SUPPLEMENTARY MATERIAL

Potential of South African Medicinal plants targeting the reduction of Aβ42 Protein as a treatment of Alzheimer's disease

TABLE S1: ^{1}H NMR and ^{13}C NMR data for Crotoxigenin-3-*O*-*β*-digitalopyranosyl-(1-4)-*O*-*β*-*digitoxopyanoside* [A] in methanol-d4 compared to those reported by (Rakotondramanga et al., 2016) in DMSO-d6

Position	**δH (observed ppm), *J* (Hz)**	**δH (literature ppm), *J* (Hz)**	**δC (observed ppm)**	**δC (literature ppm)**
Aglycone				
1	2.2 (2H, m)	2.42 (2H, dt, J=13.5,3.5,3.5)	31.3	31.4
2	1.86, 1.63 (2H, m)	2.12, 1.62 (2H)	28.7	32.5
3	4.08 (1H, br.s)	4.02 (1H, br,s)	72.09	76.0
4	1.44 (2H, m)	1.44 (2H)	20.6	35.6
5	1.86 (1H, m)	1.66 (1H)	34.5	42.83
6	1.84, 1.53 (2H, m)	1.88, 1.25 (2H)	27.9	30.3
7	1.83, 1.59 (2H, m)	1.71, 1.22 (2H)	24.6	28.2
8	1.88 (1H, m)	1.65 (1H)	41.61	48.5
9	2.20 (1H, m)	1.74 (1H)	29.5	42.79
10	-	-	50.8	51.36
11	1.61 (2H, m)	1.46,1.19 (2H)	21.4	21.7
12	1.54, 1.63 (2H, m)	1.49, 1.35 (2H)	39.4	39.4
13	-	-	49.68	49.4
14	-	-	84.7	85.0
15	1.77 (2H, m)	2.41, 1.62 (2H)	21.7	26.8
16	2.2, 1.9 (2H, m)	2.11, 1.87 (2H)	26.5	27.7
17	2.8 (1H, dd, J=8.7, 5.9)	2.76 (1H, dd, J=9.4,5.4)	50.8	50.9
18	0.98 (3H, s)	0.87 (3H,s)	14.8	15.6
19	9.44 (1H, s)	9.97 (1H,s)	206.7	208.3
20	-	-	176.0	174.4
21	5.06 (1H, dd, J=18.4, 1.68), 4.9 (1H, dd, J= 18.2, 1.79)	5.01 (1H, dd, J=18.2,1.8) 4.80 (1H, dd, J=18.2,1.8)	73.9	73.5
22	5.94 (1H, br.s)	5.89 (1H, br.s)	116.4	117.8
23	-	-	177.2	174.6
Sugar Moieties				
Digitoxose				
1′	4.95 (1H, m)	4.94 (1H, br.dd, J=1.9,1.2)	95.7	95.3
2′	1.99, 1.77 (2H, m)	1.97, 1.73 (2H)	37.5	37.3
3	4.30 (1H, q, J=6.17, 3.0)	4.24 (1H, q, J=6.4,3.2)	67.4	66.8
4'	3.26 (1H, dd, J=9.4, 2.8)	3.22 (1H, dd, J=9.3,2.9)	82.7	82.8
5'	3.87 (1H, m)	3.85 (1H)	67.1	67.9
6'	1.30 (3H, d, J=6.2)	1.36 (3H, d, J=6.5)	15.5	16.5
Digitalose				
1"	4.37 (3H, d, J=7.8)	4.32 (1H, d, J=7.8)	104.7	
2"	3.58 (1H, dd, J= 9.7, 7.7)	3.7 (1H, dd, J=9.5,7.8)	69.9	70.3
3"	3.15 (1H, dd, J=9.6, 3.1)	3.27 (1H, dd, J= 9.5,3.3)	82.9	83.4
4"	3.89 (1H)	3.22 (1H)	68.3	67.8
5"	3.66 (1H, dd, J=12.5, 6.7)	3.63 (1H, dd, J=6.1,1.4)	70.09	70.6
6"	1.32 (3H, d, J=6.2)	1.32 (3H, d, J=6.2)	17.13	18.2
3"-OCH3	3.48 (3H, s)	3.52(3H, s)	55.9	57.7

