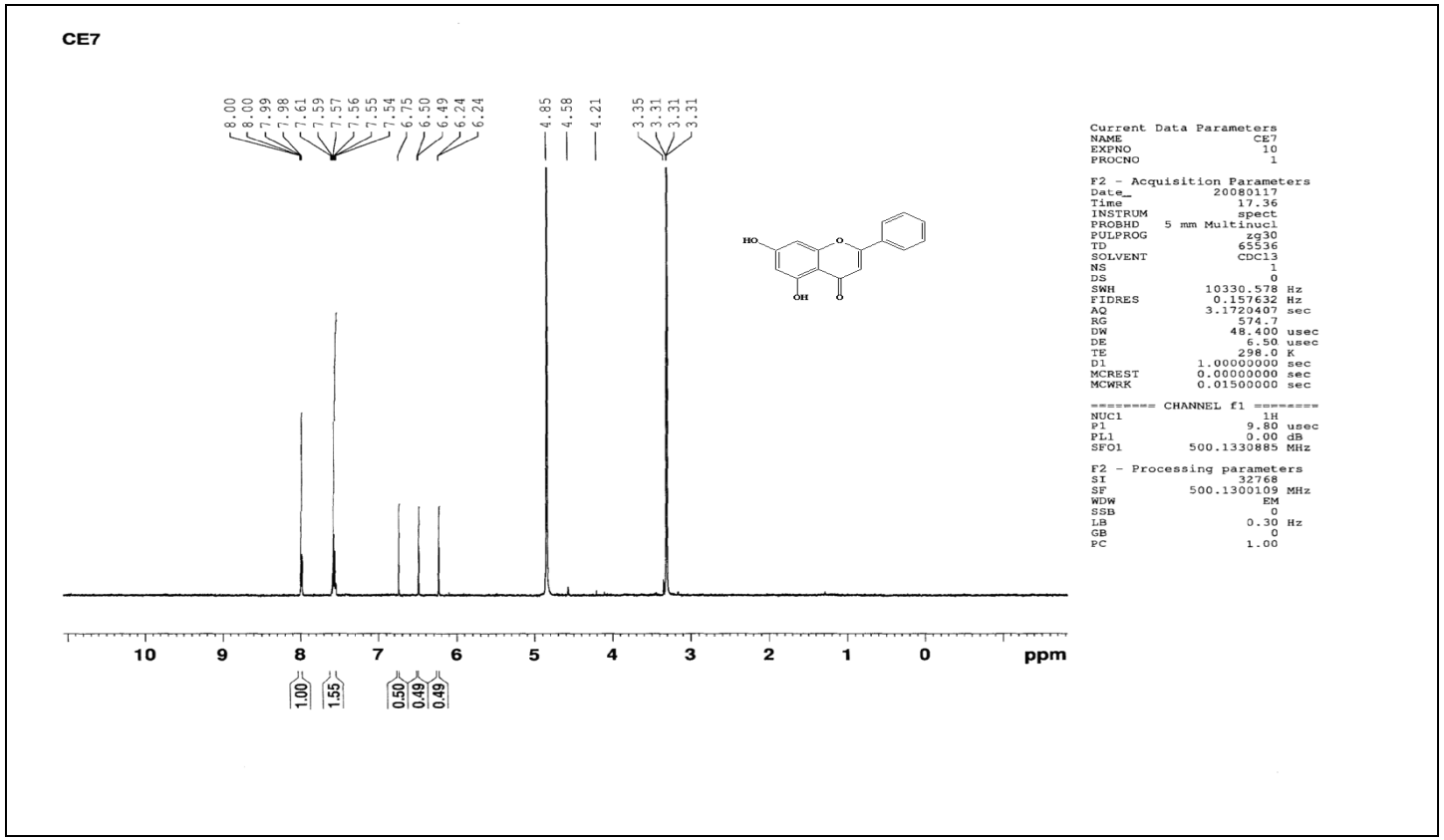

Figure 6.5 (b): ^{1}HNMR: (500 MHz, CD_3OD): of CE 7 (Chrysin) isolated from methanolic extract of *Clausena excavate*

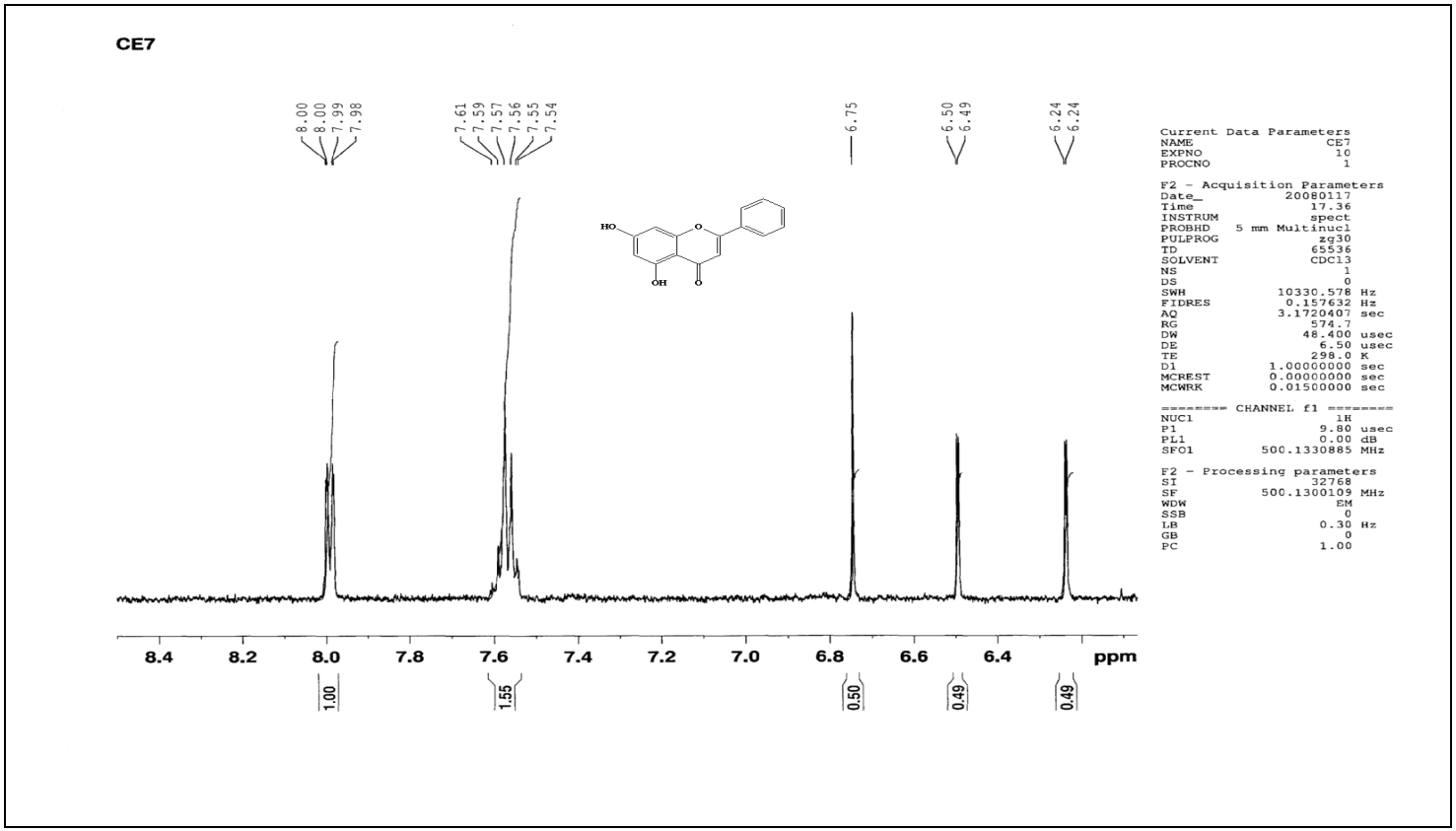
