Supplementary Materials

Comprehensive LC-MS/MS-based phytochemical perspectives and osteogenic effects of *Uraria crinita*

Yi-Tzu Lin[†,a], Yi-Wen Mao[†,b], Zuha Imtiyaz[a], Wen-Fei Chiou[c], and Mei-Hsien Lee[a,d,e,*]

[a] PhD Program for Clinical Drug Development of Herbal Medicine, College of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan; d339102003@tmu.edu.tw (Y.T.L.); d339105004@tmu.edu.tw (Z.I.)

[b] School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan; cutemaomao2007@gmail.com (Y.W.M.)

[c] National Research Institute of Chinese Medicine, Ministry of Health and Welfare, Taipei 11221, Taiwan; wfchiou@nricm.edu.tw (W.F.C.)

[d] Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan

[e] Center for Reproductive Medicine & Sciences, Taipei Medical University Hospital, Taipei 11031, Taiwan

* Corresponding author: Mei-Hsien Lee

E-mail address: lmh@tmu.edu.tw (M.H.L.)

Tel.: +886-2-2736-1661 ext. 6151

[†] These authors contributed equally to this work.

Content

hydroxygenistein (**4**) by UPLC/Q-TOF-MS/MS.

Figure S11 (A) Extracted ion chromatogram, (B) MS, and (C) MS/MS spectra of genistein (**5**) by UPLC/Q-TOF-MS/MS.

Figure S12 (A) Extracted ion chromatogram, (B) MS, and (C) MS/MS spectra of 5,7-dihydroxy-3′,5′-dihydroxyisoflavone (**6**) by UPLC/Q-TOF-MS/MS.

