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Research Article

Isolation and Identification of Steroid Triterpenoids from the Polar and Non-Polar Fractions of *Caralluma attenuate* (Wight) Roots.

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ABSTRACT
The present investigation aimed at to determine the probable phytochemical components from the non-polar to polar extracts of *Caralluma attenuata*. The compound extracted was subjected to UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT and SEPT. The resultant spectral data was used to elucidate the compound. The first compound was named as CAR-1 which gave a positive result with Liebermann-Burchard reaction. Spectral data showed that it contains thirty eight carbon resonances, side chain contains ten carbons and one terminal double bond, primary hydroxyl groups, and eight methyls, fifteen methylenes, ten methynes and five quarternary carbons. From the mass spectrum of CAR-1 the peak at m/z 555 $(M+H)^+$ is considered as a base peak with molecular formula $C_{38}H_{66}O_2$. The second compound was named as CAR-2 which is also a white crystalline solid and gave a positive result with Liebermann-Burchard reaction. Spectral data showed that CAR-2 contains 38 carbon resonances. In that side chain contains ten carbons which has α,β-unsaturated alcohol. From the mass spectrum of CAR-2 the peak at m/z 570 is considered as the molecular ion peak with molecular formula $C_{38}H_{66}O_3$. The results indicating the compounds isolated are steroid/terpenoid.

Keywords:

INTRODUCTION

Phytochemical characterization of plant material is important as it relates to the therapeutic actions. It is perhaps obvious that different species of plant would have different chemical constituents. However these differences can extent to different varieties or even the same variety grown in different location or harvested at a different time. Different parts of plant such as leaves, bark, seeds, roots, flowers and pods can also have different active constituents. The genus *Caralluma* belongs to the family Asclepiadaceae.*Caralluma spp* is a succulent plant native to Asia, the Mediterranean, and Africa[1].Literature survey reveals that *Caralluma* species are a good source of steroids and their glycosides. Not many *Caralluma* (not even a dozen!), have been investigated, earlier for their chemical constituents or for biological activity, the world over. All *Carallumas* investigated so far, yielded a number of new steroidal glycosides. The latest example is the isolation of twenty new steroidal glycosides from *C. negevensis*. Some of the steroidal glycosides isolated were obtained in good yields. For example, Carumbelloside-I was obtained from *C. umbellata*, in a yield of 0.3 – 0.5% w/w. This enabled semi-synthetic reactions to be carried out and biological screening also was made possible. For example, Carumbelloside-I was converted into 14(-Hydroxy progesterone, in just two steps. This chemical exhibited anti-fertility and cytotoxic activities, when they are tested. Recently, five new steroidal pregnane glycosides were isolated from *C. stalagmifera* and three known compounds from n-butyl alcoholic fraction of alcoholic extract of *C. indica*[2]. In view of the above-mentioned facts, phytochemical work on *Caralluma* appeared worthwhile tasks. It is likely that some of them constitute a very good source of steroids and might yield them in higher amounts than the plants examined in the past. It is also possible that, some of these compounds have interesting biological actions. Oxypregnane glycosides are reported to possess cytotoxic activity[3,4]. The chemical investigation of *C. adscendens* var *adscendens* one flavonoid glycoside and three steroidal / triterpenoidal glycosides were isolated. A number of nonpolar setroidal glycosides were isolated from the nonpolar (CHCl3 or ether) fractions of other Carallumas like *C.resseliana*, *C.penicillata* and *C.negevensis.* In view of this it was felt that the nonpolar i.e., hexane, benzene, acetone fraction of *Carallumas* should be investigated for the presence of Steroid / triterpenoids. The glycosides were isolated from the polar fractions i.e., ethylacetate and n-butyl alcohol fractions. In the non-polar i.e., hexane, benzene fractions glycosides were not present. But steroid / triterpenoids are present. All along our attention was on the isolation and identification of steroid / triterpenoids from the non-polar

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Table 1: Fractions of the respective extract of *C. attenuate* root.

Fractions	Eluent	Nature of the product	Spot no.	Rf value
1-4	Benzene	Yellow waxy material	No spot	Not examined further
5-11	Benzene	White solid	1	0.437
12-27	Benzene	White solid	2	0.30
27-30	Benzene: Methanol (95:5)	Pale brown residue	-	Not examined further
31-36	Benzene : Methanol (90:10)	Pale brown residue	-	Not examined further
37-50	Methanol	Pale brown residue	No Characteristic spot	Not examined further

fractions of different *Carallumas*. No attempt was made to extract the compounds from the roots of *C. attenuata* starting from n-hexane, benzene, acetone, methanol i.e., the extractions were carried out from the non-polar end ~~and~~ to the polar end. An attempt was made to extract the compounds from *C. attenuata* root using nonpolar solvents like n-hexane, benzene, acetone with a view to isolate compounds from different extracts and assign the structures using Mass, ^{1}H NMR, IR, ^{13}C NMR spectra and to search the biological activity of the compounds.

MATERIALS AND METHODS

Chemicals used

Solvents like n-Hexane (SQ) (Qualigen) Benzene (SQ) (Qualigen), Benzene (AR) (Finar), Acetone (SQ) (Qualigen), Acetone (AR) (Finar), Methanol (SQ) (Qualigen) were used and were purified according to the procedures given[5]. Silicagel 60-120 and 200 mesh, silica gel G (ACNE) were used for column and thin layer chromatography and sulfuric acid is used as spray reagent.

Instruments used

The ^{1}HNMR Spectra on amx 400 MHz, ^{13}C NMR Spectra, DEPT and SEFT spectra were recorded on amx 100 MHz. The mass spectra were recorded on esquire 3000 plus (ESI type). We got all the spectral data from Instrumentation and NMR facility, Indian Institute of Science, Bangalore.

Extraction and Isolation

Chemical examination of the roots of *C. attenuata* plant Plant material was collected from Tirumala forest of Seshachalam hills, Tirupati, Chittoor District, Andhra Pradesh and has been identified by Dr K. MadhavaChetty, Department of Botany, Sri Venkateswara University, Tirupati, India. The roots were carefully separated, washed and were air dried for 60 days under shade and powdered in a wearing blender using a mixer and passed through a 24-mesh sieve. The powder was stored at 4^{0}C for further analysis. About 100 gm *Caralluma attenuata* root powder was extracted with n-Hexane (1.5L) in Soxhlet extractor at reflux temperature for 18 hours. The n-Hexane extract of the roots of was filtered and then concentrated on water bath to give a green semi solid (18 gm). This was dissolved in minimum amount of benzene (100 ml). The plant material was then re-extracted with benzene (1L), in Soxhlet extractor at reflux temperature for 10 hours. The Benzene extract was filtered and then concentrated on water bath and yielded a greenish semi-solid, weighed about 3 gm. The plant material was then re-extracted successively with acetone (1L), methanol (1L) in the Soxhlet extractor at reflux temperature. These extracts were filtered and concentrated on water bath and examined separately. The acetone extract was filtered and concentrated on water bath yielded a greenish semi solid weighed about 2gm. The methanol extract was filtered and concentrated in water bath yielded a semisolid weighed about 2.5gm.

Detection by TLC

TLC plates were made by using a homogenous suspension of silica gel prepared by mixing 40 g of 200 mesh, silicagel G (ACNE) in about 85 ml distilled water. The suspension was then poured into **S**tahl TLC (UND PLAN model) spreader, which was adjusted to 0.25 mm thickness. Carrier plates (20 cm x 5 cm) of the same thickness were laid in a row on a template and coated in a single passage of the spreader over them. These plates were left on the template for air drying the transparency of the layer disappeared and dried at 110°C for 30 min and kept in desiccators.

Chemical Tests: The following chemical tests were carried out on different prepared extracts to know the nature of the compounds present in them.

Liebermann-Burchard Test

The extract / compound was dissolved in acetic anhydride, heated to boiling, cooled and then one ml of concentrated Sulphuric acid was added along the sides of the test tube. A positive reaction is indicated by appearance of red, pink or violet colour at the junction of the liquids[6].

Salkowski Test

The extract / compound was dissolved in Chloroform Sulphuric acid and then1ml of concentrated Sulphuric acid was added. Red colour precipitate indicates the presence of steroids/Triterpenoids[7].

n-HEXANE EXTRACT

The semi-solid (18 gm) obtained from n-Hexane extract. The concentrated n-Hexane extract was subjected to thin layer chromatography using silica gel and performed Liebermann Burchard test as mentioned earlier.

BENZENE EXTRACT

The Benzene extract was concentrated on water bath, yielded a greenish semi-solid (3 gm). The compound was used Liebermann Burchard test and TLC was performed for this extract as mentioned earlier.

ACETONE EXTRACT

The acetone extract was filtered and concentrated on water bath yielded a greenish semi solid (2gm). TLC was performed for this extract as mentioned earlier.

METHANOL EXTRACT

The methanol extract was filtered and concentrated in water bath yielded a semisolid (2.5g). TLC was performed for this extract as mentioned earlier. The n-Hexane, Benzene, Acetone and Methanol extracts were spotted at 2 cm from the edge of the TLC plate. The chromatogram was developed in a mixture of suitable solvent systems

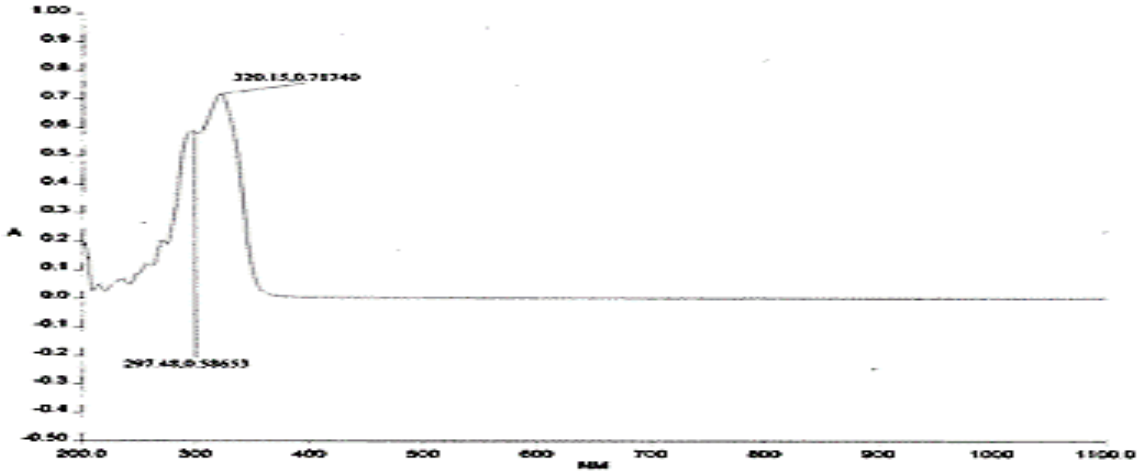


Figure 1: The UV spectrum of CAR-1.

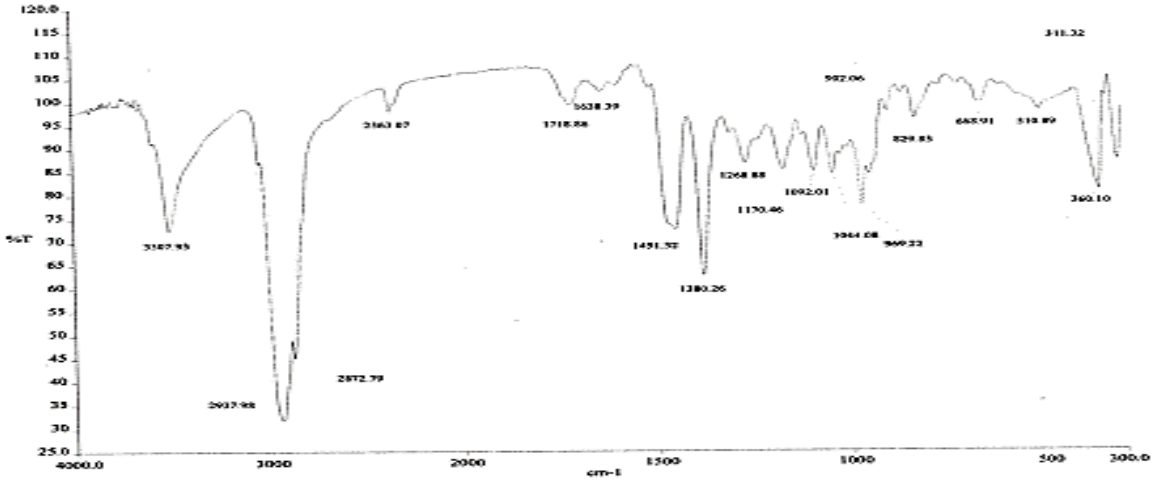


Figure 2: The IR spectrum of CAR-1.

