Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Bioactivity guided isolation of oxypregnane-oligoglycosides (Calotroposides) from the root bark of *Calotropis gigantea* as potent anticancer agents

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Contents

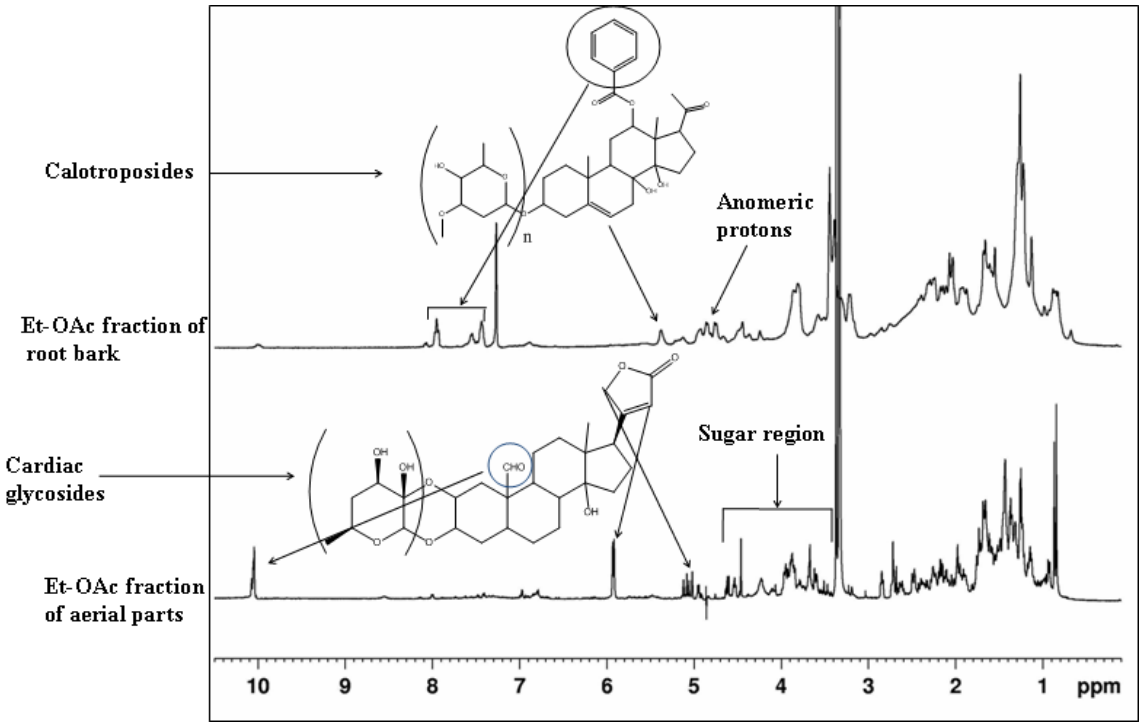

Figure S1: Stacked plot of ^{1}H NMR spectra of ethyl acetate fractions (Et-OAc) of aerial parts and root bark of *Calotropis gigantea*.

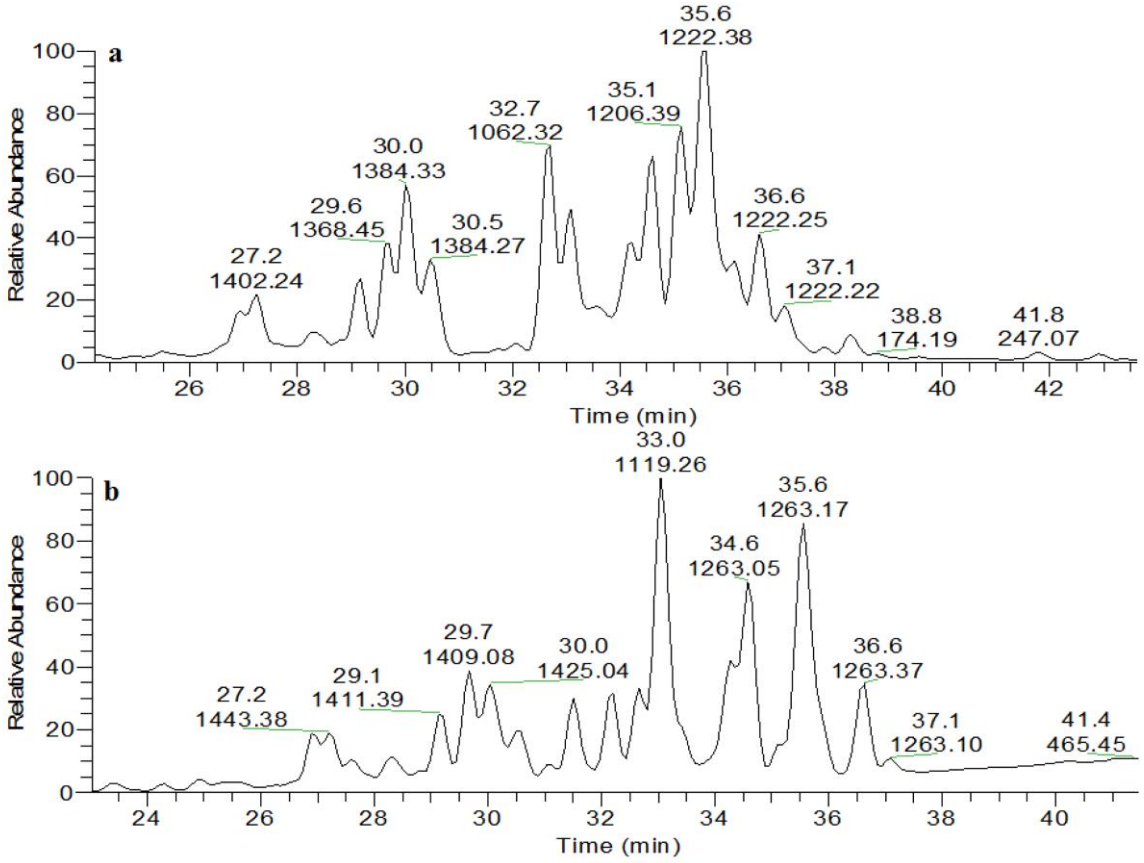

Figure S2: (a) LC-MS (positive mode) spectrum and (b) LC-MS (negative mode) spectrum of ethyl acetate (F02-CPRB-2) fraction.

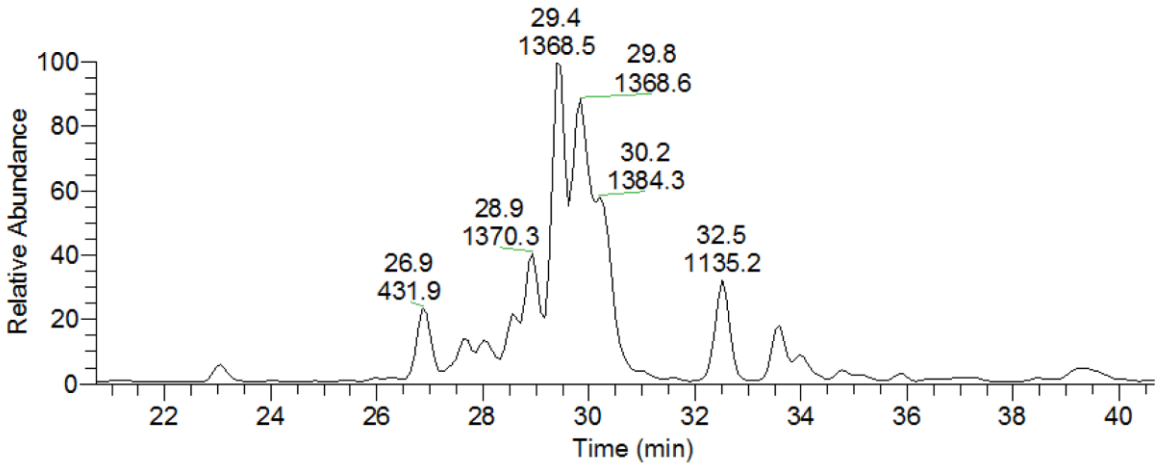

Figure S3: LC-MS (positive mode) spectrum of sub-fraction F02-7.