TABLE S2: [1]H NMR and [13]C NMR data for desglucouzarin [B] in methanol-d4 compared to those reported by (Gohar et al., 2000) in DMSO-d6

Position	**δH (observed ppm), *J* (Hz)**	**δH (literature ppm), *J* (Hz)**	**δC (observed ppm)**	**δC (literature ppm)**
1	1.07, 1.82 m	0.92,1.75 m	34.6	36.67
2	1.65, 1.89 m	1.75 m	33.9	29.07
3	3.80 m	3.05 m	78.2	76.41
4	1.77 m	1.14, 1.63 m	32.1	33.97
5	1.45 m	0.95 m	41.6	43.77
6	1.41 m	1.13, 1.23 m	26.9	28.53
7	1.91 m	0.92, 1.94 m	26.6	27.27
8	1.67 m	1.42 td (J=2.93)	41.8	40.81
9	1.83 m	0.87 td (J=2.93)	36.2	49.41
10	-	-	34.7	35.46
11	1.49, 1.83 m	1.18, 1.4 m	20.8	20.82
12	1.54 m	1.3, 1.42 m	39.6	38.89
13	-	-	49.8	48.64
14	-	-	85.3	83.69
15	1.79, 2.2 m	1.55t, 1.92t	31.9	32.18
16	2.22 m	1.75, 1.96 m	26.4	26.38
17	2.88 dd (J=9.1, 5.9)	2.72 dd (J=9.6, 5.4)	50.8	50.15
18	0.96 s	0.72 s	22.3	15.73
19	0.92 s	0.75 s	15.1	12.01
20	-	-	177.1	176.46
21	4.95 , 5.01 dd, J=1.49, 18.4	4.88 m, 4.95 dd (J=1.47, 18.32)	73.7	73.2
22	5.92 s	5.89 s	116.8	116.26
23	-	-	176.1	173.96
1’	4.4 d (J=7.80)	4.2 d (J=8.06)	100.8	100.68
2’	3.18 m	2.85 m	73.9	73.51
3’	3.31 m	3.10 m	76.7	76.80
4’	3.32 m	3.01 m	70.2	70.15
5’	3.39 m	3.50 m	76.5	76.41
6’	3.90 m, 3.67 dd (J=5.2, 12)	3.35 m, 3.65 ddd (J=2, 5.4, 12)	61.3	61.15

[1] s: singlet, d: doublet, m: multiplet, dd: doublet-doublet, Chemical shifts are interchangeable due to the different solvents used.

Table S3: Tentative identification of compounds obtained from ESI-MS negative mode of DCM:MeOH extract of *Cussonia paniculata* (leaf)

Peak	RT (min)	Acquired [M-H]- m/z	Formula of Possible structure	Theoretical [M-H]- m/z	Calculated accurate mass (Da)	Possible structure	Mass error (ppm)	MS/MS Data (Fragments)	Confirmation with a standard		Reference
									RT (min)	[M-H]- m/z	
1′	5.60	609.1469	$C_{27}H_{30}O_{16}$	609.1455	610.1533	Rutin	-2.2	301.0353, 300.0292, 271.0259	5.60	609.1454	(Sousa et al., 2014)
2′	10.15	987.5145	$C_{49}H_{80}O_{20}$	987.5164	988.5243	Clethroidoside B	1.9	471.3481, 469.1565	-	-	http://www.ebi.ac.uk/chebi/
3′	10.36	1029.5261	$C_{51}H_{82}O_{21}$	1029.5270	1030.5348	Pseudoprostodioscin	0.8	471.3488, 469.1578	-	-	http://www.ebi.ac.uk/chebi/
4′	10.82	793.4369	$C_{42}H_{66}O_{14}$	793.4374	794.4452	Spinasaponin A	0.6	631.3863	-	-	http://www.ebi.ac.uk/chebi/

Table S4: Tentative identification of compounds obtained from ESI-MS negative mode of DCM:MeOH extract of *Schotia brachypetala* (leaf)