(Benzene: ethyl acetate 97:3; Chloroform: methanol 90:10; Ethyl acetate: methanol: water 81:11:8; Chloroform: methanol 70:30) and dried at room temperature. The spots were visualized with UV light at 365 nm. The dried TLC plates were then sprayed with Methanol Sulphuric acid reagent. The Rf values of the coloured spots were recorded.

Column Chromatography of Hexane extract

Silica gel column chromatography was carried out with Hexane extract of *C. attenuata* root for the isolation of the compounds. n-Hexane extract (180gm) was subjected to silica gel column chromatography (10-40µL of 150 gm) and the column was eluted successively with 2 L Benzene followed by Benzene: Methanol (95:5), Benzene: Methanol (90:10) and altogether 50 fractions (50mL each) were collected.

RESULTS AND DISCUSSION

When solvent extracts of n-Hexane, Benzene, Acetone and Methanol was tested for Liebermann Burchard test, a characteristic pink colour was developed with n-Hexane and Benzene but not with Methanol and Acetone extracts. Both n-Hexane and Benzene extracts showed similar kind of spots on TLC but no spot with the other two extracts, and there was no further examination with these extracts (Table-1). Silica gel column chromatography was performed with n-Hexane extract and no spot has been observed from fractions 1-4. Fractions 5-11 on concentration followed by crystallization from benzene gave a white crystalline solid and it gave pink colour with Liebermann-Burchard test indicating that it might be a Terpenoid/Steroid. It was designated as CAR-1. Fractions 12-27 on concentration followed by crystallization from benzene gave white crystalline solid. It was designated as CAR-2. It gave positive Liebermann-Burchard tests. It may be another Steroid / Terpenoid.

Structural elucidation of CAR-1

CAR-1 is a white crystalline solid melts at 144^0C. It gave a positive Liebermann-Burchard reaction, Salkowski test. The colour of the spot on TLC plate resembled to that of a Steroid / Terpenoid.

U.V. Spectral data of CAR-1

The U.V. Spectrum is presented in Fig.1 It showed maximum at 320.15 and 297.48 nm absorption in U.V. region.

IR Spectral data of CAR-1

IR data of CAR-1: 3507.93, 2937.98, 2872.79, 2363.07, 1718.86, 1451.32, 1380.26, 1268.88, 1170.46,1092.01,1044.08, 969.22, 829.83,668.91,510.89,360.10,311.32. The IR spectrum is presented in Fig. 2 and absorption peaks were presented in Table-2. The IR spectrum of CAR-1 showed a peak at

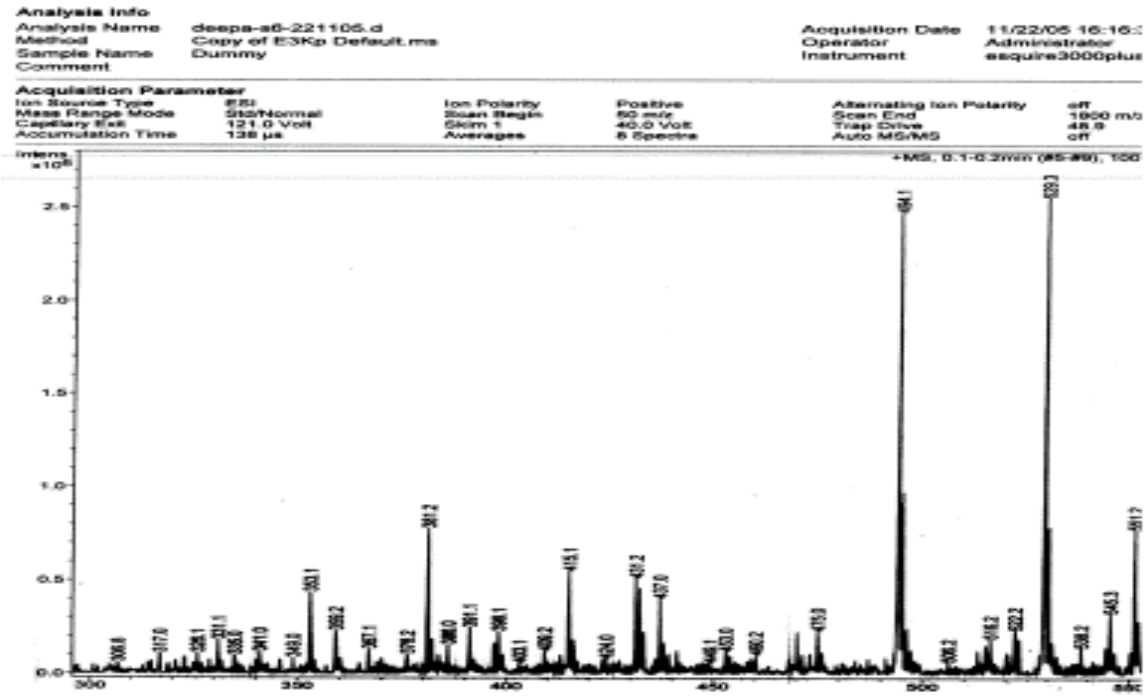


Figure 3: Mass spectrum of CAR-1.

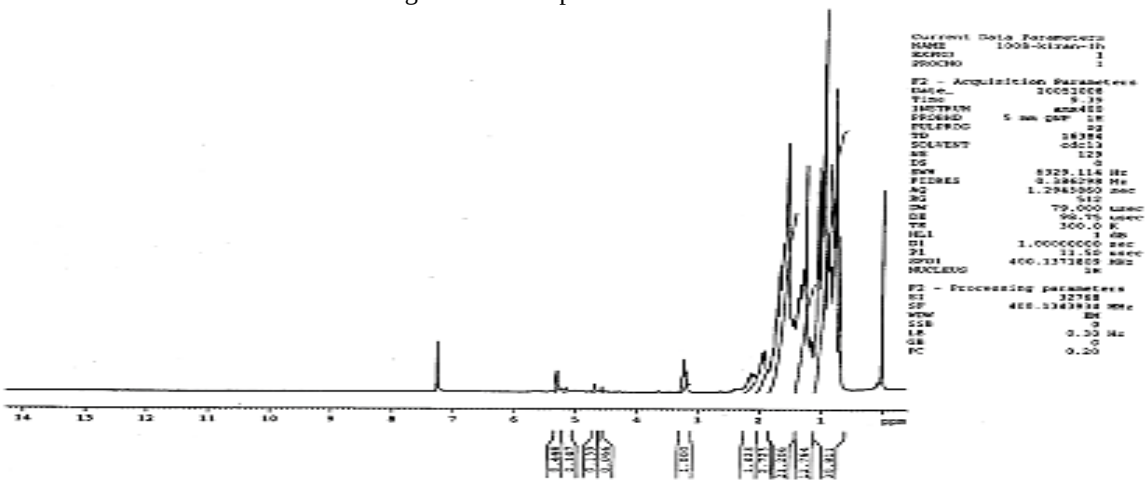


Figure 4: ^{2}H NMR of CAR-1.

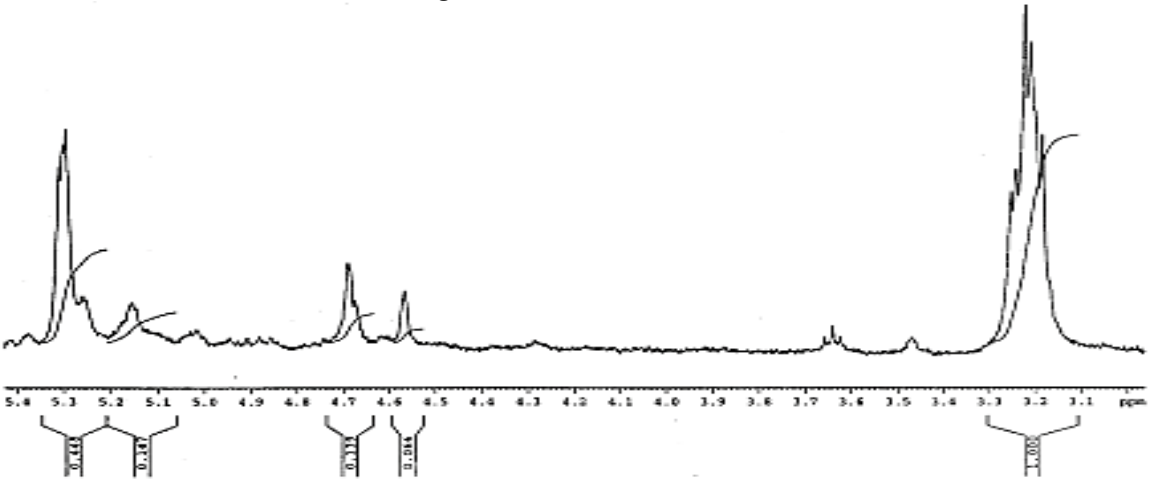


Figure 4a: ^{2}H NMR of CAR-1.

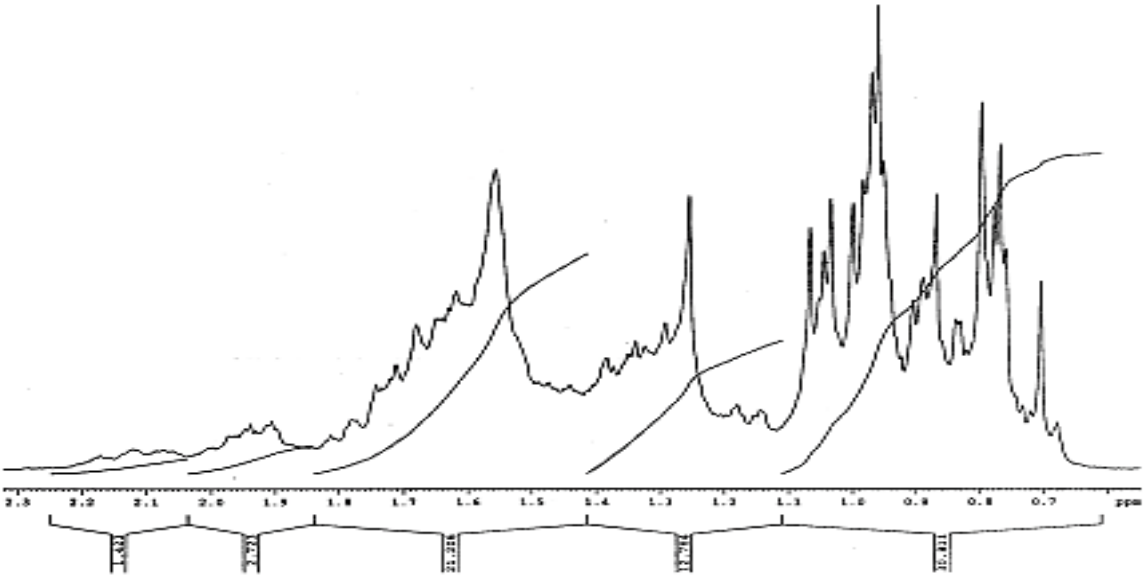


Figure 4b: ^{2}H NMR of CAR-1.

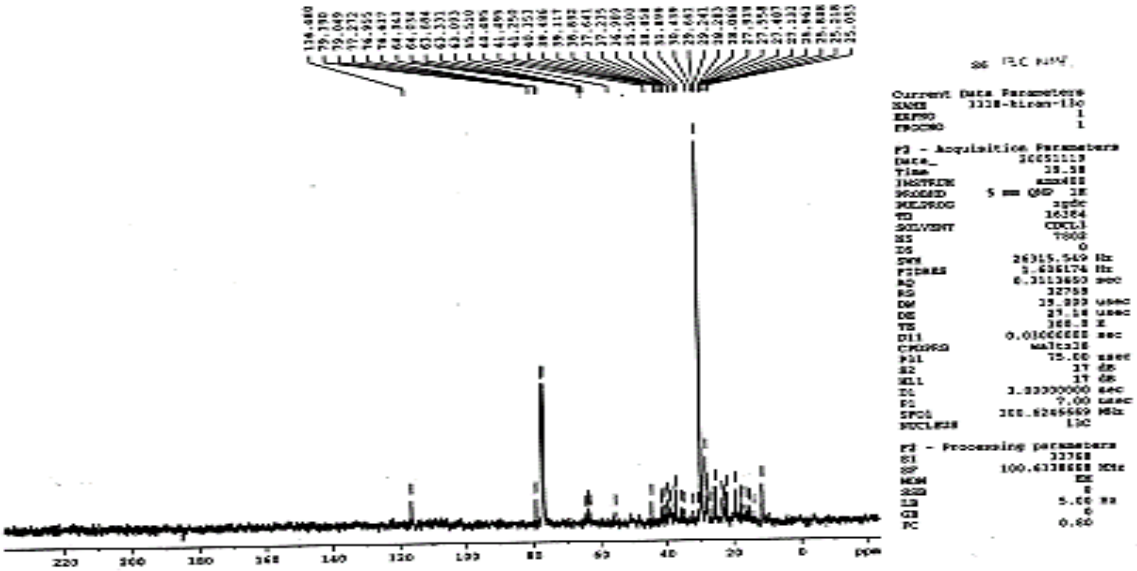


Figure 5: ^{23}C NMR spectrum of CAP-1.