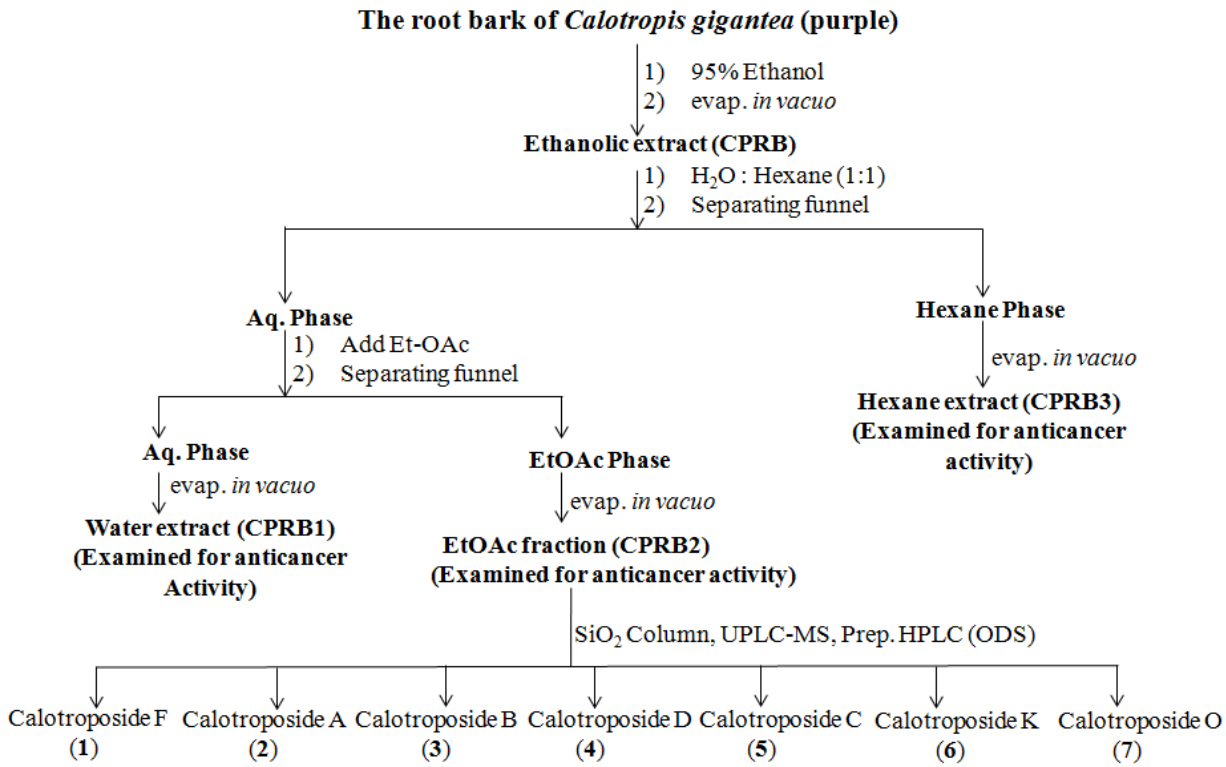

Figure S4: Flow chart for isolation of compounds 1- 7

Table S1: LC-MS gradient elution system for chromatographic separation on Waters (THERMO ODS-2, 250X4.6 mm, 5 µm) analytical column with 0.800 mL/min.

S. No.	Time (min)	A (Acetonitrile), %	B (5 mM Ammonium Acetate buffer), %
1	0	5	95
2	30	70	30
3	40	80	20
4	50	80	20
5	55	5	95
6	60	5	95

Spectroscopic data of compounds 1, 2, 3, 4 and 5

Calotroposide F (1): (12-*O*-benzoyldeacetylmetaplexigenin-3-*O*-β-D-oleandropyrano-syl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside): white amorphous solid, m.p. 185-188 °C, $[\alpha]_D$ -12.5° (c=1.2, in MeOH at 22 °C). IR ($CHCl_3$) cm^{-1} 3512, 3019, 1676, 1403, 1215, 1058. UV (MeOH) nm (log ε) 230 (4.56), 273 (3.15), 280 (3.01). ^1H NMR ($CDCl_3$) δ (ppm) 1.11, 1.85 (1H both m, CH_2-1), 2.03, 1.90 (1H both m, CH_2-2), 3.55 (1H, m, H-3), 2.22, 2.41 (1H both m, CH_2-4), 5.37 (1H, brs, H-6), 2.01, 2.23 (1H both m, CH_2-7), 1.61 (1H, m, H-9), 2.01, 1.53 (1H both m, CH_2-11), 4.84 (1H, m, H-12), 2.13, 1.87 (1H both m, CH_2-15), 2.08, 2.17 (1H both m, CH_2-16), 2.89 (1H, m, H-17), 1.54 (3H, s, CH_3-18), 1.12 (3H, s, CH_3-19), 2.05 (3H, s, CH_3-21) (pregnanone moiety), 7.94 (2H, d, *J*=7.5 Hz, H-2',6'), 7.44 (2H, t, *J*=7.5 Hz, H-3',5'), 7.55 (1H, t, *J*=7.5 Hz, H-4') (benzoyl moiety), 4.84, 4.66, 4.44, 4.74 (1H each, dd, J_1= 9.72 Hz, J_2=1.51 Hz) (anomeric protons), 1.62-1.70 (2H X 4 = 8H, C-2 protons of sugars), 3.75-3.86 (4H, C-3 protons of sugars), 3.16-3.21 (4H, C-4 protons of sugars), 1.26-1.36 (4H, C-5 protons of sugars), 1.50-1.60 (12H, 4 X CH_3 C-6 protons of sugars) 3.32, 3.34, 3.35, 3.37 (12H, 4 X CH_3 C-3-OMe protons of sugars). ^{13}C NMR ($CDCl_3$) (see **Table S2**), ESI-MS *m/z* 1043 $[M-H]^-$ and 1067 $[M+Na]^+$, ESI-HRMS *m/z* 1067.5554 $[M+Na]^+$ (calculated), 1067.5525 (found), molecular formula: $C_{56}H_{84}O_{18}$.

Calotroposide A (2): (12-*O*-benzoyllineolon-3-*O*-β-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside): A white amorphous solid, m.p. 160-167 °C, $[\alpha]_D$ +2.6° (c=1.2, in MeOH at 22 °C). IR ($CHCl_3$) cm^{-1} 3409, 3018, 1676, 1402, 1215, 1058, UV (MeOH) nm (log ε) 230 (4.56), 273 (3.15), 280 (3.01). ^1H NMR ($CDCl_3$) δ (ppm) 1.09, 1.85 (1H both m, CH_2-1), 1.91, 2.03 (1H both m, CH_2-2), 3.57 (1H, m, H-3), 2.25, 2.40 (1H both m, CH_2-4), 5.38 (1H, brs, H-6), 2.23 (2H, m, CH_2-7), 1.61 (1H, m, H-9), 2.01, 1.53 (1H both m, CH_2-11), 4.85 (1H, m, H-12), 2.12, 1.88 (1H both m, CH_2-15), 2.07, 2.17 (1H both m, CH_2-16), 2.88 (1H, m, H-17), 1.51 (3H, s, CH_3-18), 1.13 (3H, s, CH_3-19), 2.04 (3H, s, CH_3-21) (pregnanone moiety), 7.94 (2H, d, *J*=7.4 Hz, H-2',6'), 7.43 (2H, t, *J*=7.4 Hz, H-3',5'), 7.55 (1H, t, *J*=7.5 Hz, H-4') (benzoyl moiety), 4.85, 4.65, 4.42, 4.73, 4.86 (1H each dd, J_1= 9.72 Hz, J_2=1.51 Hz) (anomeric protons), 1.10-1.40 (2H X 5 = 10H, C-2 protons of sugars), 3.79-3.86 (5H, C-3 protons of sugars), 3.21-3.27 (6H, C-4 protons of sugars), 1.26-1.36 (5H, C-5 protons of sugars), 1.57-1.65 (15H, 5 X CH_3 C-6

protons of sugars) 3.30-3.35(15H, 5 X CH_3 C-3-OMe protons of sugars). ^{13}C NMR ($CDCl_3$) (see **Table S2**), ESI-MS *m/z* 1187 $[M-H]^-$, 1206 $[M+NH_4]^+$ and 1211 $[M+Na]^+$, ESI-HRMS *m/z* 1211.6340 $[M+Na]^+$ (calculated), 1211.6285 (found), molecular formula: $C_{63}H_{96}O_{21}$.