Peak	RT (min)	Acquired [M-H]- m/z	Formula of Possible structure	Theoretical [M-H]- m/z	Calculated accurate mass (Da)	Possible structure	Mass error (ppm)	MS/MS Data (Fragments)	Confirmation with a standard		Reference
									RT (min)	[M-H]- m/z	
1	5.37	463.0875	$C_{21}H_{20}O_{12}$	463.0876	464.0954	Myricetin-3-O-alpha-L-rhamnopyranoside	2.1	317.0262, 271.0243, 179.0014			(Saldanha et al., 2013)
2	5.64	463.0872	$C_{21}H_{20}O_{12}$	463.0876	464.0954	Isoquercetin	0.8	301.0344, 300.0272, 271.0257	5.67	463.0872	(Sánchez-Rabaneda et al., 2003), (Zhou et al., 2011)
3	6.22	447.0946	$C_{21}H_{20}O_{11}$	447.0927	448.1005	Quercetin-3-O-rhamnoside	-4.2	301.0352, 300.0436, 271.0420, 255.0608			(Soong & Barlow, 2005), (Sánchez-Rabaneda et al., 2003)
4	7.12	301.0356	$C_{15}H_{10}O_{7}$	301.0348	302.0426	Quercetin	-2.6	151.0066, 121.0306, 107.0152	7.09	301.0384	(Sánchez-Rabaneda et al., 2003)

Fig. S1: Galantamine did not change the level of Aβ42. APPsw-transfected HeLa cells were incubated with indicated concentrations of galantamine for 8 h. The level of Aβ42 was measured from the conditioned media by using specific ELISA methods. The level of Aβ42 was not changed by galantamine (n = 6).

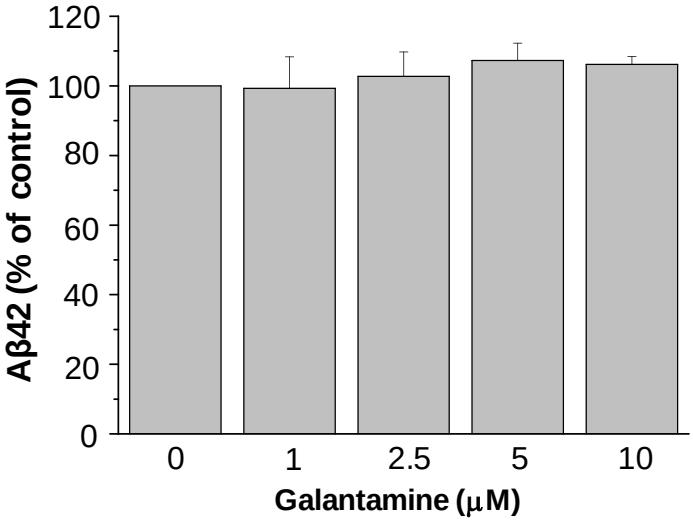


Fig. S2: Effect of fractions on the production of Aβ42 in APPsw-transfected HeLa cells. 15 Fractions were obtained from leaves of X. undulatum by HPLC. Cells were incubated with 50 μg/ml extract and 10 μg/ml fractions for 8 h, and the level of Aβ42 was measured from the conditioned media by specific ELISA methods. The two fractions, AT-1-49N and AT-1-49O, potently decreased the secreted level of Aβ42 (n = 2). *, P<0.05; **, P<0.01; ***, P<0.001.