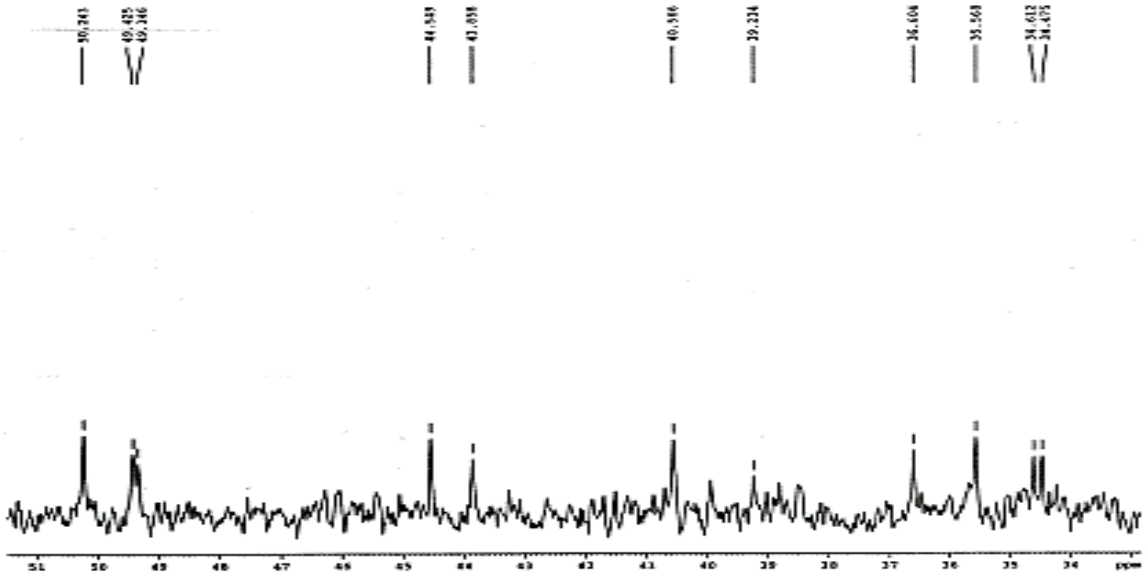


Figure 5a: ^{23}C NMR spectrum of CAR-1

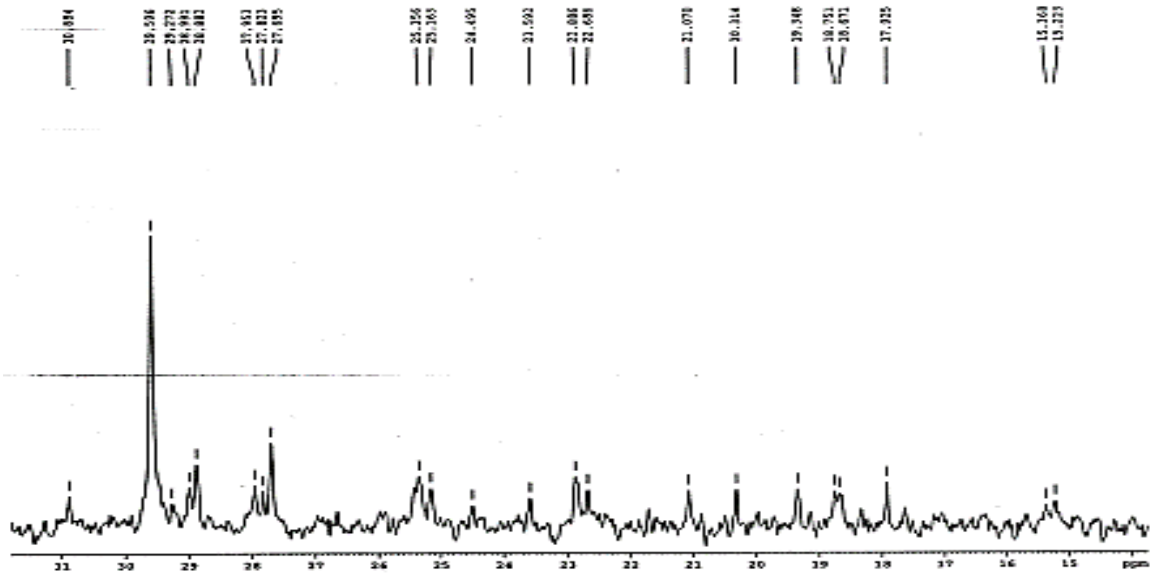


Figure 5b: ^{23}C NMR spectrum of CAR-1.

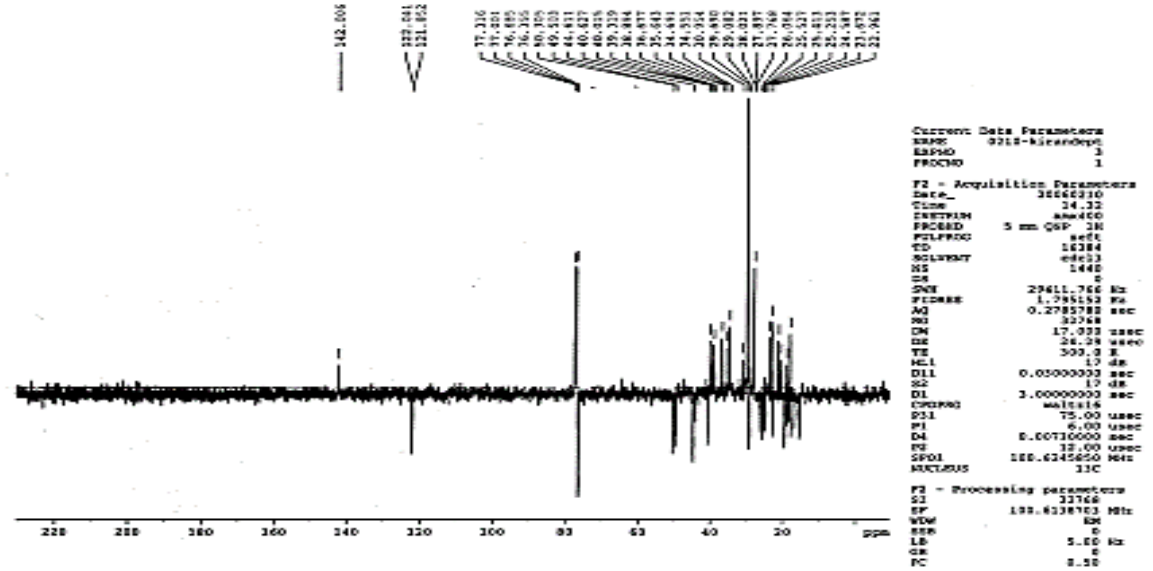


Figure 6: SEFT ^{13}C NMR spectrum of CAR-1.

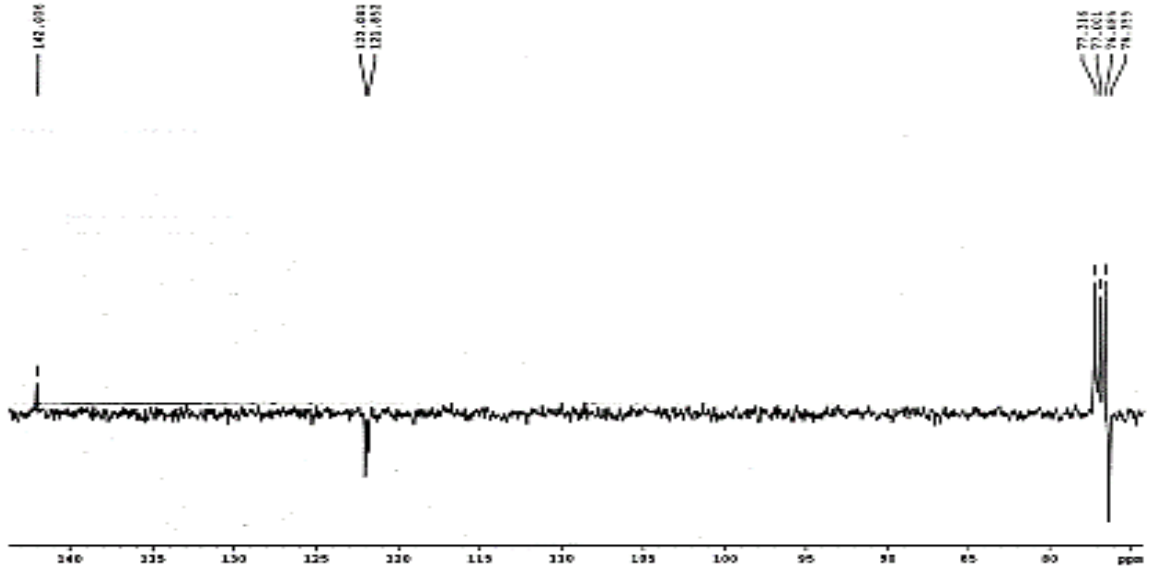


Figure 6a: SEFT ^{33}C NMR spectrum of CAR-1.