Calotroposide B (3): (12-*O*-benzoyldeacetylmetaplexigenin-3-*O*-*β*-D-cymaropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside): A white amorphous solid, m.p. 185-190 °C $[\alpha]_D$ +13.4° (c=1.2, in MeOH at 22 °C). IR ($CHCl_3$) cm^{-1} 3402, 3018, 1676, 1402, 1215, 1058. UV (MeOH) nm (log ε) 231 (4.06), 271 (3.05), 280 (3.01). ^1H NMR ($CDCl_3$) *δ* (ppm) 1.09, 1.85 (1H both m, CH_2-1), 2.03, 1.89 (1H both m, CH_2-2), 3.56 (1H, m, H-3), 2.25, 2.41 (1H both m, CH_2-4), 5.34 (1H, brs, H-6), 2.01, 2.24 (1H both m, CH_2-7), 1.60 (1H, m, H-9), 2.02, 1.53 (1H both m, CH_2-11), 4.87 (1H, m, H-12), 2.12, 1.88 (1H both m, CH_2-15), 2.07, 2.20 (1H both m, CH_2-16), 1.51 (3H, s, CH_3-18), 1.12 (3H, s, CH_3-19), 2.05 (3H, s, CH_3-21) (pregnanone moiety), 7.95 (2H, d, *J*=7.5 Hz, H-2',6'), 7.44 (2H, t, *J*=7.5 Hz, H-3',5'), 7.54 (1H, t, *J*=7.5 Hz, H-4') (benzoyl moiety), 4.83, 4.65, 4.40, 4.73, 4.85 (1H each dd, J_1= 9.72 Hz, J_2=1.51 Hz) (anomeric protons), 1.12-1.40 (2H X 5 = 10H, C-2 protons of sugars), 3.79-3.86 (5H, C-3 protons of sugars), 3.21-3.26 (6H, C-4 protons of sugars), 1.26-1.35 (5H, C-5 protons of sugars), 1.61-1.67 (15H, 5 X CH_3 C-6 protons of sugars) 3.34, 3.35, 3.36, 3.37,3.41 (15H, 5 X CH_3 C-3-OMe protons of sugars). ^{13}C NMR ($CDCl_3$) (see **Table S2**), ESI-MS *m/z* 1203 $[M-H]^-$, 1222 $[M+NH_4]^+$ and 1227 $[M+Na]^+$, ESI-HRMS *m/z* 1227.6290 $[M+Na]^+$ (calculated), 1227.6273 (found), molecular formula: $C_{63}H_{96}O_{22}$.

Calotroposide D (4): **(**12-*O*-benzoyllineolon-3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside): A white amorphous solid, m.p. 175-178 °C, $[\alpha]_D$ -16.8° (c=1.1, in MeOH at 22 °C). IR ($CHCl_3$) cm^{-1} 3407, 3016, 1711, 1274, 1090. UV (MeOH) nm (log ε) 230 (4.56), 273 (3.15), 280 (3.01). ^1H NMR ($CDCl_3$) *δ* (ppm) 1.11, 1.83 (1H both m, CH_2-1), 2.03, 1.98 (1H both m, CH_2-2), 3.55 (1H, m, H-3), 2.24, 2.30 (1H both m, CH_2-4), 5.39 (1H, brs, H-6), 2.01, 2.25 (1H both m, CH_2-7), 1.66 (1H, m, H-9), 2.01, 1.53 (1H both m, CH_2-11), 4.92 (1H, m, H-12), 2.10, 1.87 (1H both m, CH_2-15), 2.15, 2.25 (1H both m, CH_2-16), 2.89 (1H, m, H-17), 1.51 (3H, s, CH_3-18), 1.12 (3H, s, CH_3-19), 2.01 (3H, s, CH_3-21) (pregnanone moiety), 7.91 (2H, d, *J*=7.5 Hz, H-2',6'), 7.45 (2H, t, *J*=7.5 Hz, H-3',5'), 7.52 (1H, t, *J*=7.5 Hz, H-4') (benzoyl moiety), 4.84, 4.66, 4.42, 4.71, 4.85 (1H each dd, J_1=9.72 Hz, J_2=1.51 Hz) (anomeric protons), 1.62-

1.77 (2H X 5 = 10H, C-2 protons of sugars), 3.75-3.86 (5H, C-3 protons of sugars), 3.21-3.25 (6H, C-4 protons of sugars), 1.25-1.35 (5H, C-5 protons of sugars), 1.61, 1.65 (15H, 5 X CH_3, C-6 protons of sugars) 3.31, 3.32, 3.33, 3.37, 3.38 (15H, 5 X CH_3 C-3-OMe protons of sugars). ^{13}C NMR ($CDCl_3$) (see **Table S2**), ESI-MS m/z 1187 $[M-H]^-$, 1206 $[M+NH_4]^+$ and 1211 $[M+Na]^+$, ESI-HRMS m/z 1211.6340 $[M+Na]^+$ (calculated), 1211.6335 (found), molecular formula: $C_{63}H_{96}O_{21}$.

Calotroposide C (5): (12-*O*-benzoyldeacetylmetaplexigenin-3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside): A white amorphous solid, m.p. 191-196 °C, $[\alpha]_D$ -2.5° (c=1.3, in MeOH at 22 °C). IR ($CHCl_3$) cm^{-1} 3400, 3019, 1654, 1385, 1083. UV (MeOH) nm (log ε) 230 (4.06), 273 (3.05), 280 (3.01). ^{1}H NMR ($CDCl_3$) δ (ppm) 1.11, 1.85 (1H both m, CH_2-1), 2.03, 1.98 (1H both m, CH_2-2), 3.56 (1H, m, H-3), 2.24, 2.31 (1H both m, CH_2-4), 5.35 (1H, brs, H-6), 2.01, 2.26 (1H both m, CH_2-7), 1.65 (1H, m, H-9), 2.00, 1.50 (1H both m, CH_2-11), 4.91 (1H, m, H-12), 2.10, 1.88 (1H both m, CH_2-15), 2.14, 2.23 (1H both m, CH_2-16), 1.60 (3H, s, CH_3-18), 1.13 (3H, s, CH_3-19), 2.02 (3H, s, CH_3-21) (pregnanone moiety), 7.92 (2H, d, *J*=7.5 Hz, H-2',6'), 7.41 (2H, t, *J*=7.5 Hz, H-3',5'), 7.54 (1H, t, *J*=7.5 Hz, H-4') (benzoyl moiety), 4.82, 4.65, 4.42, 4.73, 4.67 (1H each dd, J_1= 9.72 Hz, J_2=1.51 Hz) (anomeric protons), 1.61-1.71 (2H X 5 = 10H, C-2 protons of sugars), 3.77-3.87 (5H, C-3 protons of sugars), 3.22-3.26 (6H, C-4 protons of sugars), 1.20-1.35 (5H, C-5 protons of sugars), 1.61, 1.70 (15H, 5 X CH_3 C-6 protons of sugars) 3.353,.35, 3.36, 3.37, 3.38 (15H, 5 X CH_3 C-3-OMe protons of sugars). ^{13}C NMR ($CDCl_3$) (see **Table S2**), ESI-MS *m/z* 1203 $[M-H]^-$, 1222 $[M+NH_4]^+$ and 1227 $[M+Na]^+$, ESI-HRMS m/z 1227.6290 $[M+Na]^+$ (calculated), 1227.6278 (found), molecular formula: $C_{63}H_{96}O_{22}$.

Table S2: ^{13}C chemical shift values (ppm) of calotroposides (1-7)

Position of Carbon	1	2	3	4	5	6	7
1	39.0	39.0	38.8	39.0	39.0	38.9	38.9
2	32.1	31.6	33.3	31.6	32.1	32.1	33.4
3	77.1	72.8	76.9	77.2	77.1	77.3	77.6
4	38.5	38.4	38.4	38.4	38.4	38.7	38.9
5	140.7	140.8	141.3	141.4	140.8	140.7	141.3
6	117.8	117.5	117.3	117.8	117.8	117.5	117.8
7	33.3	33.4	33.5	34.7	34.2	35.1	35.2
8	74.5	73.8	72.3	72.1	73.1	74.1	74.2
9	43.8	44.2	43.7	44.2	43.8	43.7	43.8
10	37.2	36.5	36.5	36.5	36.5	36.6	34.0
11	22.3	22.1	23.5	22.5	21.5	21.9	21.7
12	74.1	73.4	73.1	73.4	73.4	73.3	73.3
13	57.2	57.3	57.3	57.3	57.3	55.6	53.6
14	88.2	88.2	88.0	88.2	88.2	88.2	88.2
15	29.7	29.8	29.7	29.8	29.9	29.5	29.5
16	32.1	32.1	31.9	32.1	32.1	32.1	32.1
17	58.5	57.5	91.4	58.5	91.6	59.8	58.2
18	9.7	9.7	9.5	9.7	9.7	9.9	9.7
19	18.9	19.0	18.8	19.0	19.0	19.3	19.0
20	209.6	209.8	209.6	209.8	209.5	209.6	209.9
21	27.5	27.6	27.3	27.6	27.5	27.5	24.5