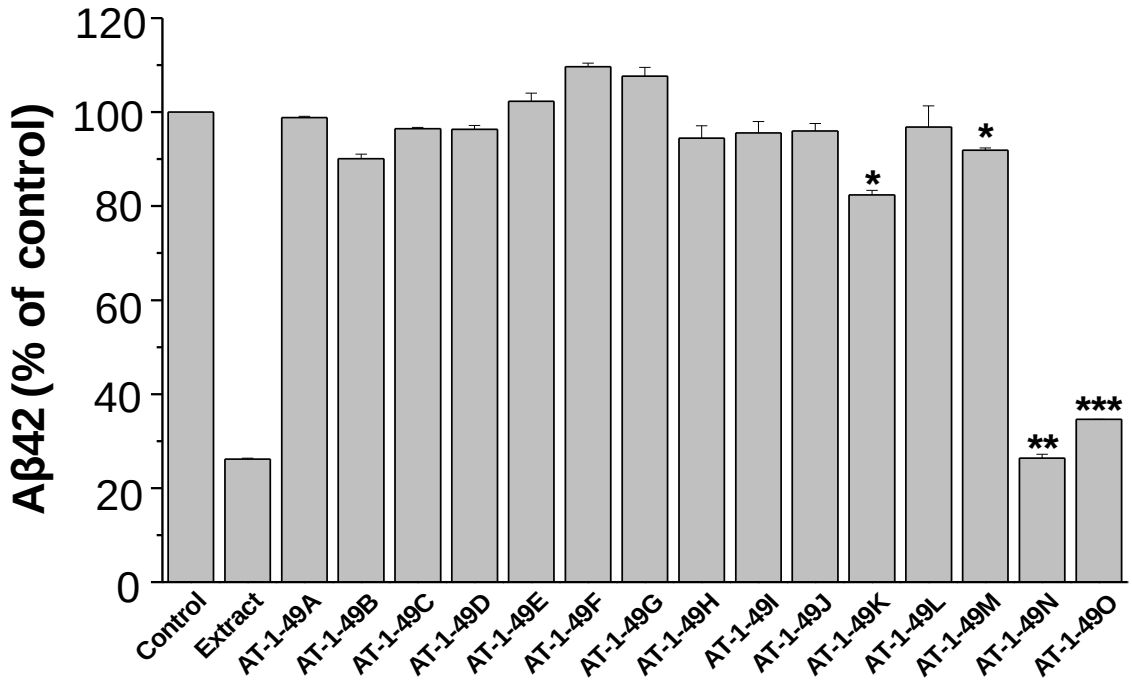


Fig. S3: HPLC chromatogram of Compound [A] isolated from fraction AT-1-49N of *X.undulatum* extract

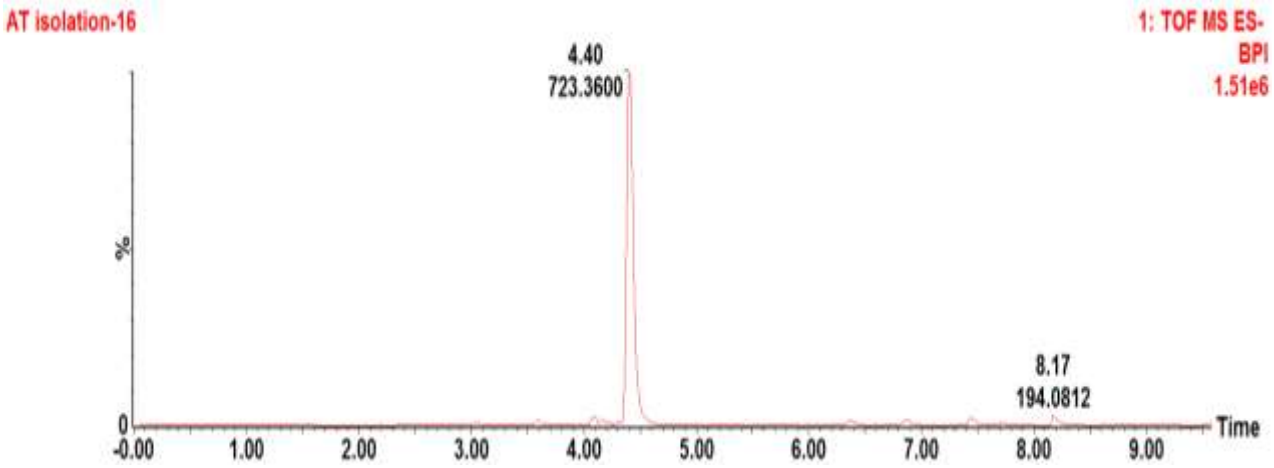


Fig. S4: HPLC chromatogram of Compound [B] isolated from fraction AT-1-49O of *X.undulatum* extract