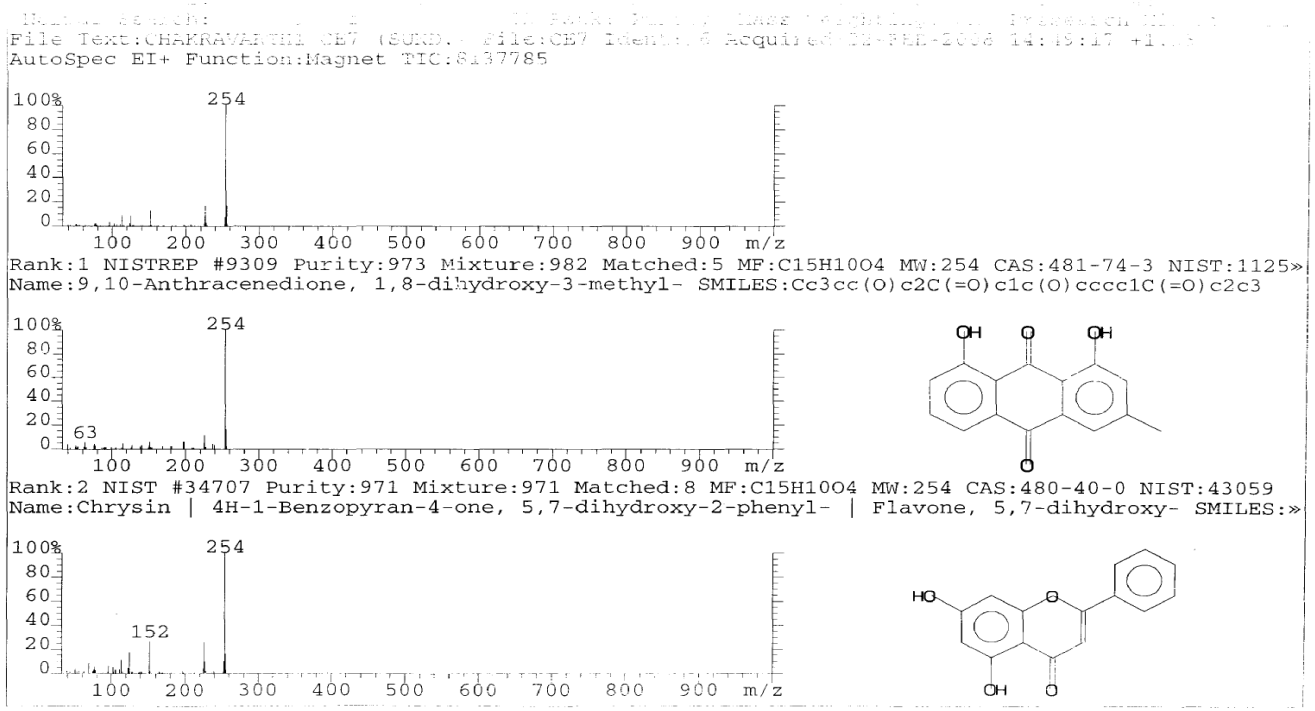
Figure 6.5 (c): Mass of CE 7 (Chrysin) (library match of fragmentation pattern) isolated from methanolicextracts of *Tetractomia roxburghiana*