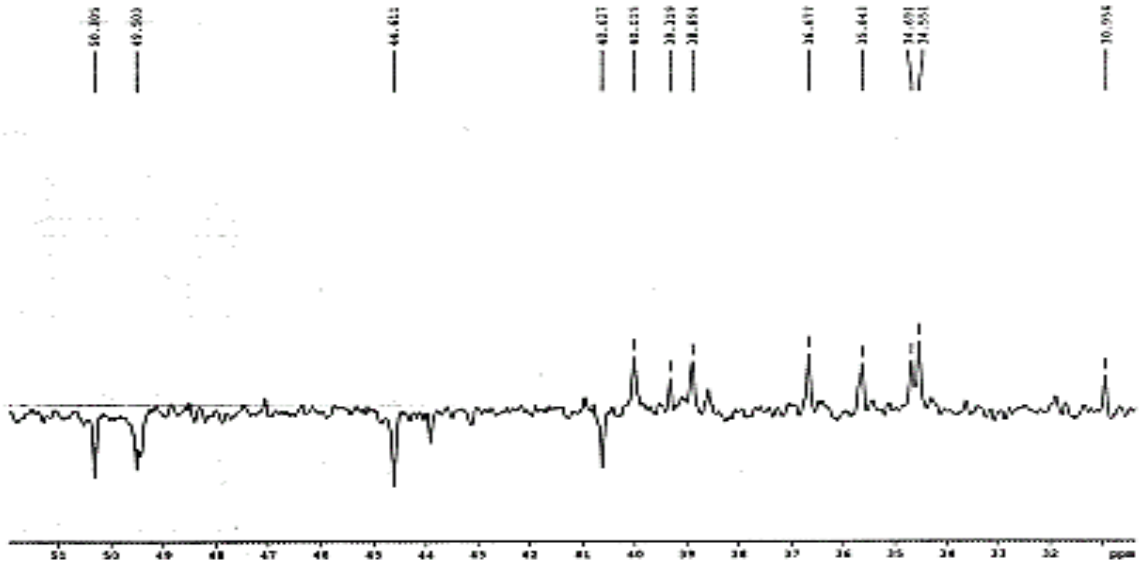


Figure 6b: SEFT ^{23}C NMR spectrum of CAR-1

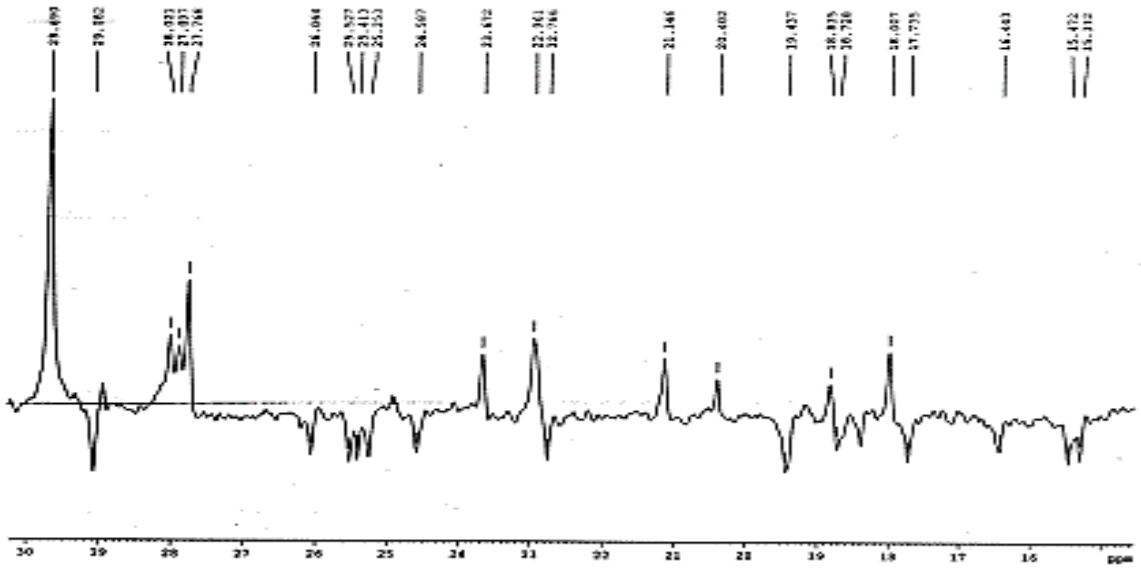


figure 6c: SEFT ^{13}C NMR spectrum of CAR-1

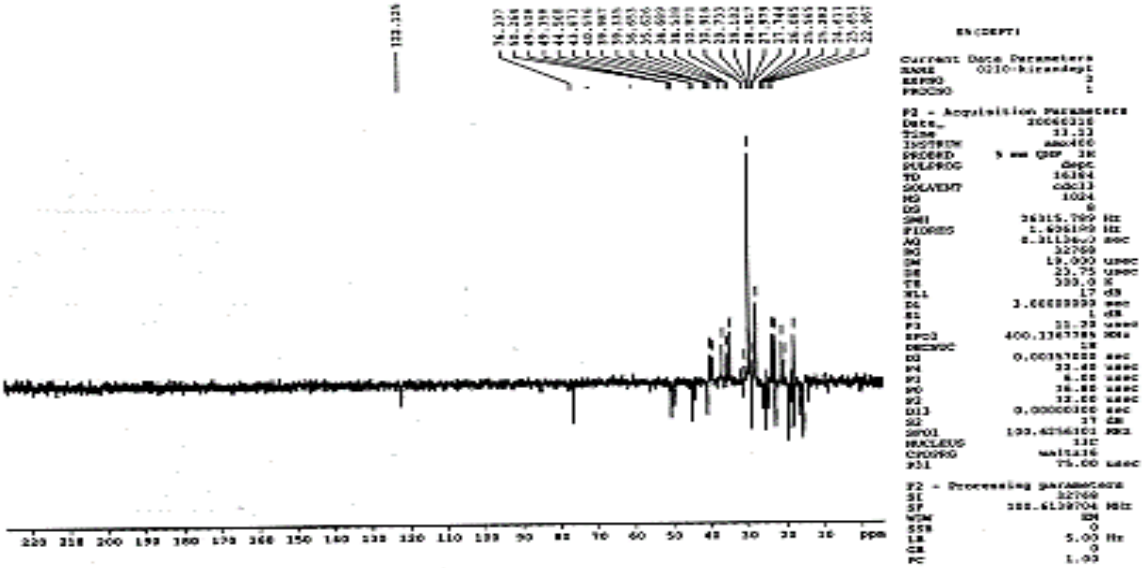


Figure 7: DEPT ^{23}C NMR spectrum of CAR-1.

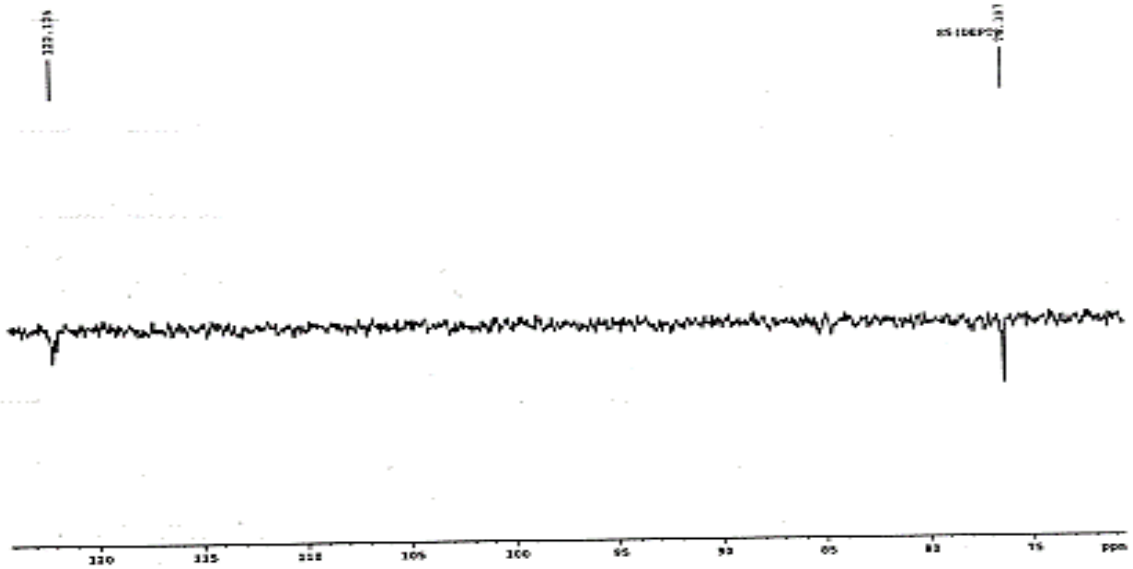


figure 7a: DEPT ^{23}C NMR spectrum of CAR-1.

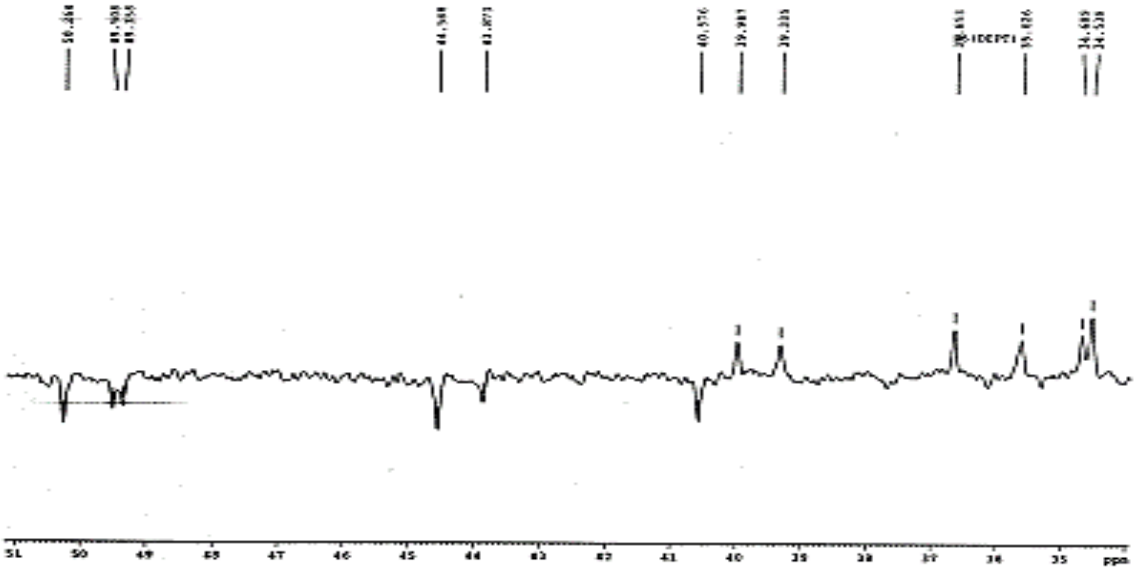


Figure 7b: DEPT ^{23}C NMR spectrum of CAR-1.

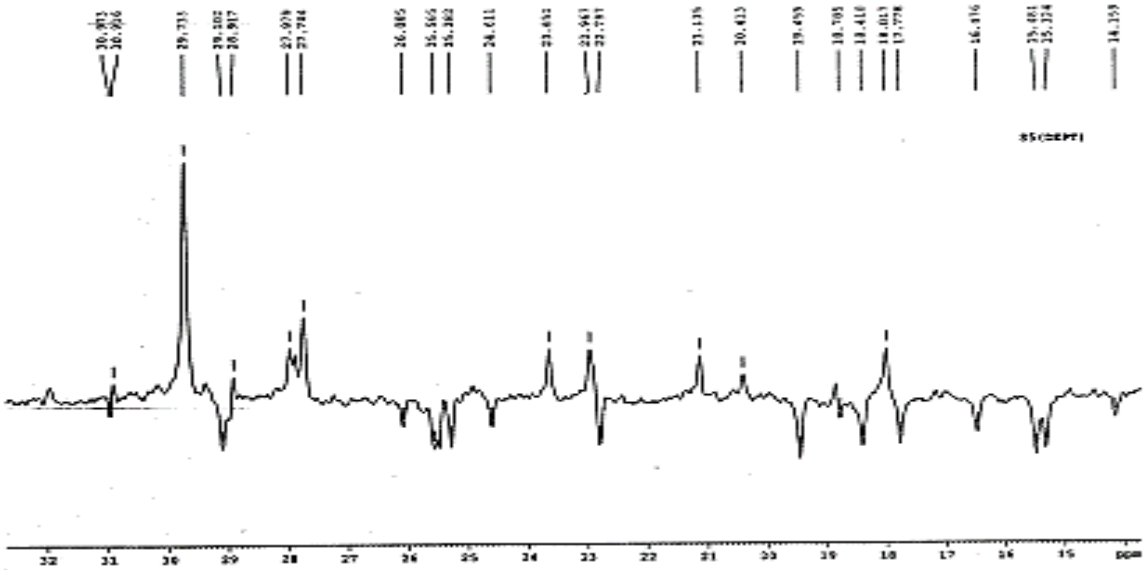


Figure 7c: DEPT ^{23}C NMR spectrum of CAR-1.

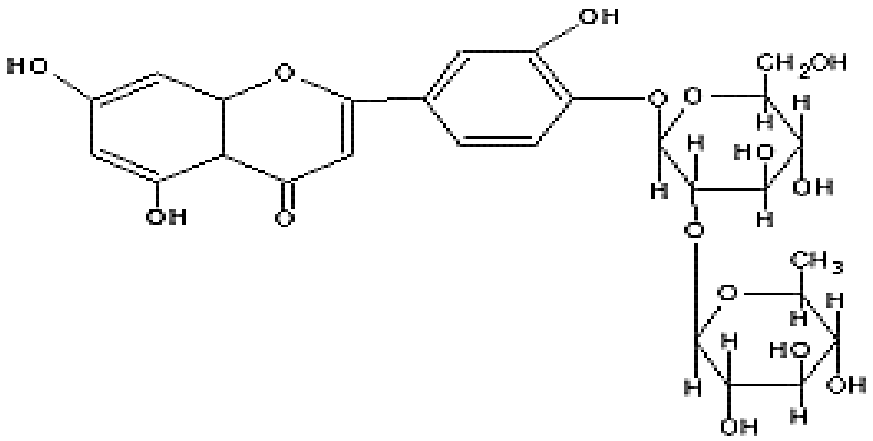


figure 8: CAI was identified as luteclin-4'-O-[α- (L-rhamnophranosyl-(1→2) β-D-ghocopyranoside)].

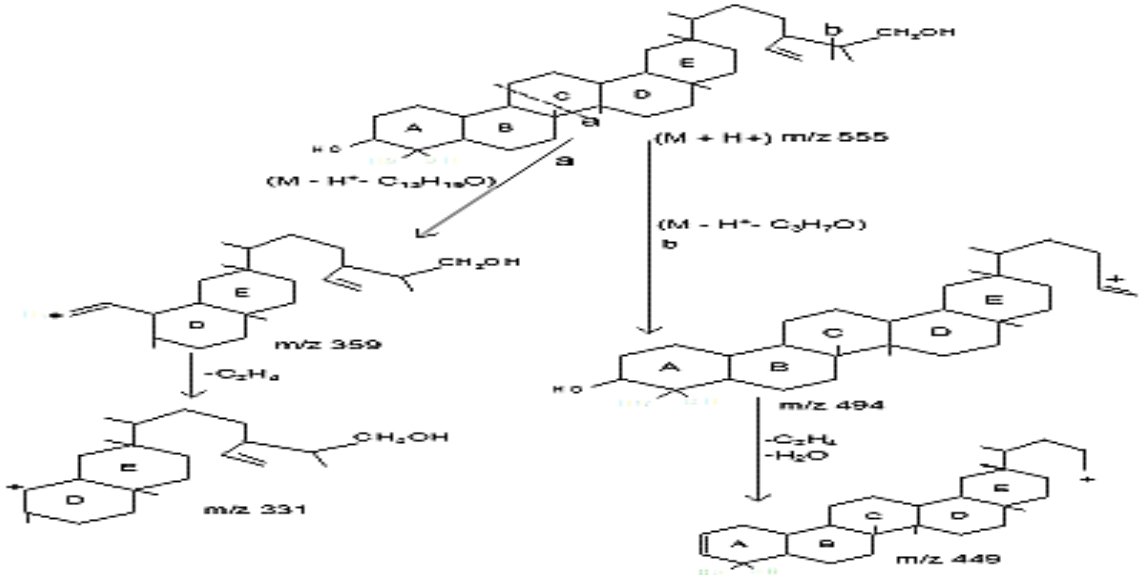


Chart 1: Mass fragmentation pattern of CAR-1 molecule.