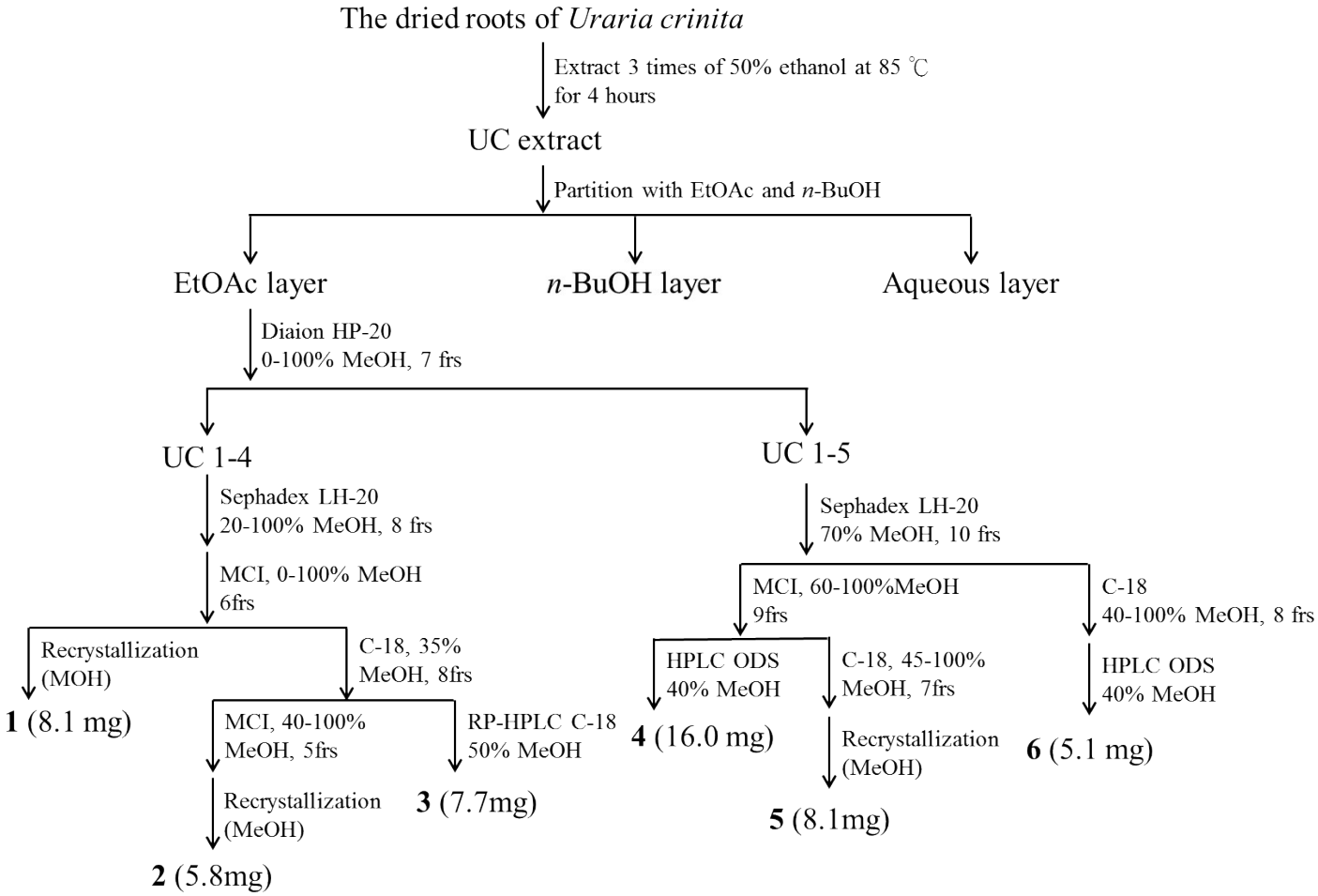


Chart S1 Isolation and purification of compounds from *U. crinita*.

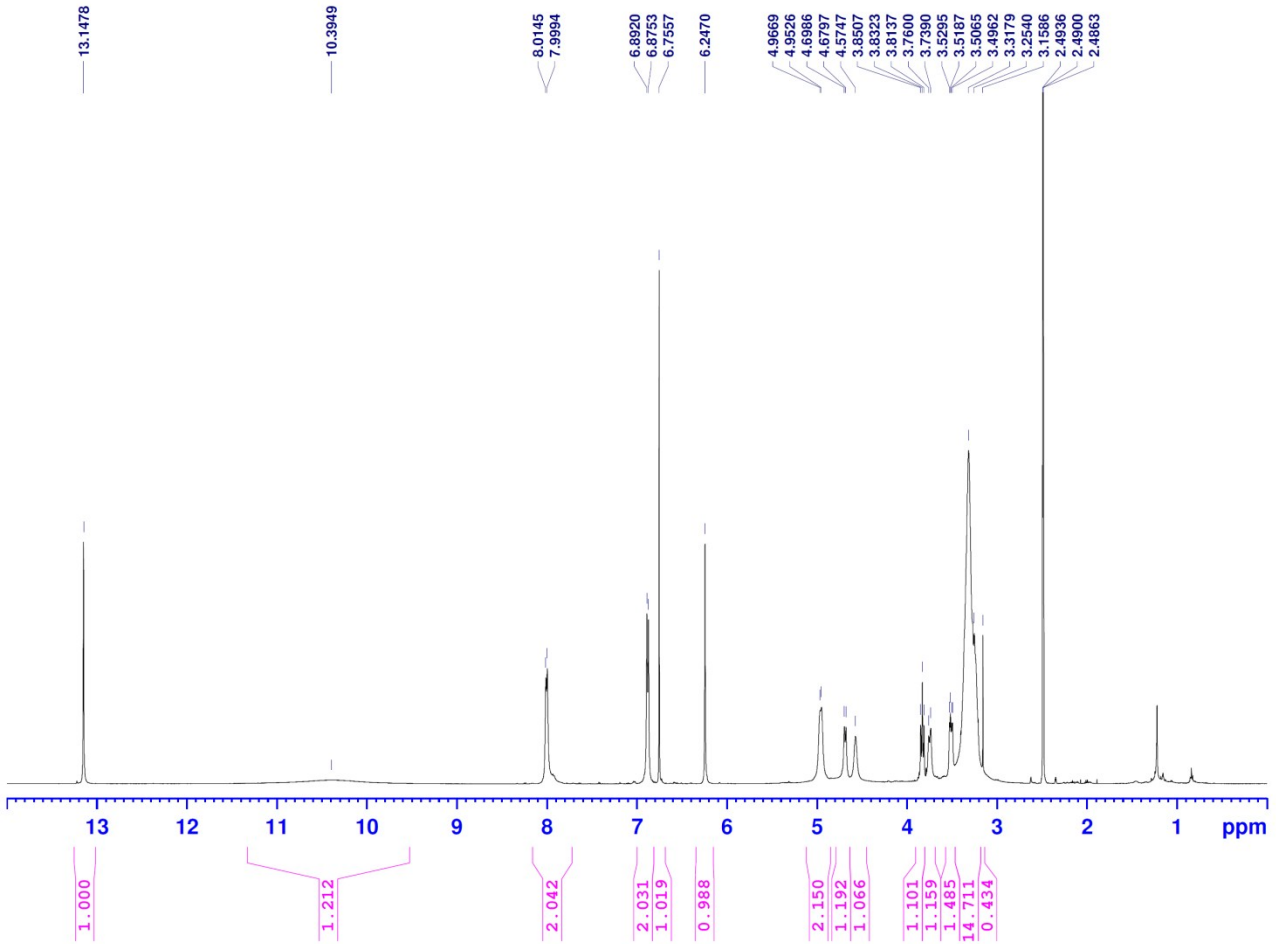
Table S1 HPLC-TQ-MS/MS conditions for the isolated compounds from a 50% ethanol extract of *U. crinita*.