1'	130.0	130.2	130.2	130.4	130.0	130.3	130.3
2'	128.4	128.5	128.3	128.5	128.5	128.6	128.5
3'	129.6	129.7	129.5	129.7	129.7	129.7	129.7
4'	133.0	133.1	132.9	133.3	133.1	133.1	133.3
5'	129.6	129.7	129.5	129.7	129.7	129.7	129.7
6'	128.5	128.6	128.4	128.6	128.6	128.5	128.6
7'	165.3	165.4	165.2	165.5	165.5	165.3	165.5
Cym 1'	96.2	96.3	96.1	96.3	96.2	96.2	96.2
2'	37.2	36.6	35.5	36.6	35.6	35.6	34.4
3'	77.4	77.4	77.4	77.4	77.4	77.4	77.4
4'	82.6	82.8	82.5	82.6	82.6	82.6	82.6
5'	68.4	71.1	71.7	71.3	71.9	71.8	71.8
6'	18.9	18.7	18.0	18.5	18.1	18.3	18.5
C-3'-OMe	58.3	58.5	59.7	58.2	59.9	58.3	59.8
Cym 1''	99.8	99.9	99.7	99.8	99.9	99.8	99.8
2''	35.6	36.5	35.1	32.5	32.3	33.4	37.4
3''	78.0	78.0	79.1	78.0	78.0	78.0	78.0
4''	82.5	82.7	82.4	82.7	82.7	82.4	82.7
5''	68.7	68.7	68.5	68.7	68.7	68.4	68.7
6''	18.6	18.5	18.2	18.4	18.4	18.6	18.5
C-3''-OMe	58.1	58.4	58.0	58.4	58.4	58.2	58.5
Ole 1'''	100.2	100.3	100.2	100.4	100.3	100.3	100.3
2'''	37.4	34.6	36.3	34.6	36.3	36.6	36.6

3'''	79.3	79.3	79.3	79.3	79.3	79.3	79.1
4'''	82.3	82.6	82.3	82.7	82.2	82.4	82.4
5'''	71.1	72.5	72.3	72.5	72.5	72.5	72.5
6'''	18.3	18.5	18.4	18.4	18.4	18.4	18.3
C-3'''-OMe	57.2	57.3	58.2	57.3	58.2	57.2	58.3
Ole 1''''	101.5	101.5	101.3	101.5	101.5	101.5	101.5
2''''	36.4	34.0	35.5	34.0	35.8	34.6	35.6
3''''	78.0	79.3	79.1	79.1	79.1	79.3	79.3
4''''	80.8	82.4	82.5	82.5	82.7	82.7	82.7
5''''	72.5	72.5	73.2	71.9	73.4	71.2	72.6
6''''	18.1	18.4	18.6	18.1	18.7	18.3	18.7
C-3''''-OMe	56.7	57.3	56.6	55.6	56.8	56.5	55.6
Cym or Ole 1'''''		98.4	98.3	101.5	101.5	98.4	101.5
2'''''		35.8	37.3	35.7	37.5	38.9	38.9
3'''''		75.6	75.5	75.6	75.6	75.6	75.6
4'''''		80.9	80.7	80.9	80.9	80.9	80.9
5'''''		71.1	71.1	71.1	71.1	71.2	71.2
6'''''		19.0	18.6	18.4	18.5	18.1	18.1
C-3'''''-OMe		56.8	55.4	56.8	55.6	56.8	56.5
glu , 1''''''						99.6	99.6
2''''''						71.6	72.3
3''''''						74.0	74.9
4''''''						67.2	67.1

5''''''						71.8	71.8
6''''''						56.5	56.4

Copies of NMR and HR-MS spectra of compounds 6 and 7

1D, 2D NMR and HR-MS spectra of compound (6) (12-*O*-benzoylisolineolon-3-*O*-*β*-D-Cymaropyranosyl-(1-4)-*β*-D-cymaropyranosyl-(1-4)-*β*-D-Oleandropyranosyl-(1-4)-*β*-D-Oleandropyranosyl-(1-4)-*β*-D-cymaropyranosyl (1-4) -*β*-D-glucopyranoside):