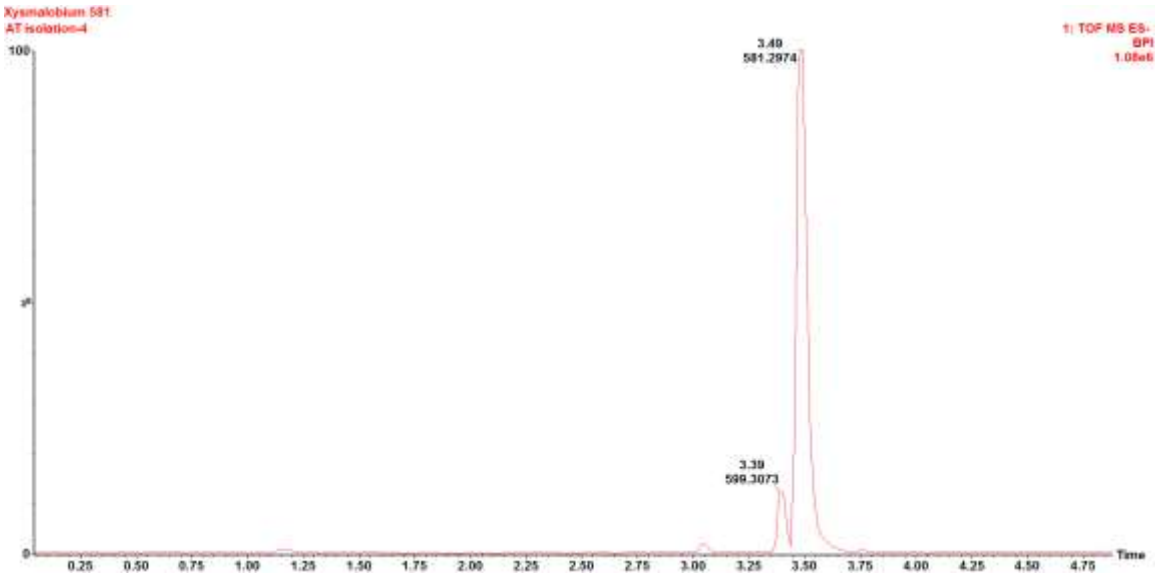


Fig. S5: Compound A and B decreased cell viability. APPsw-transfected HeLa cells were incubated with 10 μM of compound A or B for 8 h. Cell viability was measured using an EZ-Cytox kit. Compound A and B at high concentration (10 μM) induced cytotoxicity (n = 6). **, P<0.01.

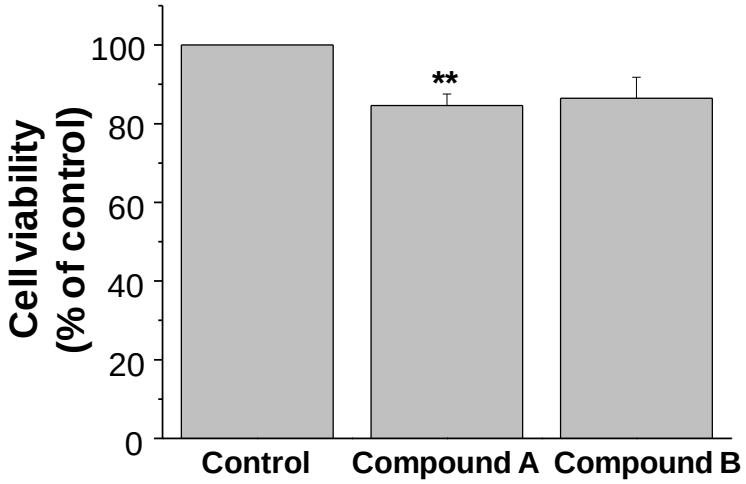


Fig. S6: Comparison of the accurate mass, retention time and the mass fragmentation pattern of the pure standard (a) and identified compound Rutin (b) from *Cussonia paniculata* leaf extracts