Compounds No.	**MRM transitions (m/z)**		**MS/MS parameters [a] (volts)**					
	Precursor ion	**Product ion**	**DP**	**FP**	**EP**	**CE**	**CEP**	**CXP**
Vitexin (**1**)	431	311	-110	-200	-10	-28	-19.30	-54
	431	283	-110	-200	-10	-45	-19.30	-50
Salicylic acid (**2**)	137	93	-30	-200	-10	-20	-11.95	-20
	137	65	-30	-200	-10	-40	-11.62	-14
Apigenin 6-*C*-*β*-D-apiofuranosyl(1→2)-*α*-D-xylopyranoside (**3**)	533	383	-100	-200	-10	-30	-21.85	-50
	533	341	-100	-200	-10	-40	-21.85	-50
2′-Hydroxygenistein (**4**)	285	217	-80	-200	-10	-26	-15.65	-40
	285	199	-80	-200	-10	-30	-15.65	-40
Genistein (**5**)	269	159	-50	-200	-10	-40	-15.25	-35
	269	133	-50	-200	-10	-45	-15.25	-20
5,7-Dihydroxy-3′,5′-dihydroxyisoflavone (**6**)	285	257	-100	-200	-10	-30	-15.65	-50
	285	229	-100	-200	-10	-40	-15.65	-45

[a] De-clustering potential (DP), focusing potential (FP), entrance potential (EP), collision energy (CE), collision cell entrance potential (CEP), collision cell exit potential (CXP).

Figure S1

(A)