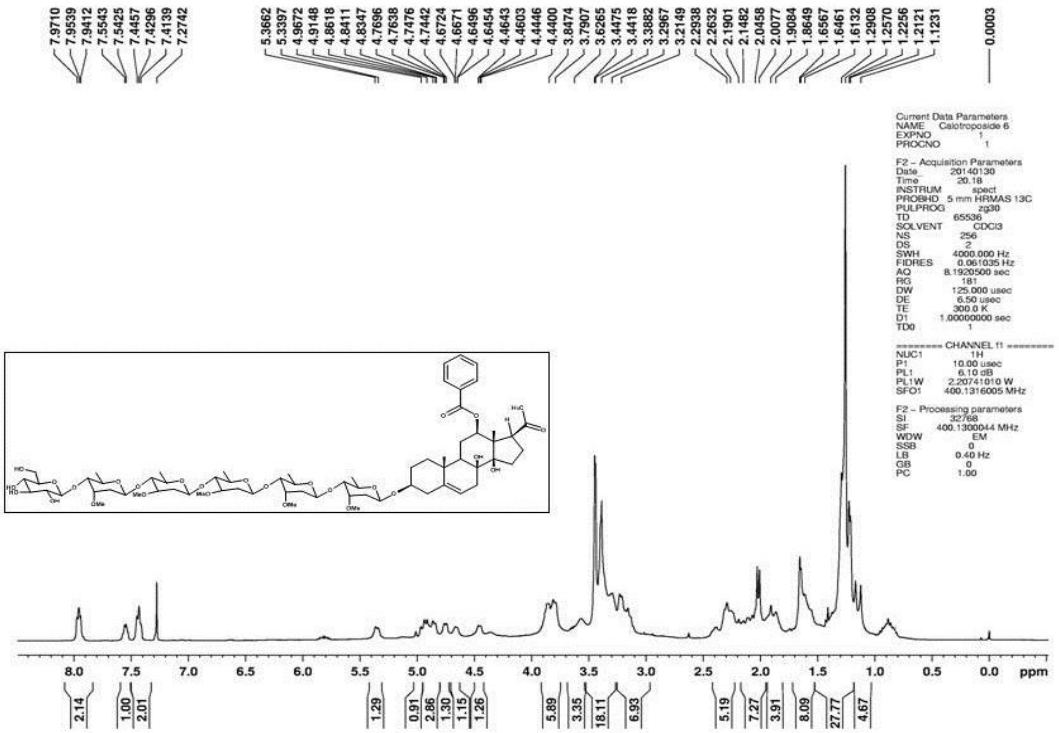

Figure S5: Proton spectrum of compound 6

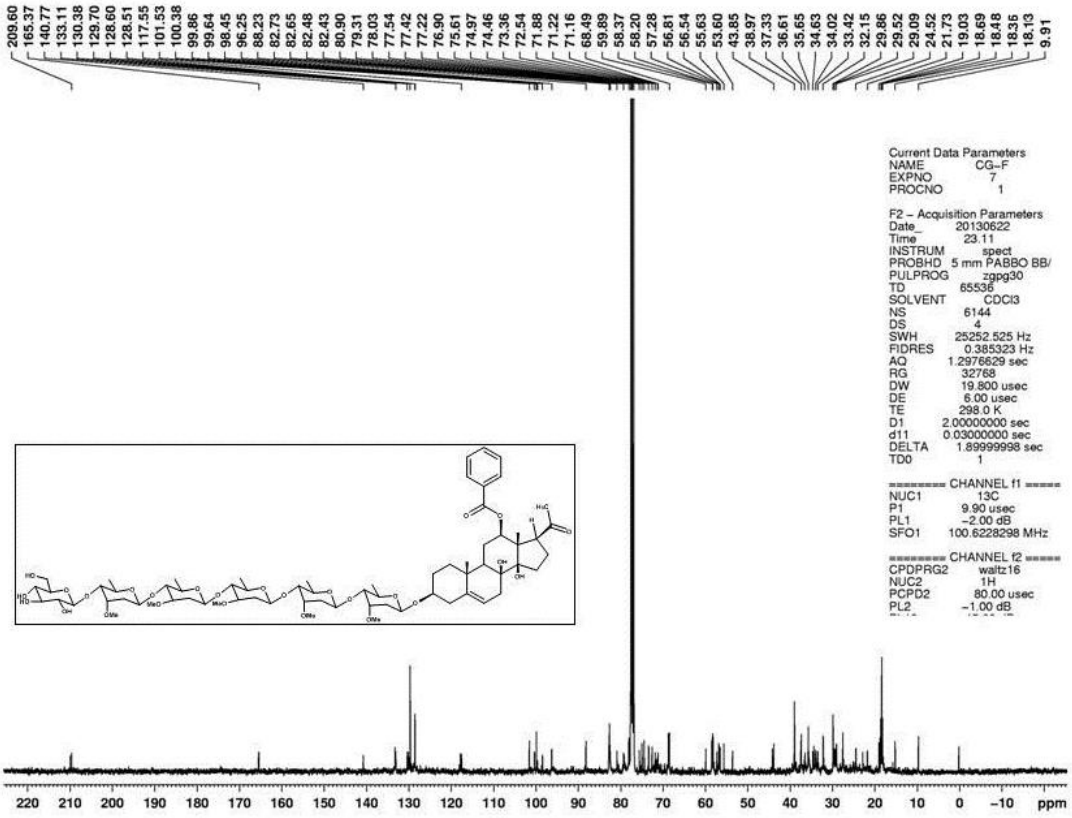

Figure S6: Carbon spectrum of compound 6

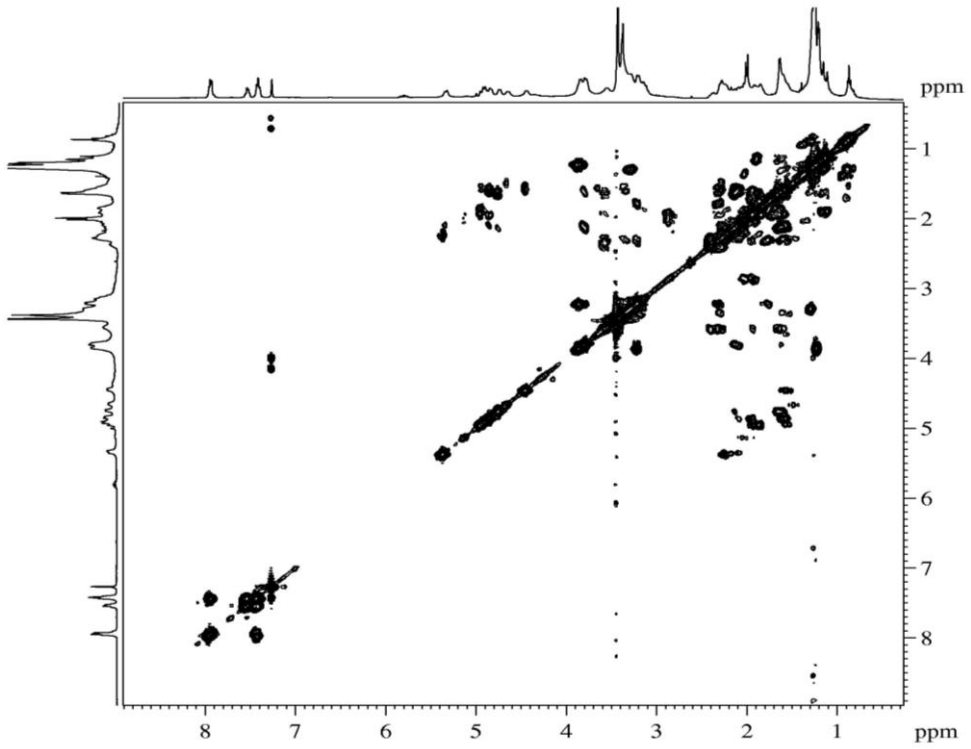

Figure S7: COSY spectrum of compound 6

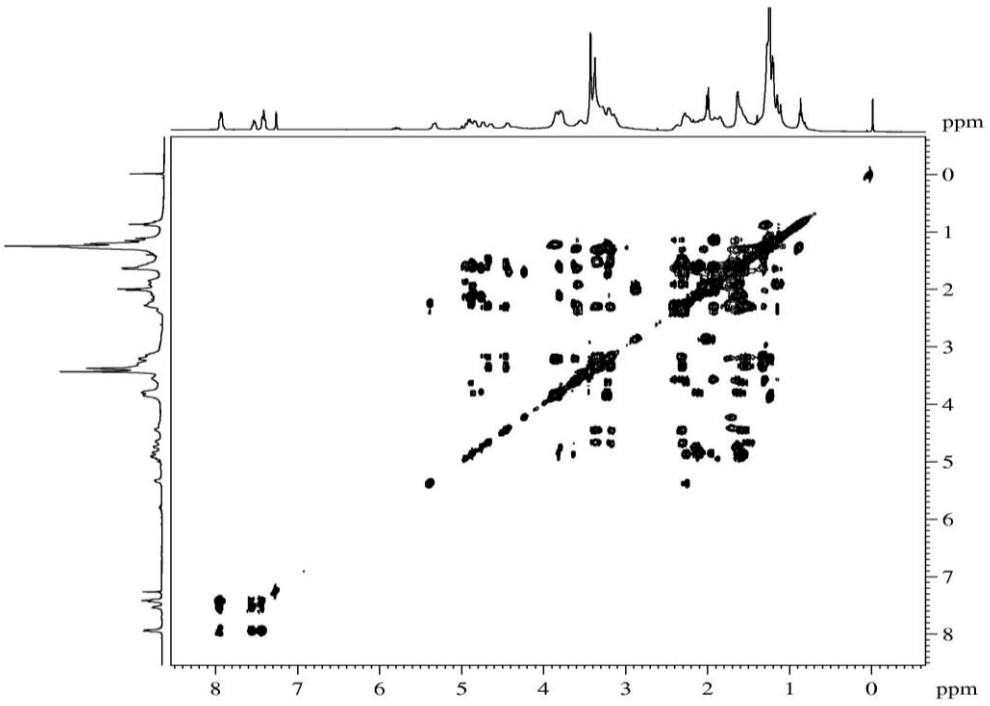

Figure S8: TOCSY spectrum of compound 6

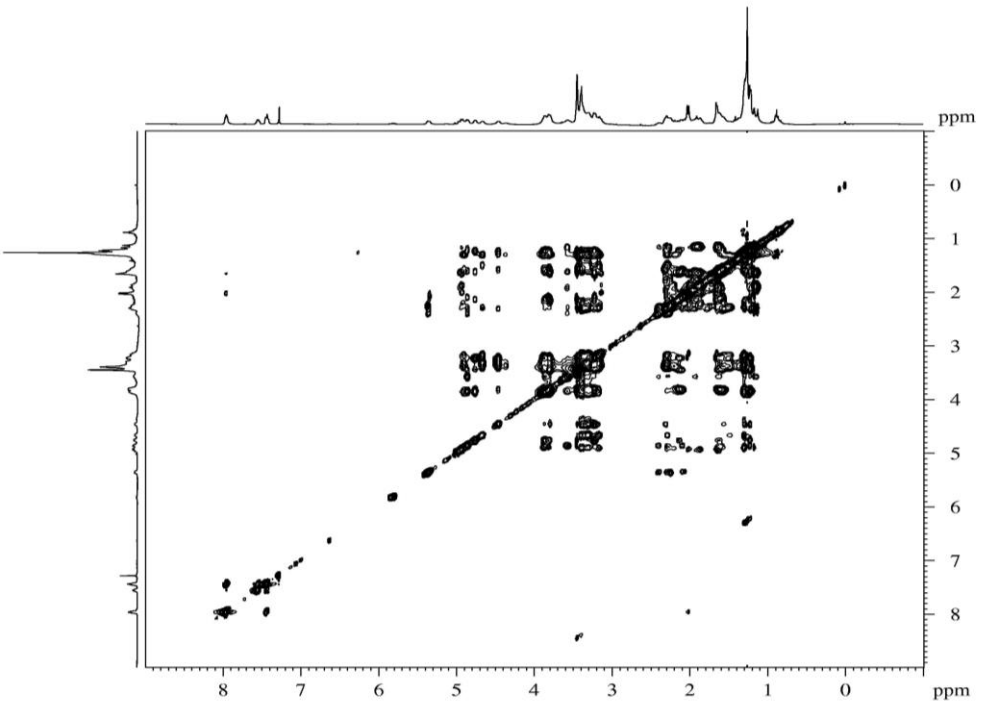

Figure S9: NOESY spectrum of compound 6

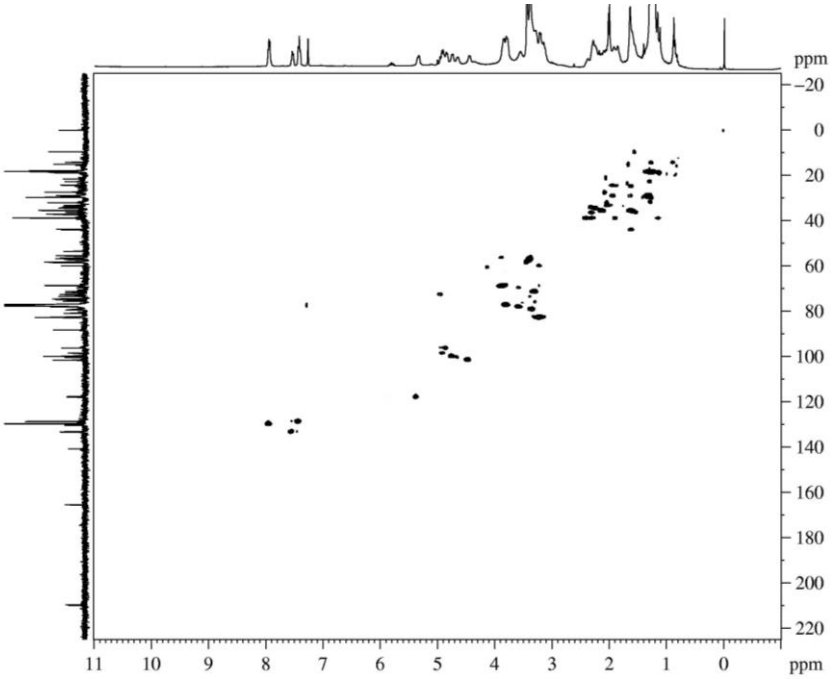