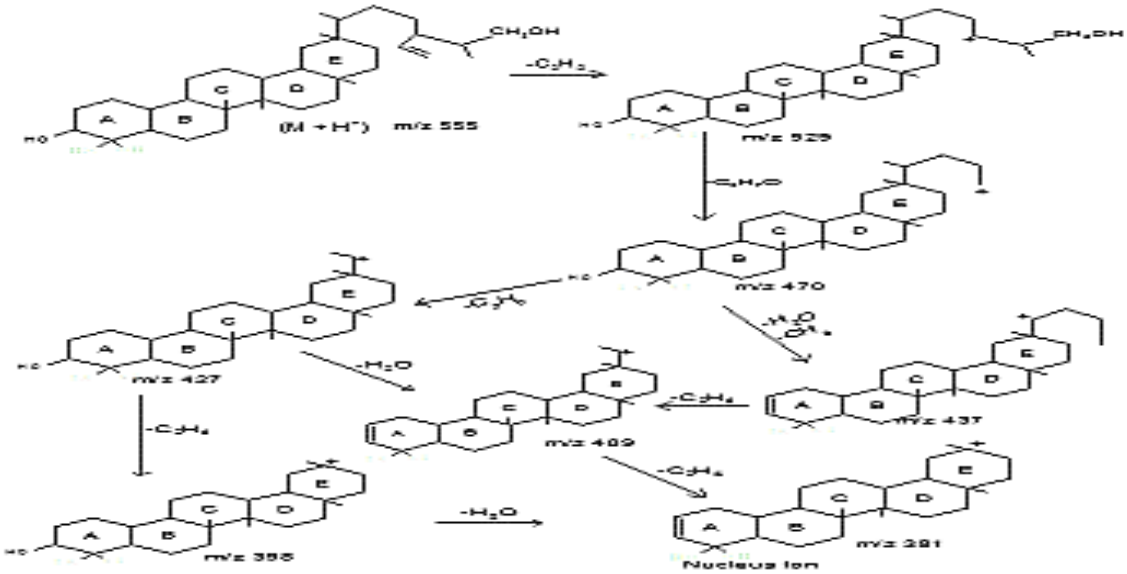


Chart 1: Mass fragmentation pattern of CAR-1 molecule.

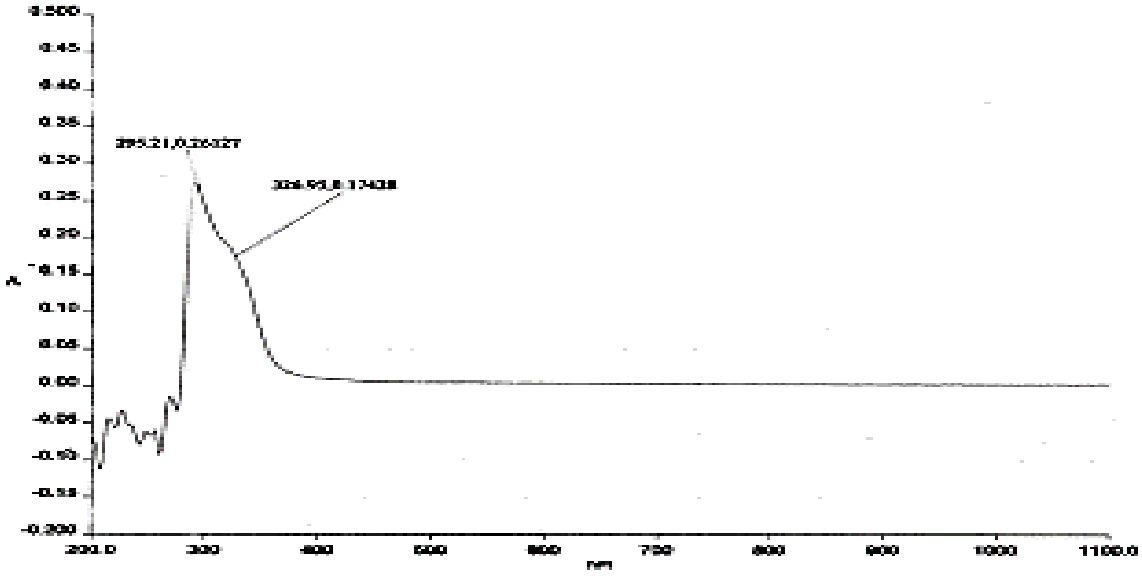


Figure 9: The UV spectrum of CAR-2.

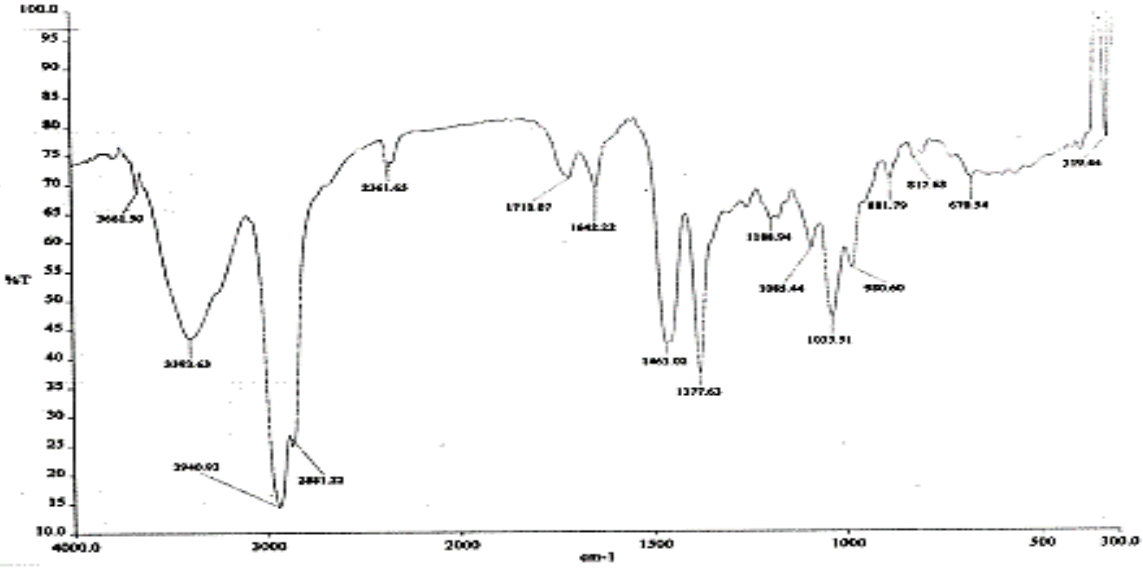


figure 10: The IR spectrum of CAR-2.

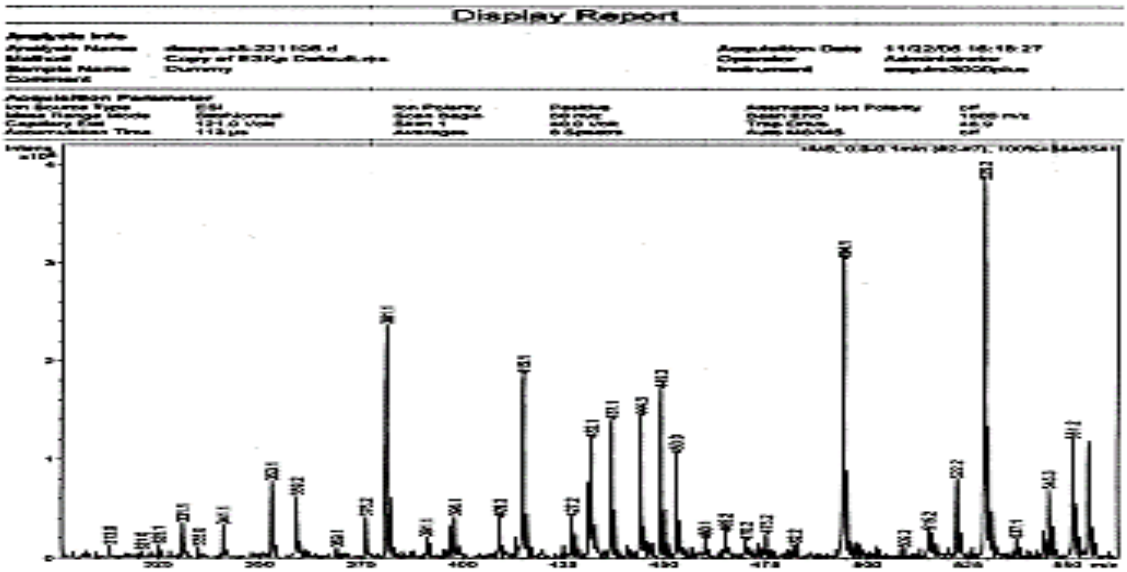


figure 11: Mass spectrum of CAR-2.

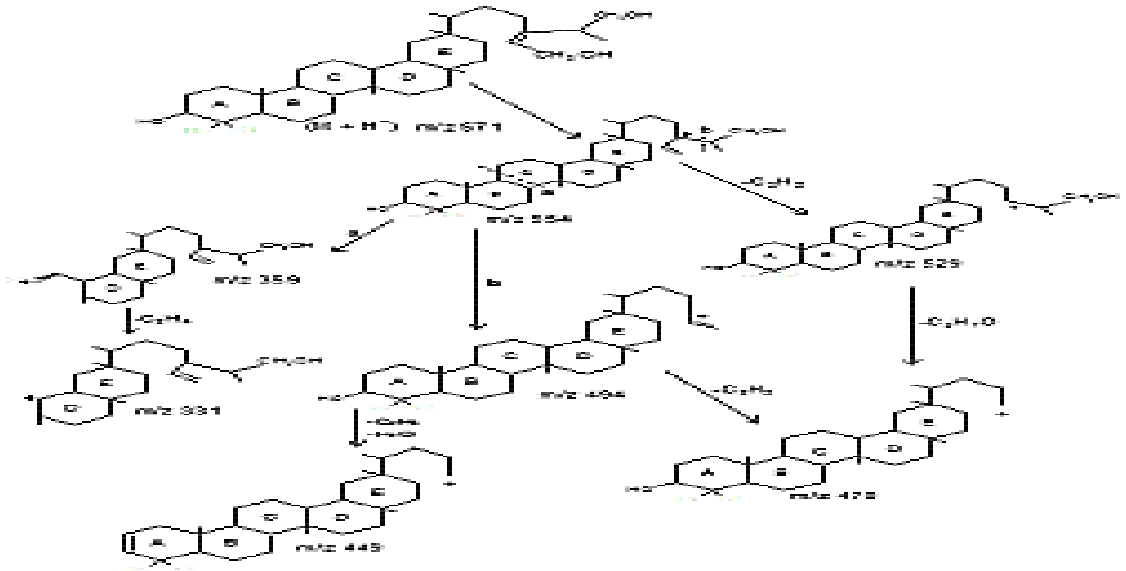
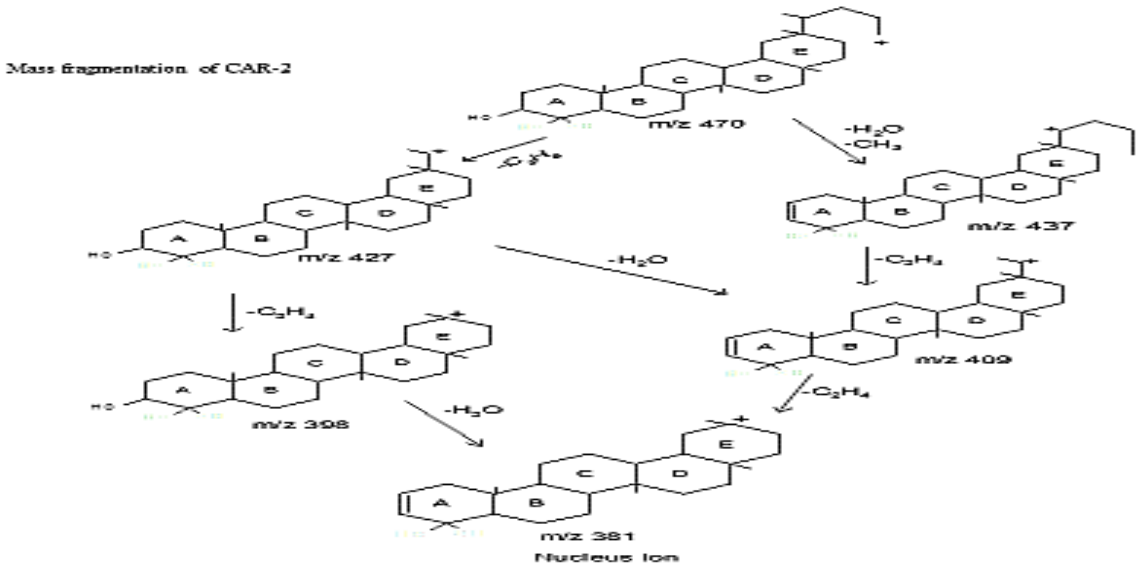


Chart 2: Mass fragmentation of CAR-2.