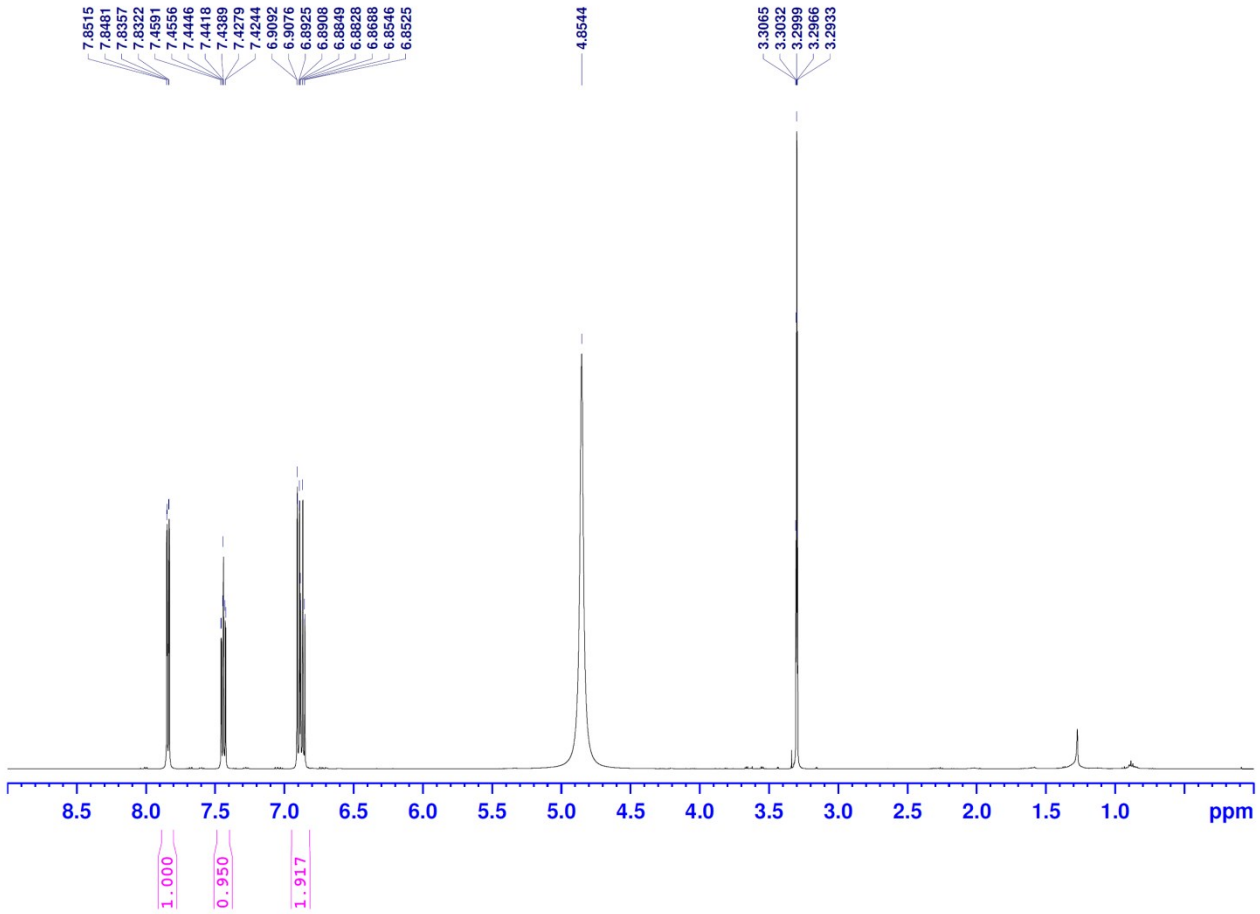

13.1478
10.3949
8.0145
7.9994
6.8920
6.8753
6.7557
6.2470
4.9669
4.9526
4.6986
4.6797
4.5747
3.8507
3.8323
3.8137
3.7600
3.7390
3.5295
3.5187
3.5065
3.4962
3.3179
3.2540
3.1586
2.4936
2.4900
2.4863
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.000
1.212
2.042
2.031
1.019
0.988
2.150
1.192
1.066
1.101
1.159
1.485
14.711
0.434

(B)

Vitexin (**1**). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H 3.25 (1H, m, H-5"), 3.27 (1H, m, H-3"), 3.32 (1H, m, H-4"), 3.51 (1H, dd, J = 11.0, 5.3 Hz, H-6"a), 3.75 (1H, d, J = 11.0 Hz, H-6"b), 3.83 (1H, t, J = 9.3 Hz, H-2"), 4.68 (1H, d, J = 9.5 Hz, H-1"), 6.25 (1H, s, H-6), 6.76 (1H, s, H-3), 6.88 (2H, d, J = 8.4 Hz, H-3', 5'), 8.01 (2H, d, J = 8.4 Hz, H-2', 6'), 12.15 (1H, brs, OH).