Figure S10: HSQC spectrum of compound 6

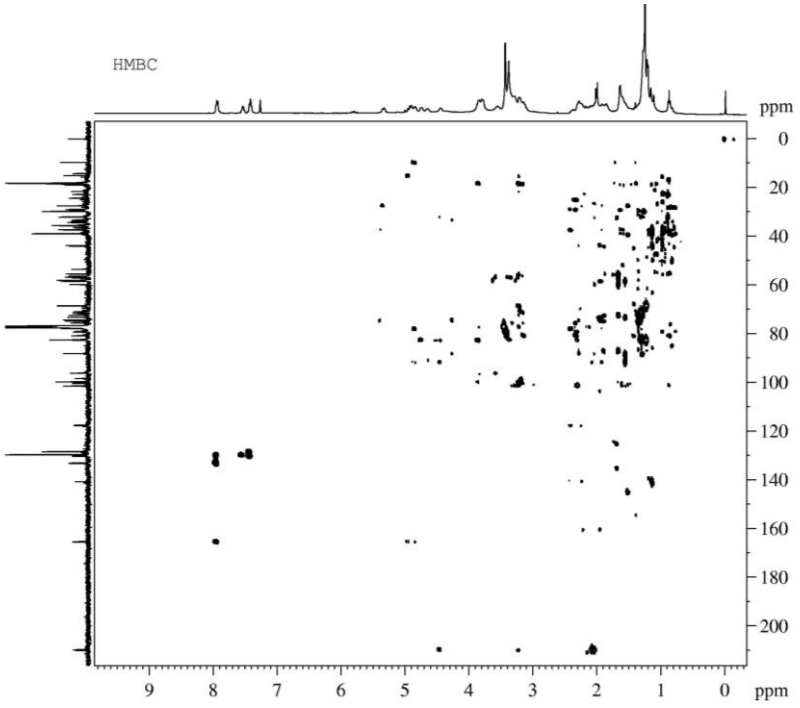

Figure S11: HMBC spectrum of compound 6

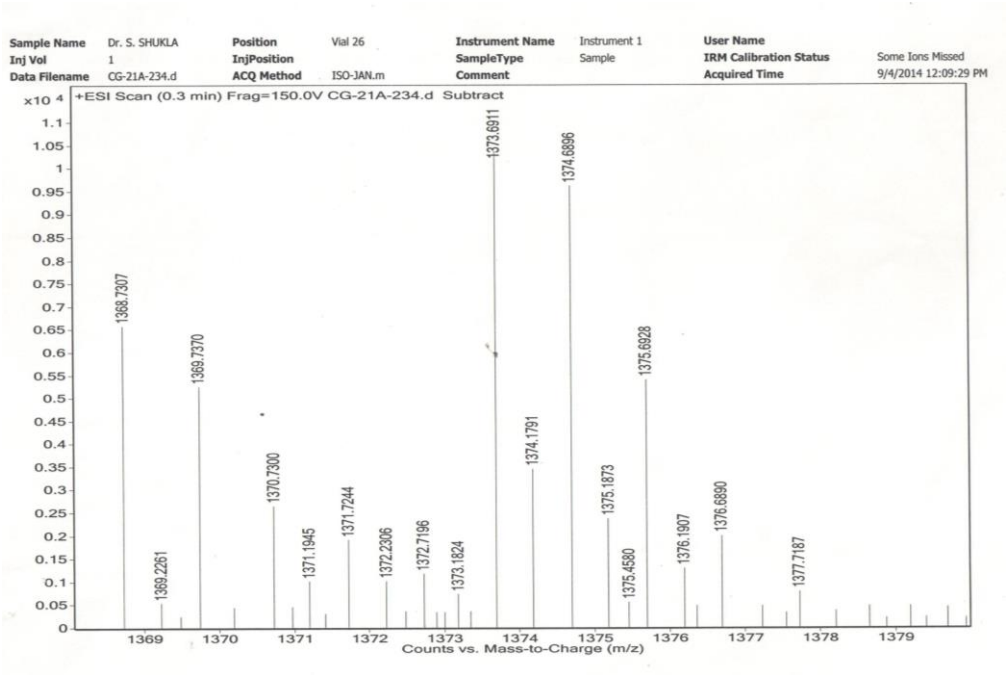

Figure S12: HR-MS spectrum of compound 6

1D and 2D NMR and HR-MS spectra of compound (7) 12-*O*-benzoylisolineolon-3-*O*-*β*-D-Cymaropyranosyl-(1-4-*β*-D-cymaropyranosyl (1-4)-*β*-D-Oleandropyranosyl (1-4)-*β*-D-Oleandropyranosyl (1-4) -*β*-D-Oleandropyranosyl (1-4) -*β*-D- glucopyranoside:

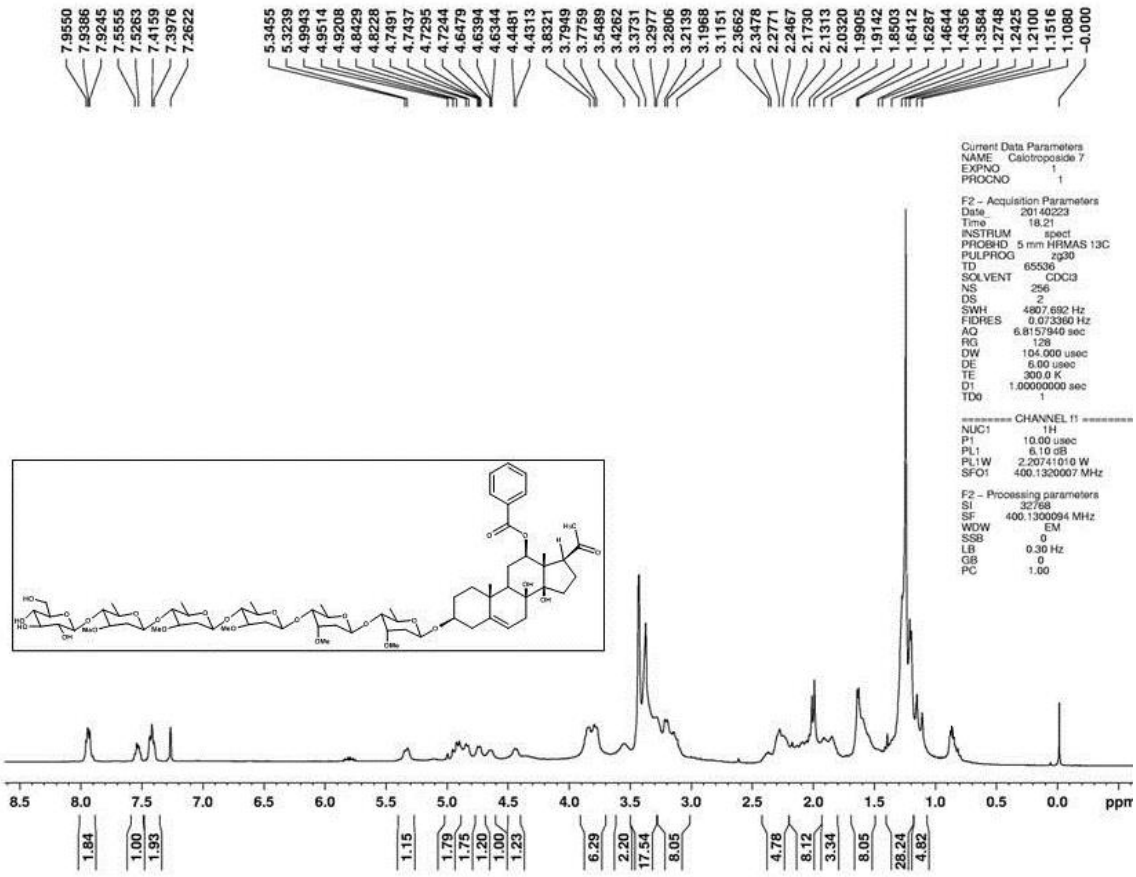

Figure S13: Proton spectrum of compound 7

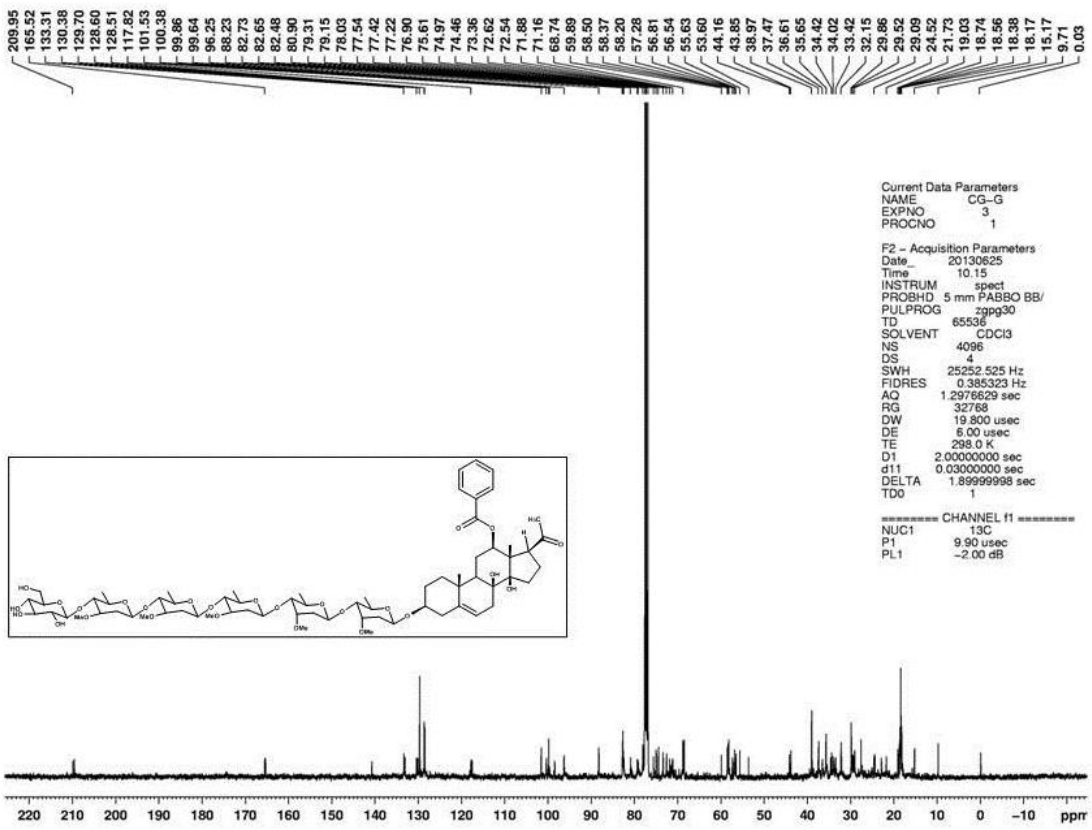

Figure S14: Carbon spectrum of compound 7

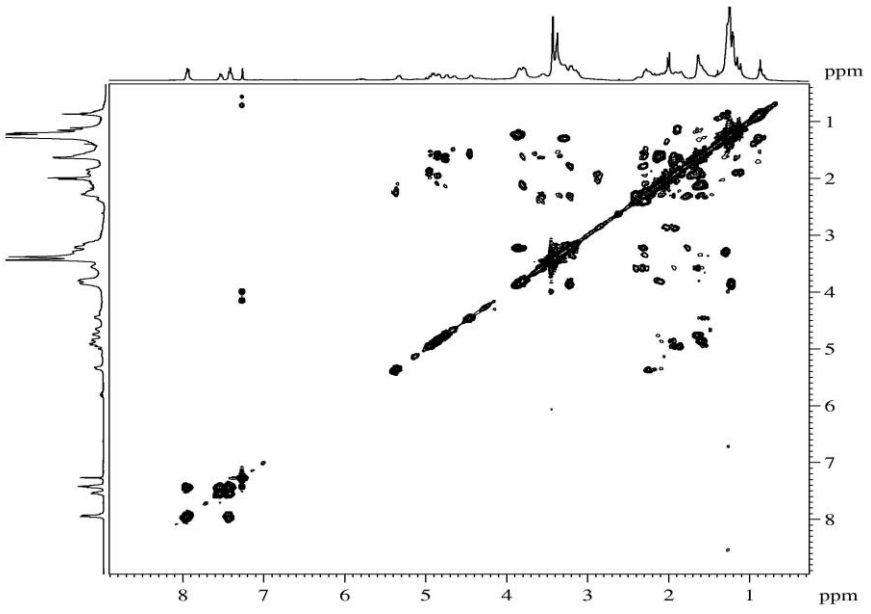

Figure S15: COSY spectrum of compound 7

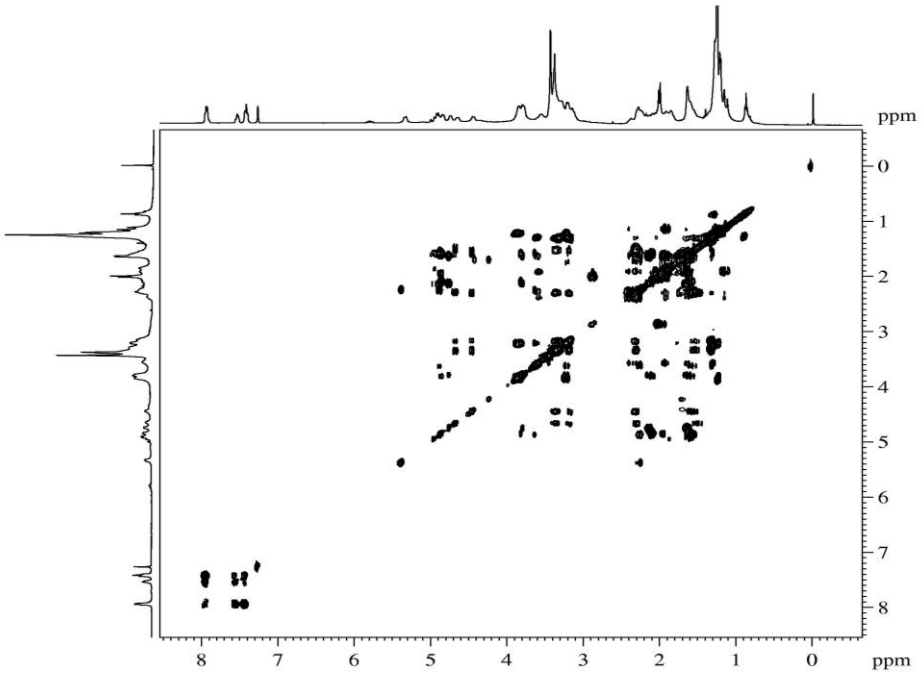

Figure S16: TOCSY spectrum of compound 7