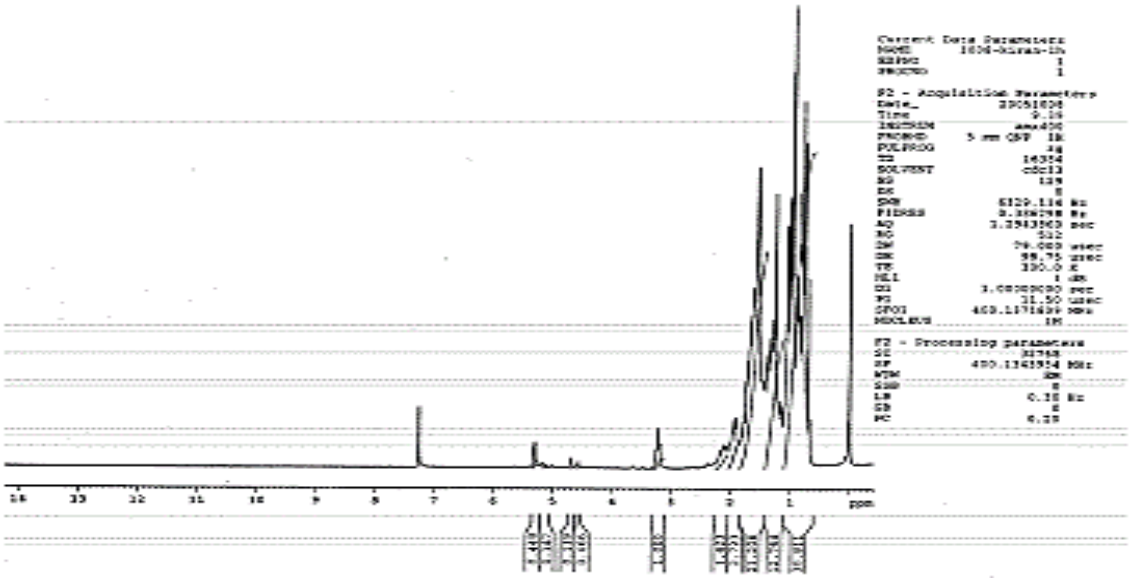


Figure 12: The ^{2}H NMR spectrum of CAR 2.

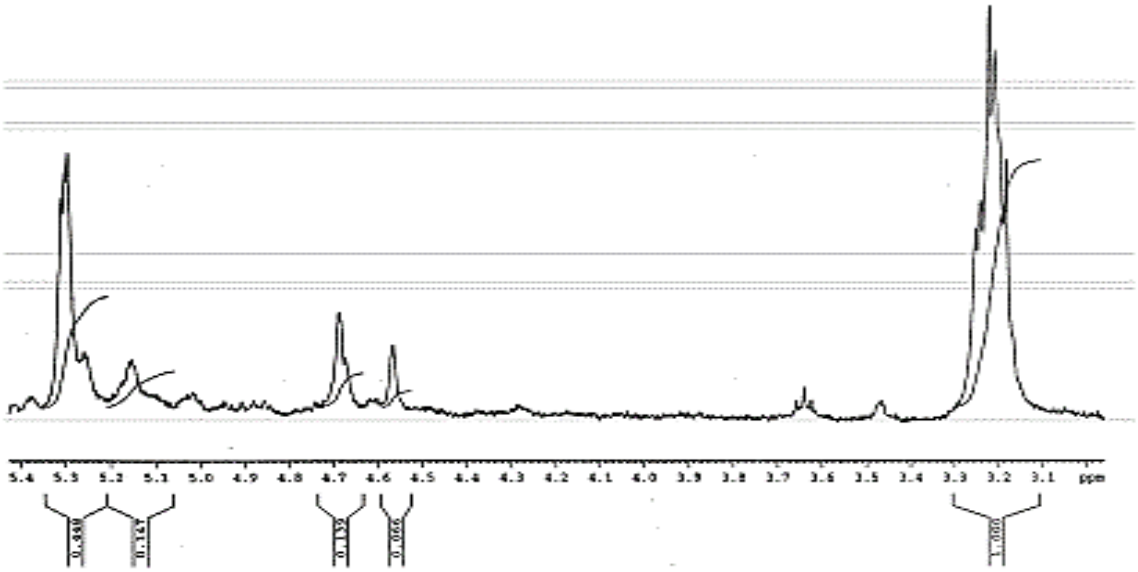


Figure 12a: The 1H NMR spectrum of CAR-2.

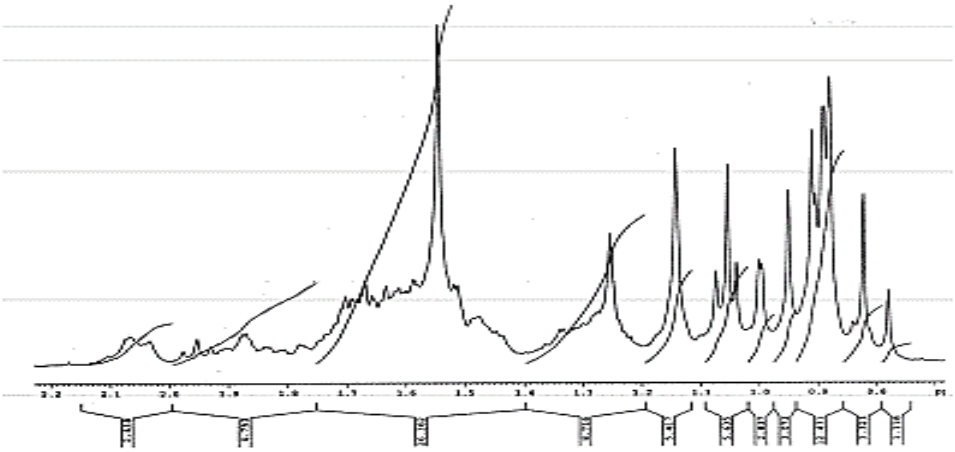


Fig: 12b The 1H NMR Spectrum of CAR 2

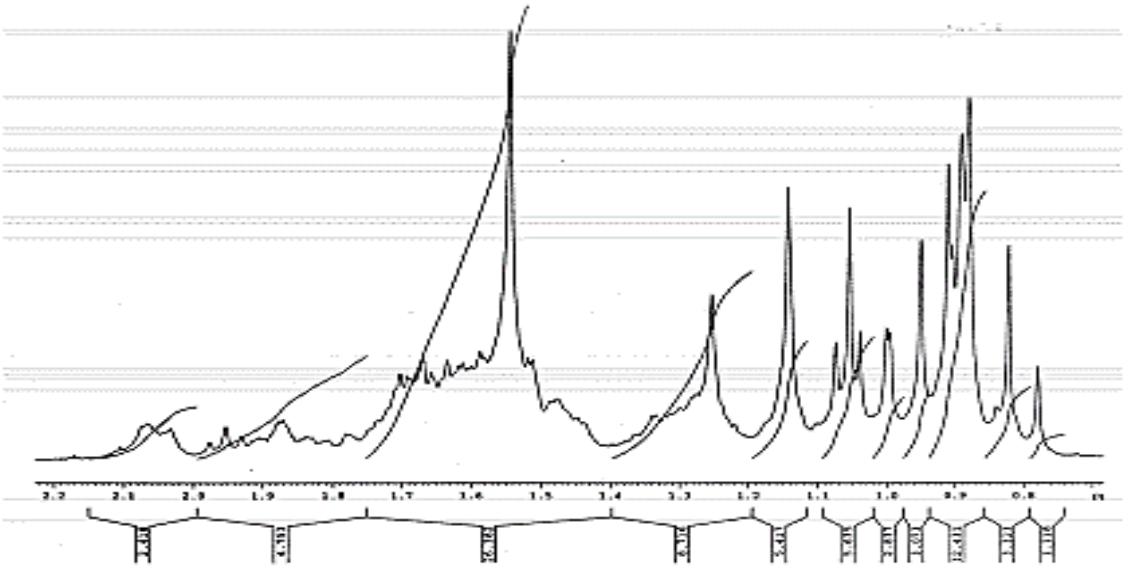


Figure 12c: The 1H NMR spectrum of CAR 2.

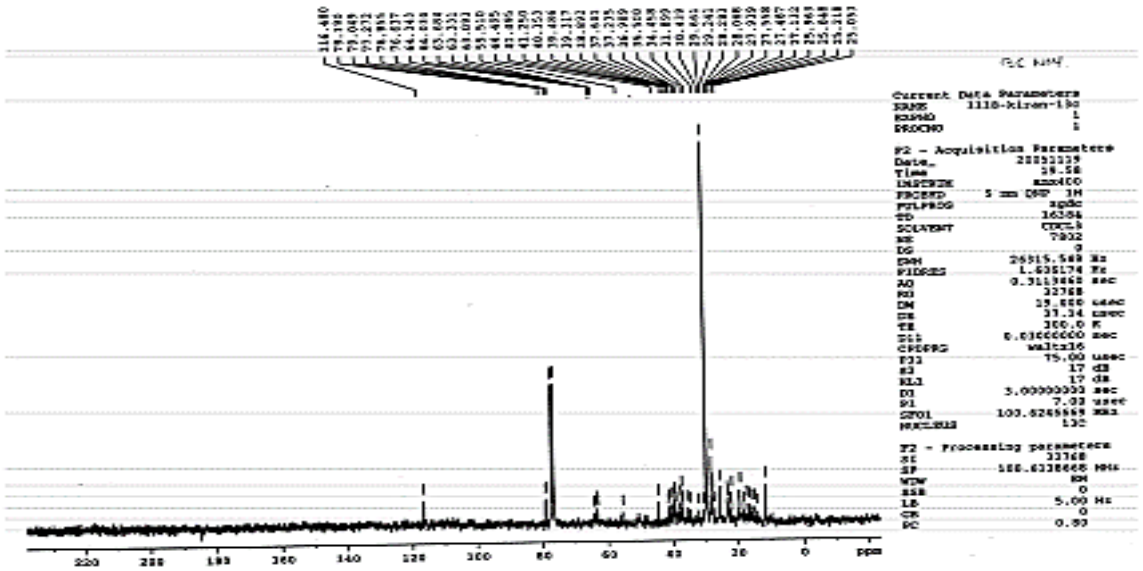


Figure 13: The ^{13}C NMR Spectrum of CAR-2.

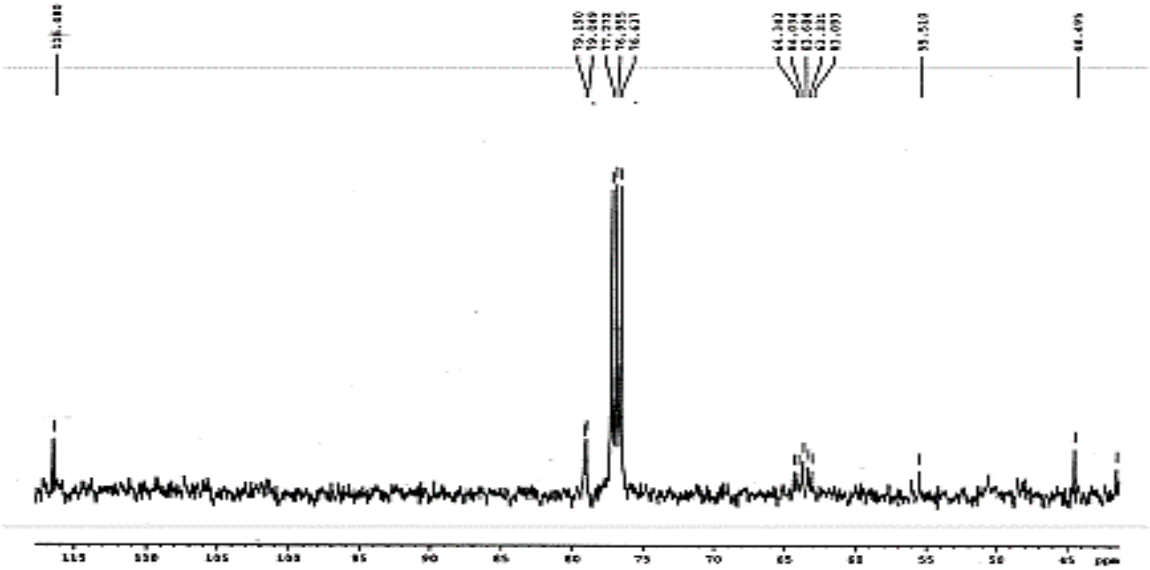


figure 13a: The ^{23}C NMR Spectrum of CAR-2.