(a)

(b)

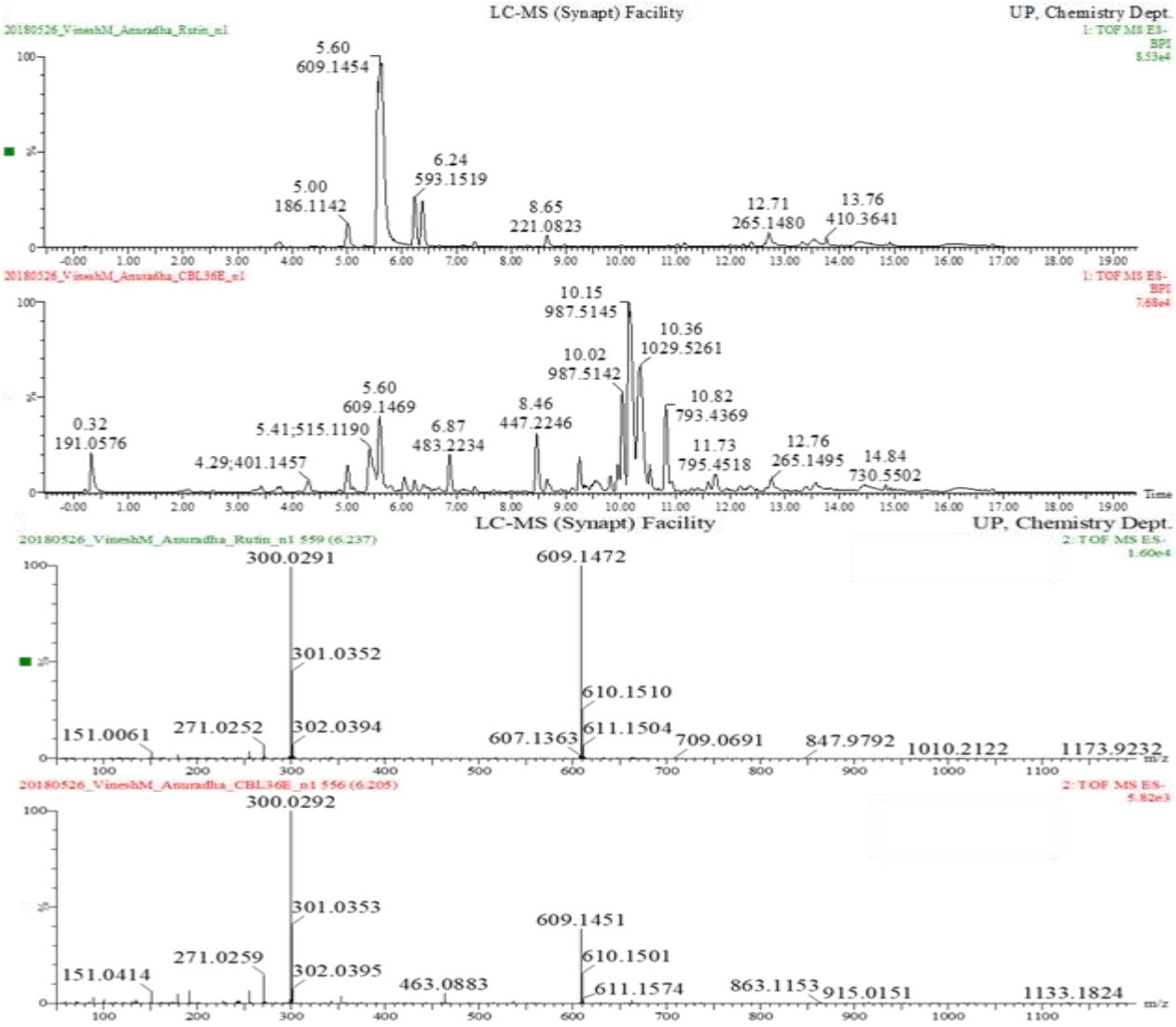


Fig. S7: Comparison of the accurate mass, retention time and the mass fragmentation pattern of the pure standard (a) and identified compound Isoqurcetin (b) from *Schotia brachypetala* leaf extract