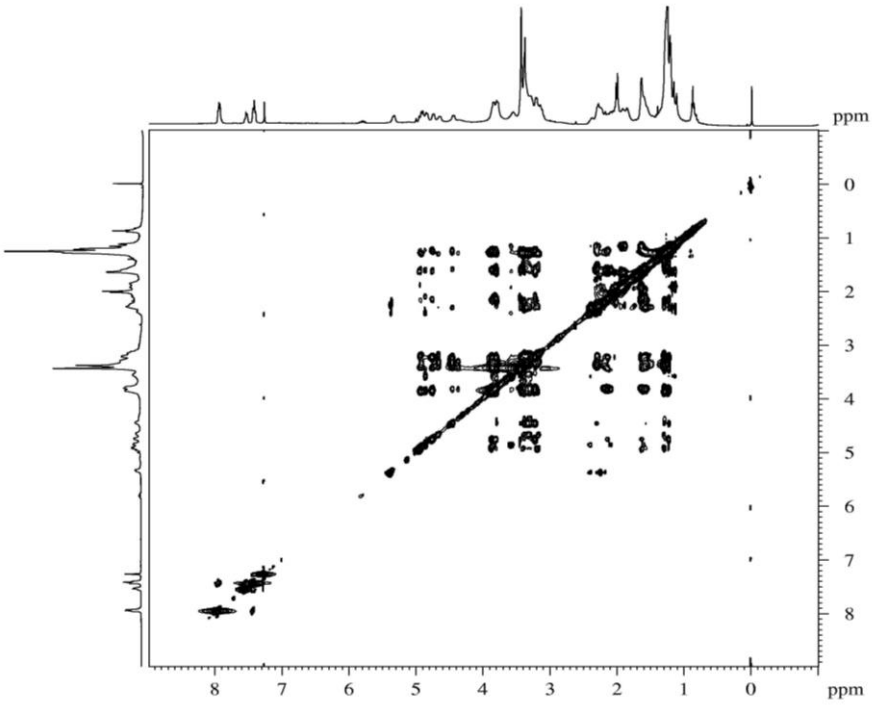

Figure S17: NOESY spectrum of compound 7

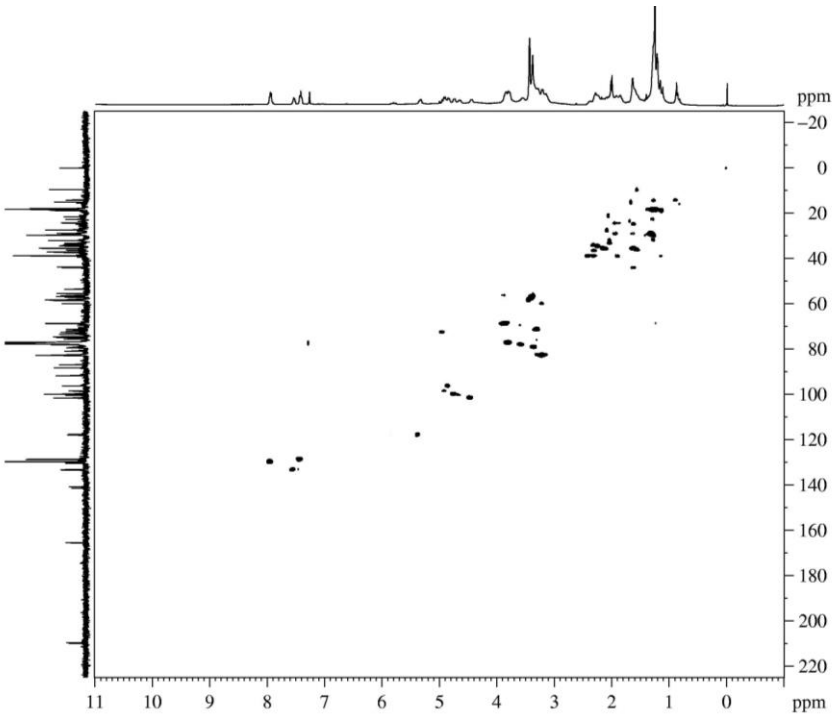

Figure S18: HSQC spectrum of compound 7

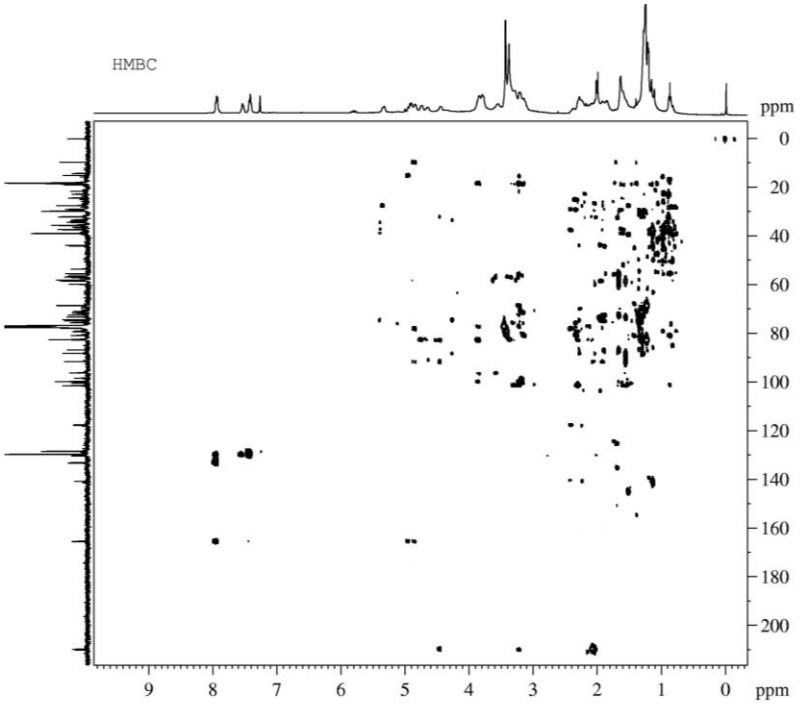

Figure S19: HMBC spectrum of compound 7

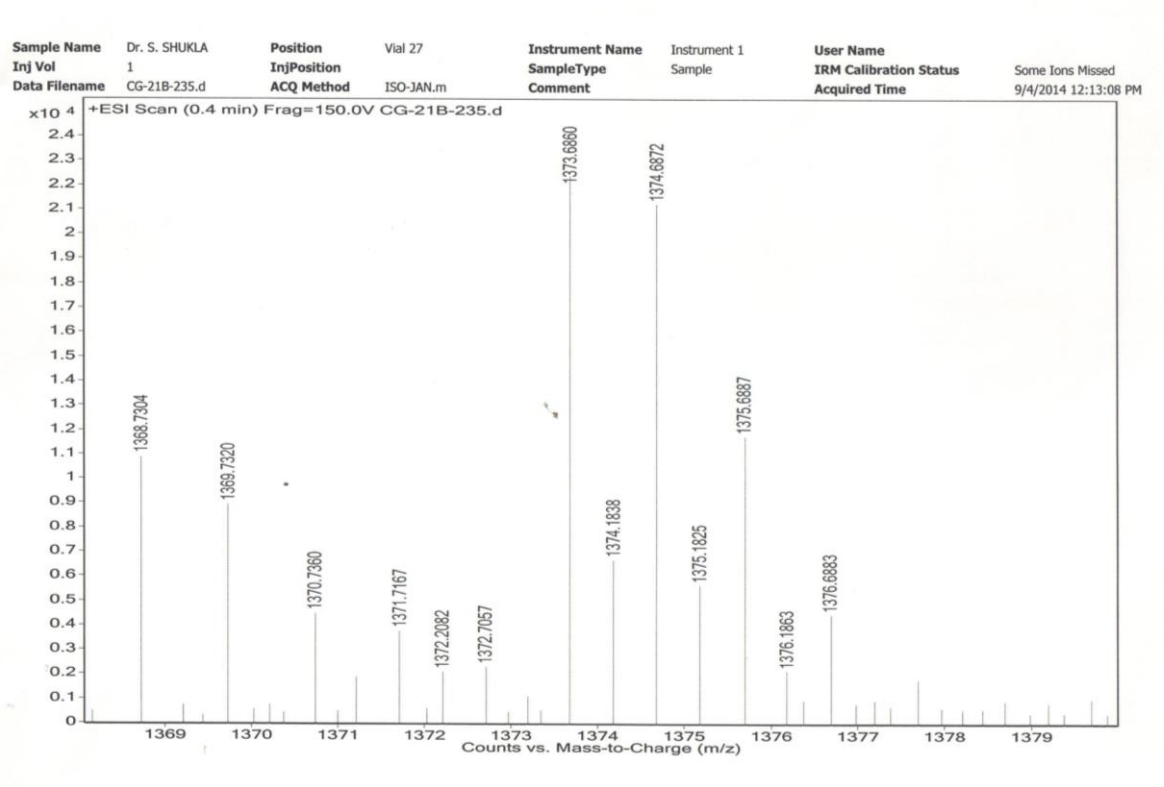

Figure S20: HR-MS spectrum of compound 7