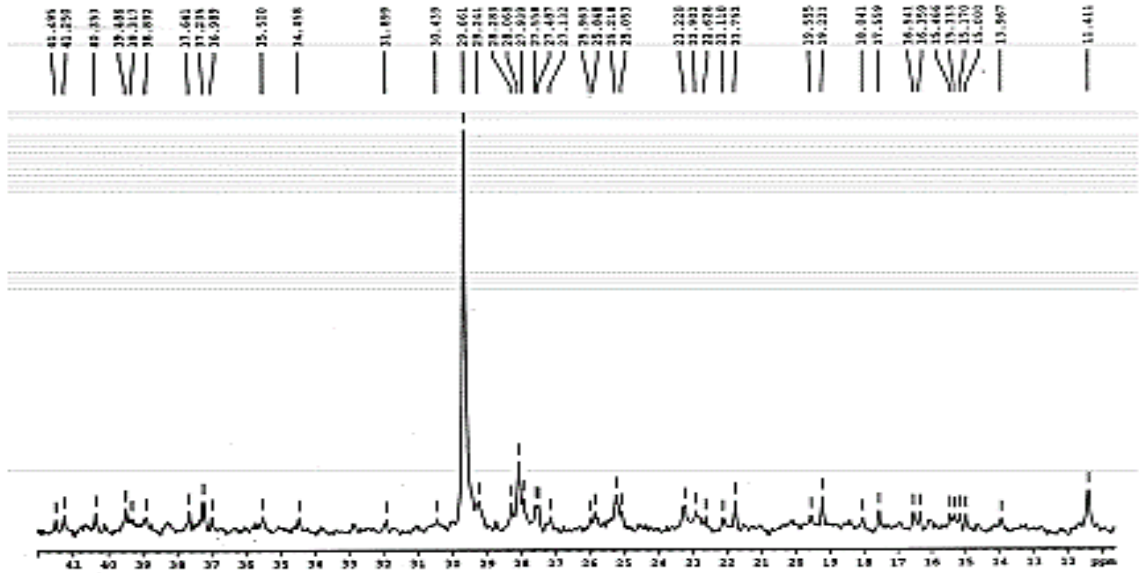


Figure 13b: The ^{13}C NMR Spectrum of CAR-2.

Table 2: The important IR absorption peaks of CAR-1.

Absorption cm^{-1}	Group assignment
3508	O-H Stretching vibration, the peak is sharp
2938	C-H stretching in CH_2 group
2873	C-H stretching in CH_3 absorption
1639	Weak C=C stretching
1451 - 1380	Gem dimethyl vibrations
969	Out of plane C-H bending of Terminal Alkene

Table 3: The mass spectral data of CAR-1.

S. No.	*m/z* value	Ion Formed
1.	537	$[M+H-OH]^+$
2.	529	$[M+H-C_2H_2]^+$
3.	494	$[M-H-C_3H_7O]^+$
4.	470	$[M-C_5H_9O]^+$
5.	449	$[M-C_5H_{13}O_2]^+$
6.	437	$[M-C_6H_{14}O_2]^+$
7.	427	$[M-C_8H_{15}O]^+$
8.	398	$[M-C_{10}H_{19}O]^+$
9.	381	$[M-C_{10}H_{21}O_2]^+$
10.	359	$[M-C_{13}H_{19}O]^+$
11.	331	$[M-C_{15}H_{23}O]^+$

3508 cm-1 hydroxyl group and a band of 969cm-1 indicate the presence of intramolecular RCH=CH2 double bond.

Mass Spectral data of CAR-1

551.2,545.3,537,529,522.2,515,494,475.2,470,465.2,453, 449,444,437,434,432,427,415,409,399,381,376,359,353,3 41,331. Mass spectrum of CAR-1 was shown in Fig. 3. The mass spectral data are given in Table-3. From the mass spectrum it can be seen a peak at m/z 555 (m+H+) indicating a molecular weight of 554 which corresponds to the molecular formula $C_{38}H_{66}O_2$.

^{1}H NMR spectrum of CAR-1

5.3062,5.2998,4.6909,3.2538,3.2428,3.2252,3.2133,3.186 1,2.1808,2.1711,2.1355,2.1195,2.1137,2.0854,2.0827,1.9 993,1.9714,1.9304,1.9255,1.9057,1.8499,1.8418,1.8390,1 .7120,1.6812,1.6502,1.6313,1.6017,1.5969,1.5576,1.4916 ,1.4742,1.4060,1.3855,1.3392,1.3247,1.3138,1.2925,1.25 54,1.2153,1.2050,1.1954,1.1533,1.0351,1.0000,0.9845,0. 9245,0.8897,0.8694,0.8599,0.8213,0.7993. ^{1}H NMR Spectrum recorded in $CDCl_3$ (400 MHZ) is represented in Fig. 4, 4a, 4b. The corresponding ^{1}H NMR data of CAR-1is shown below. 1α0.9245 (m), 1β1.4916 (m), 2α2.1137 (m), 2β1.8418 (m), 3 3.2538, 4-,5 0.8213(d), 6α1.2050(m), 6β1.5576(m), 7α1.2925, 7β1.4916, 8-,9 1.6812(t), 10 0.8320(t),11α 1.9255(m), 11β1.9057(m),12α1.2554(m), 12β1.5969(m), 13 0.8694(d), 14-, 15α1.2153(m), 15β2.1808(m), 16α1.9714(m),16β2.1711(m), 17-, 18 3.2428(d), 19α1.3247(m),19β1.8390(m),20-, 21α1.6017(m), 21β1.3138(m), 22α1.8499(m), 22β2.0854(m), 23 1.3855(s), 24 1.1533(s), 25 1.0000(s),26 1.4060(s), 27 0.9845(s), 28 1.0351(s), 29 0.8897(d), 30α1.2554(m),30β1.5969(m), 31α1.9135(m), 31β1.8989(m), 32 1.3392(d), 33 2.1195(d),

Table 4: ^{13}C NMR Spectral data of CAR-1.

Carbon No.	Spectral data
1.	40.576
2.	29.102
3.	76.689
4.	40.566
5.	39.335
6.	25.565
7.	26.085
8.	34.612
9.	30.973
10.	27.979
11.	24.611
12.	25.282
13.	36.653
14.	29.733
15.	22.797
16.	19.455
17.	34.538
18.	28.917
19.	50.264
20.	27.695
21.	49.508
22.	44.568
23.	29.733
24.	18.017
25.	20.413
26.	21.135
27.	23.651
28.	27.744
29.	35.626
30.	30.916
31.	43.873
32.	39.987
33.	34.689
34.	76.397
35.	22.967
36.	142.006
37.	122.116
38.	18.785

34α3.2252(m),34β 2.0827, 35 1.3741(d) ,36 5.3062(d), 37α4.6909(m), 37β3.1861(m),38 1.0460(d). The following observations are made from the analysis of ^{1}H NMR Spectrum of CAR-1 Signals (0.6 to 1.8 are characteristic for pentacyclictriterpene Moiety. The peaks at (0.9845, (1.0000, (1.3051, (1.1533, (1.4060 and (1.3855 singlets indicate the presence of six methyl groups. These peaks are characteristic to the protons at C-27, C-25, C-28, C-24, C-26 and C-23 of a pentacyclictriterpenoid moiety respectively. The peaks at (4.7 and (5.3 is the characteristic of olefinic double bond.

Peaks at (3.0 to (3.5 indicated the presence of hydroxyl group 3(-OH. The other –CH2, -CH protons present in the compound appeared as multiplet present at 0.8 to 2.1.

^{13}C NMR data of CAR-1

121.967,77.215,76.898,76.580,76.263,50.243,49.425,49.3 46,44.549,43.858,40.56,39.234,36.604,35.568,34.612,34. 475,30.884,29.596,29.272,28.991,28.88227.953,27.823,2 7.695,25.356,25.163,24.495,23.592,22.886,22.686,21.070

Table 5: The important IR absorption peaks of CAR-2

Absorption cm-1	Group Assignment
3662	O-H. Stretching the peak is sharp
3394	O-H stretching vibration, the peak is broad, Intermolecular hydrogen bonding
2941	C-H stretching in CH2
1642	Weak C=C stretching
1463-1378	Gem dimethyl groups
980	Trans olefinic

Table 6: The prominent mass spectral data of CAR-2.

S. No.	*m/z* VALUE	ION FORMED
1.	555	[M+H-OH]+
2.	529	[M+H-C2H3O]+
3.	494	[M+H+-C3H6O]+
4.	470	[M+H+-C5H8O]+
5.	437	[M+H+-C6H14O2]+
6.	427	[M+H+-C8H14O]+
7.	409	[M+H+-C8H16O2]+
8.	398	[M+H+-C10H18O]+
9.	381	[M+H+-C10H20O2]+
10.	359	[M+H+-C13H20O2]+
11.	331	[M+H+-C15H24O2]+

20.314,19.348,18.751,18.671,17.925,15.368,15.223 The ^{13}C NMR spectrum of CAR-1 was recorded at 100 MHZ in $CDCl_3$ is presented in Fig. 5, 5a, 5b. The Corresponding ^{13}C NMR Spectral data of CAR-1 is given in Table-4.

SEFT data of CAR 1

142.006,122.041,121.852,77.316,77.001,76.685,76.355,50.305,49.503,44.611,40.627,40.015,39.319,38.894,36.677,35.643,34.691,34.551,30.954,29.691,29.082,28.021,27.897,27.768,26.064,25.527,25.413,25.253,24.587,23.672,22.961,22.766,21.146,20.402,19.437,18.835,18.738,18.007,17.735,16.443,15.472,15.312 SEFT ^{13}C NMR spectrum of CAR-1 was recorded at 100 MHZ in $CDCl_3$ and is represented by Fig.6, 6a, 6b, 6c.

DEPT data of CAR – 1

122.125,76.397,50.264,49.508,49.358,44.568,43.873,40.576,39.987,39.335,36.653,35.626,34.689,34.538,30.973,30.916,29.733,29.102,28.917,27.979,27.744,26.085,25.565,25.282,24.611,23.651,22.967,22.797,21.135,20.413,19.455,18.785,18.410,18.017,17.778,16.476,15.481,15.324,14.19 DEPT – ^{13}C NMR spectrum of CAR-1 was recorded at 100 MHZ in 38 carbon resonances between 142.006 to 15.324 at 100 MHZ in $CDCl_3$ is represented in Fig.7, 7a, 7b, 7c. Analysis of the resonances from ^{13}C NMR spectrum revealed the following observations. The signals at (18.017, (20.413, (21.135, (23.651, (27.744 and (29.733 are the characteristic to C-24, C-25, C-26, C-27, C-28 and C-23 methyl groups of pentacyclictriterpenoid. Out of 38 carbon resonances, 10 carbon resonances assigned to the side chain. The remaining carbon resonances can be assigned to a penta cyclic triterpenoid which constitutes the CAR-1. The signal at (76.689 is due to C-3 hydroxyl group. The signal at (76.387 is due to the presence of the hydroxyl group at C-34 of side chain. SEFT spectrum showed carbon resonances at (142.006 and (122.116. this indicated the presence of exocyclic double bond at C36 and C37. From the spectral analysis CAR-1 is identified as Pentacyclictriterpenoid with side chain containing double bond and hydroxyl group and the structure is given below supported by the mass fragmentation pattern (Chart-I & Chart-II).

Structural elucidation of CAR-2

CAR-2 is a colour less crystalline solid melting point 157^0C. It gave a positive Liebermann-Burchard reaction and Salkowski test, indicating it might be steroid / terpenoid.

U.V. Spectral data of CAR- 2

The U.V. Spectrum is presented in Fig.9. It showed maximum 326.95 and minimum 295.21 nm absorption in UV region.

IR data of CAR-2

3661.50,3393.63,2940.93,2881.22,2361.65,1710.07,1642.22,1463.02,1377.63,1085.44,1033.51,980.60,881.79,670.54,319.44.

IR Spectrum of CAR-2

The IR Spectrum is presented in Fig. 10. The important IR absorption peaks are shown in Table-5 The IR spectrum of CAR-2 showed a peak 3661 cm-1 indicates the presence of hydroxyl group and a peak at 3393cm-1 indicates another hydroxyl group.