(a)

(b)

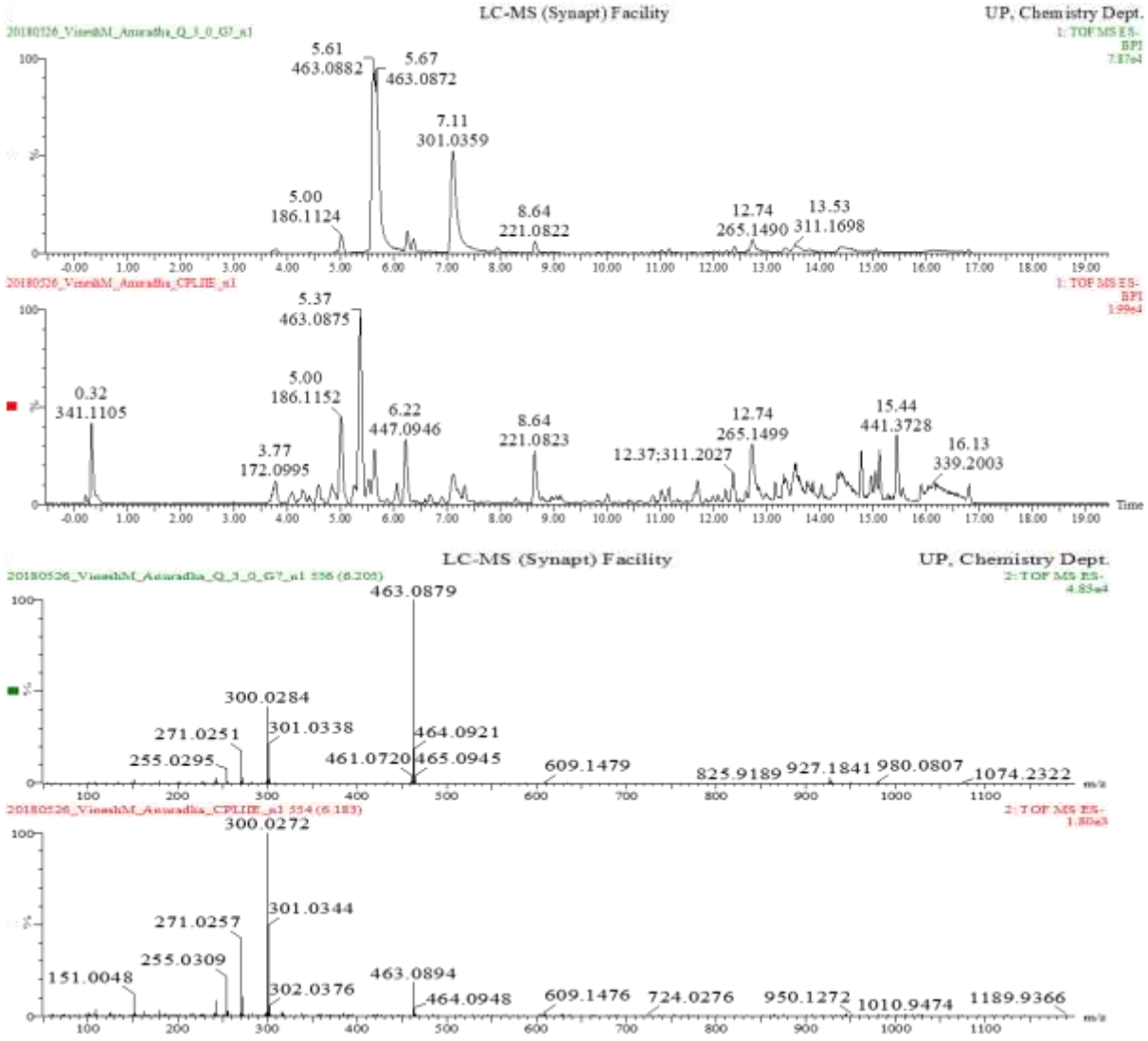
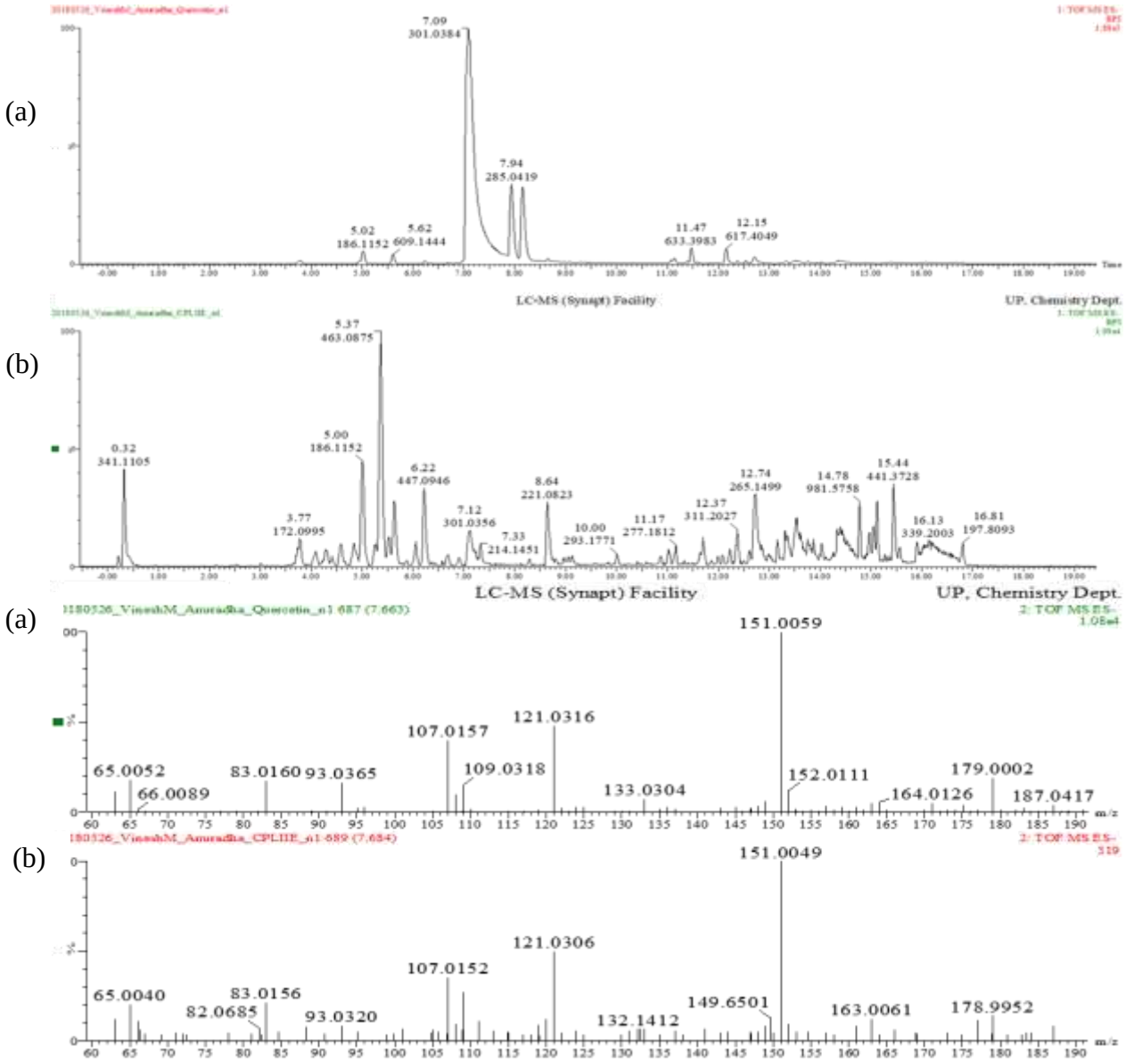


Fig. S8: Comparison of the accurate mass, retention time and the mass fragmentation pattern of the pure standard (a) and identified compound Quercetin (b) from *Schotia brachypetala* leaf extract



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