Mass spectral data of CAR-2

568,555,545,538,529,522,494,475,470,453,449,437,431,427,415,409,399,381,359,353,331. Mass spectrum of CAR-2 is shown in Fig.11. The prominent mass spectral data are given in Table-6. From the spectra it can be seen a peak at m/z 571 (m+H)+ indicating a molecular weight of 570 which corresponds to the molecular formula $C_{38}H_{66}O_3$. Other significant of positive ion peaks were observed and fragmentation pattern of the molecule is given in Chart – 2.

^1H NMR spectra of CAR-2

The ^1H NMR Spectrum recorded in CDCl3 (400 MHZ) is presented in Fig.12, 12a, 12b, 12c. The corresponding ^1H NMR data of CAR-2 is shown below. 5.6478,5.6341,3.4681,2.1045,2.0752,2.0672,2.0444,2.0328,1.9767,1.9531,1.9300,1.8986,1.8410,1.8355,1.8300,1.8056,1.7463,1.7340,1.7028,1.6698,1.5804,1.5453,0.5191,1.5125,1.4859,1.4779,1.4478,1.4379,1.3974,1.3636,1.3395,1.3339,1.3222,1.3148,1.3003,1.2767,1.2191,1.1439,1.1027,1.0399,1.0200,0.9967,0.9508,0.9113,0.8917,0.8809,0.8403,0.8226

1α0.9508(m),1β1.4859(m),2α2.1045(m),2β1.8410(m),3 3.4681(d), 4-, 5 0.8226(d 11.4), 6α1.2191(m),6β1.5191(m),7α1.3003(m),7β1.5125(m), 8 0.8403(d),9 1.6698(d), 10-,11 1.9300(d), 12α1.2536(m), 12β1.5453(m), 13 0.8917(d), 14-, 15α1.2767(m), 15β2.0752(m), 16α1.9767(m), 16β2.0672(m), 17-,18 3.4681(d), 19α1.3222(m), 19β1.8300(m), 20-,21α1.4379(m), 21β1.1027(m), 22α1.8355(m), 22β2.0444(m), 23 1.3974(s), 24 1.1439(s), 25 0.8809(s), 26 1.3636(s),27 0.9967(s),28 1.0399(s), 29 0.8917(d), 30α1.3339(m), 30β1.4478(m), 31α1.3395(m), 31β1.4779(m), 32-,33 1.5804(d), 34α3.4681(m), 34β2.0328(m),35 1.3148(s),36 5.6341(d), 37α5.6478(m),

Table 7: The[13]C NMR Spectrum of CAR-2

Carbon No.	Spectral Data
1.	40.353
2.	29.661
3.	79.190
4.	39.486
5.	55.510
6.	25.848
7.	25.963
8.	29.241
9.	30.439
10.	37.235
11.	25.053
12.	25.218
13.	36.989
14.	27.939
15.	22.902
16.	19.555
17.	34.458
18.	28.283
19.	37.641
20.	27.558
21.	31.899
22.	44.495
23.	29.661
24.	18.041
25.	16.359
26.	21.752
27.	23.220
28.	27.487
29.	35.500
30.	29.661
31.	41.250
32.	116.480
33.	64.034
34.	79.049
35.	22.110
36.	116.480
37.	63.331
38.	17.559

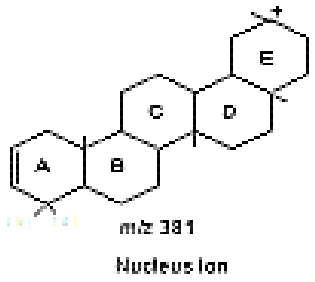


figure 14: Oteanane nucleus of CAR 1 and CAR 2**.**

37β2.0328(m),38 1.0552(s). The [1]H NMR spectra of CAR-2 is very similar to those of CAR-1 and the only difference between these two is one –OH group. The following observations are made from the analysis of [1]H NMR spectral data of CAR-2. Signals (0.6 to 1.8 are characteristic for Pentacyclictriterpene moiety. The peaks at (0.8809, (0.9967, (1.0399, (1.1439, (1.3636 and (1.3974 singlets indicate the presence of Six methyl groups. These peaks are characteristic to the proton at C-25, C-27, C-28, C-24, C-26 and C-23 of a Pentacyclictriterpenoid moiety respectively. Protons at (5.6341 (d) is characteristic of trans olefin proton signal. The signals at 2.0 to 2.1 indicated the presence of hydroxyl protons. Signal at (3 to 3.5 indicated the hydroxyl group attached to carbon containing proton.

[13]C NMR spectra of CAR-2

116.480,79.190,79.049,77.272,76.955,76.637,64.343,64.034,63.684,63.331,63.093,55.510,44.495,41.495,41.250,40.353,39.486,39.317,38.892,37.641,37.235,36.989,35.500,34.458,31.899,30.439,29.661,29.241,28.283,28.068,27.939,27.558,27.487,27.132,25.963,25.848,25.218,25.053,23.220,22.902,22.626,22.110,21.752,19.555,19.221,18.041,17.559,16.541,16.359,15.466,15.333,15.170,15.000,13.967,11.411 The ^{13}C NMR Spectrum of CAR-2 recorded at 100 MHZ in $CDCl_3$ presented in Fig.13, 13a, 13b and the corresponding ^{13}C NMR spectral data of CAR-2 in given in Table-7. The ^{13}C NMR of CAR-2 has shown 38 carbon resonances between116.480 to 11.411. Analysis of the resonances from ^{13}C NMR spectrum revealed the following observation. The signals at 16.359, 18.041, 21.752, 23.220, 27.487 and 29.661 are the characteristic to methyl groups of C-25, C-24, C-26, C-27, C-28 and C-23 of Penta cyclic triterpenoid respectively. Out of the 38 carbon resonances 10 carbon resonances were assigned to the side chains. The remaining carbon resonances can be assigned to a Penta cyclic triterpenoid which constitutes the CAR-2. The carbon resonance at 116.480 indicates the presence of double bond between C-32, C-36. The signal at 79.190 is due to hydroxyl group at C-3. The signals at 79.049 and 63.331 are due to the presence of hydroxyl groups at C-34 and C-37 of side chain respectively. From the spectral analysis CAR-2 is indicated as Penta cyclic triterpenoid with a side chain containing double bond and hydroxyl groups. The structure is given below supported by the mass fragmentation pattern of Chart – 2. From the mass fragmentation charts of CAR-1 and CAR-2, it is concluded that the difference between them is only one hydroxyl group. It is present in CAR-2 as unsaturated alcohol. Similar mass fragmentations are observed in both CAR-1, CAR-2. The nucleus peak at m/z 381 observed both in CAR-1 and CAR-2 confirms Oleanane nucleus (Fig: 14). The peak at m/z 529 with maximum intensity is also observed both in CAR-1 and CAR-2. The UV spectrum of CAR 1 showed absorption bands at λ– max 212 nm (log E4.44), 248 nm (logE4.16), 269 nm (log E 4.23) and 332 nm (log E4.23). The UV data is similar to that of 5,7,3',4'hydroxylated flavones[7]. The UV data is similar to that of the luteolin glycoside isolated from *C. tuberculata*[9,10]. IR Spectrum of CA1 showed peaks at cm^{-1} 3381 (O-H), 1655 (C=O), 1616, 1504, 1440 (C=C aromatic ring) 1072 and 1073 (C-O of sugars). The peak at 1655 cm^{-1} is characteristic of flavone carbonyl group[8]. In positive ion FABMS CA1 showed quasimolecular ion peak at m/z 595 $(M+1)^+$ and at m/z 593 $(M-1)^+$ in its negative ion FABMS, showing that its molecular ion (M^+) is at m/z 594 is the high resolution FABMS (HRFABMS)

of CA1. In this the highest mass ion is at m/z 595.04 (M+H), which corresponds to the molecular formula $C_{27}H_{30}O_{15}$. A preliminary study of the ^{1}H NMR of CA1 showed it has two hexose units on the basis of proton integration in the region δ– 3.1-3.9 ppm and due to the presence of two anomeric protons, which appeared downfield (δ- 5.2-5.3ppm) and resonated as doublets. By a consultation of literature readily assigned, the singlet at δ- 6.60 ppm is due to C-3 H, the meta coupled doublets at δ– 6.2 (J=1.8Hz) were due to C-6 and C-8 protons, the doublet at δ– 7.25(J=8.0 Hz), the multiplet at δ– 7.45, integrating for two protons were due to C-5', C-6' and C-2' protons respectively in the 5,7,3',4' hydroxylatedflavanoid skeleton. The assignment of the chemical shifts to the sugar protons is made as follows. The proton doublet at δ- 1.12 (J=7.0Hz) is a methyl which is possibly from the rhamnose sugar in CA1. The proton doublet at δ– 5.30 (J=7.5Hz) indicates glucose as the other sugar. Thus considering sugars to be D-glucose and L-rhamnose, it is possible to account for the molecular weight of 594 as due to 5,7,3',4' tetrahydroxy flavone (285) + Glucose (162)+ rhamnose (147). The clear evidence for the presence of two sugar units is from the signals at δ- 5.30 (J=7.5 Hz) and 5.20 (J=2.0 Hz) which are due to the anomeric protons of the D-glucose and L-rhamnose respectively[9.10]. The signals due to the other sugar protons cannot be readily assigned to the C-2", C-3", C-4", C-6" and C-2", C-3", C-4" protons of the glucose and rhamnose respectively as they have nearly same chemicals shifts and resonate as a complex multiplet in the region δ 3.1 – 3.9. The chemical shifts observed for CA1 were nearly same as those reported earlier for luteolin-4'-O-[α- (L-rhamnophyranosyl-(1→2) β- D- glucopyranoside)] isolated from *C.tuberculata*[9,10].Therefore, it is considered that, the glucose unit is linked β to C-4' oxygen of the luteolin (β-linkage of glucose showed J value of the anomeric proton as 7.5Hz), while the rhamnose is linked to the glucose at C-2"-O by anα linkage as the rhamnoseanomeric proton shows J=2.0 Hz in CA1. The chemical shifts (δ ppm) in the downfield region, due to the flavone carbons are same as those reported earlier for luteolin and its glycosides. The assignments are as follows : δ 164.5 (C-2), δ 103.3 (C-3), δ 181.69 (C-4), δ100.2 (C-4a), δ 162.1 (C-5), δ 99.2 (C-6), δ 164.7 (C-7), δ 94.2 (C-8), δ157.9 (C-8a), δ 119.3 (C-1'), δ 113.8 (C-2'), δ 146.2 (C-3'), δ 150.1 (C-4'), δ 116.4 (C-5') and δ 122.1 (C-6'). Some of the readily assignable sugar carbon resonances are the β linked glucose anomeric carbon at δ 100.6 (C-1") and the CH_2OH (C-6') of the glucose at δ 61.0. The – linked rhamnose methyl resonates at δ 17.9 (C-6"). The 13 C NMR spectral data is similar to that reported for luteolin-4'-O-[α- (L-rhamnophyranosyl-(1→2) β- D- glucopyranoside)] from *C.tuberculata*[8,9]. APT spectrum classifies all carbon peaks into methyl, methylene, methnic and quaternary carbons. APT spectrum of CA 1 shows the presence of one methyl, one methylene, sixteen methine and nine quaternary carbons. Thus all 27 ^{13}C NMR signals of CA1 can be assignable. HeteroCOSY spectrum is useful to confirm the proton and carbon signal assignments. In the HeteroCOSY spectrum contour peaks appear at the intersection of the proton and carbon chemical shifts. Carbons that have no directly attached protons do not show contour peaks. In the HeteroCOSY spectrum of CA1, we can observe contour peaks at the chemical shifts of rhamnose methyl proton and rhamnose methyl carbon at δ17.9 in ^{13}C and δ 1.12 in ^{1}H NMR. Further, for the glucose and rhamnoseanomeric carbons which resonate at δ 100.6 and 98.5 respectively, and the contour spots appear at the intersection of these corresponding proton chemical shifts which resonate at δ 5.30 and 5.20 respectively. Similar HeteroCOSY correlations were observed for the flavone carbons with protons attached i.e., C-3, C-6, C-8, C-2', C-5' and C-6'. From the spectral analysis CAR-2 is indicated as Penta cyclic triterpenoid with a side chain containing double bond and hydroxyl groups. The structure is given below supported by the mass fragmentation pattern of Chart – 2.

CONCLUSIONS

From the mass fragmentation charts of CAR-1 and CAR-2, it is concluded that the difference between them is only one hydroxyl group. It is present in CAR-2 asα, β-unsaturated alcohol. Similar mass fragmentations are observed in both CAR-1, CAR-2. The nucleus peak at m/z 381 observed both in CAR-1 and CAR-2 confirms Oleanane nucleus.Thus from an analysis of the IR, UV, Mass, ^{1}H NMR, ^{13}C NMR, APT and HeteroCOSY, CA1 was identified as luteolin-4'-O-[α- (L-rhamnophyranosyl-(1→2) β- D- glucopyranoside)].

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