Figure S2

(B)

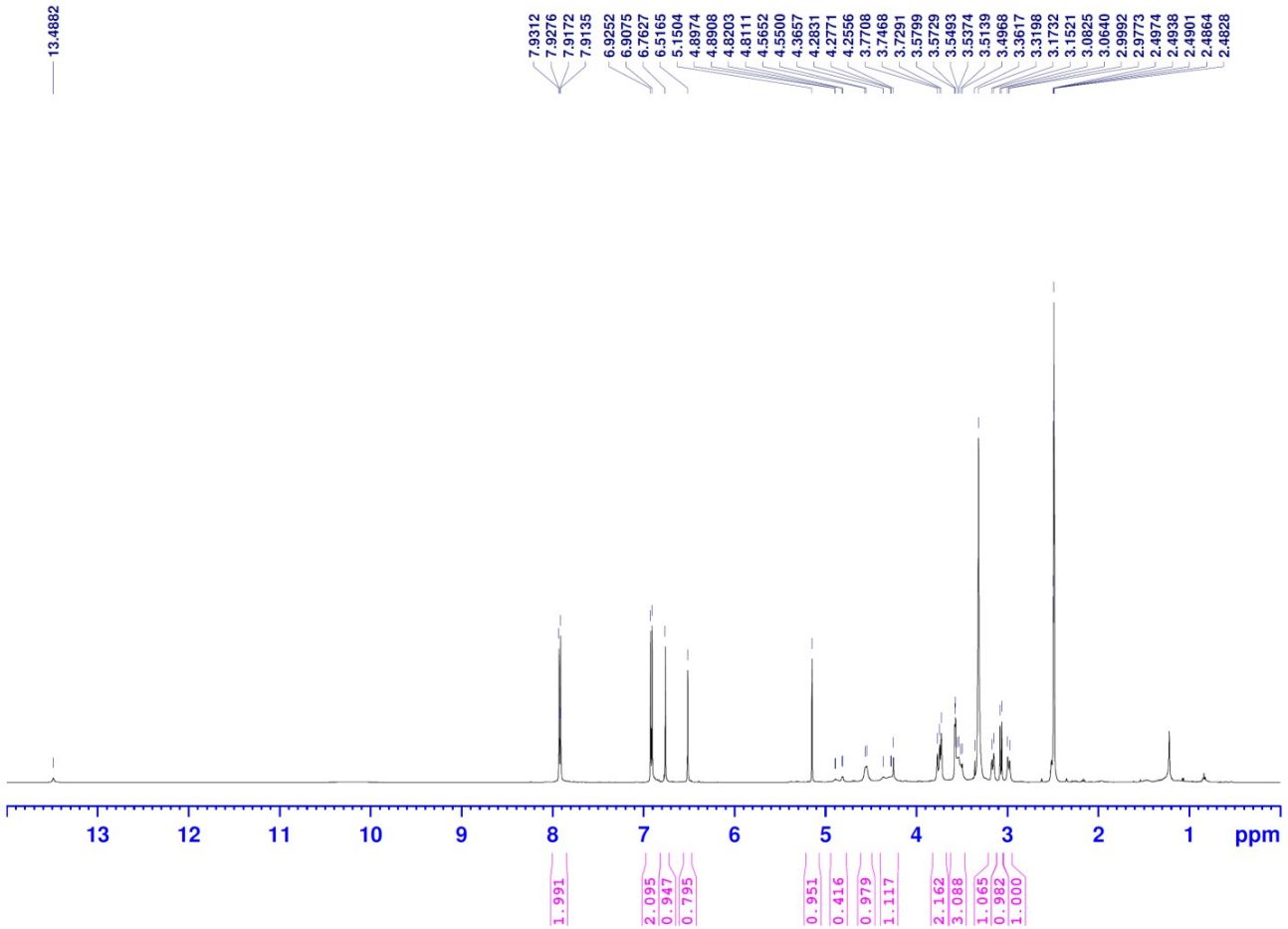

7.8515
7.8481
7.8357
7.8322
7.4591
7.4556
7.4446
7.4418
7.4389
7.4279
7.4244
6.9092
6.9076
6.8925
6.8908
6.8849
6.8828
6.8688
6.8546
6.8525
4.8544
3.3065
3.3032
3.2999
3.2966
3.2933
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.000
0.950
1.917

(B)

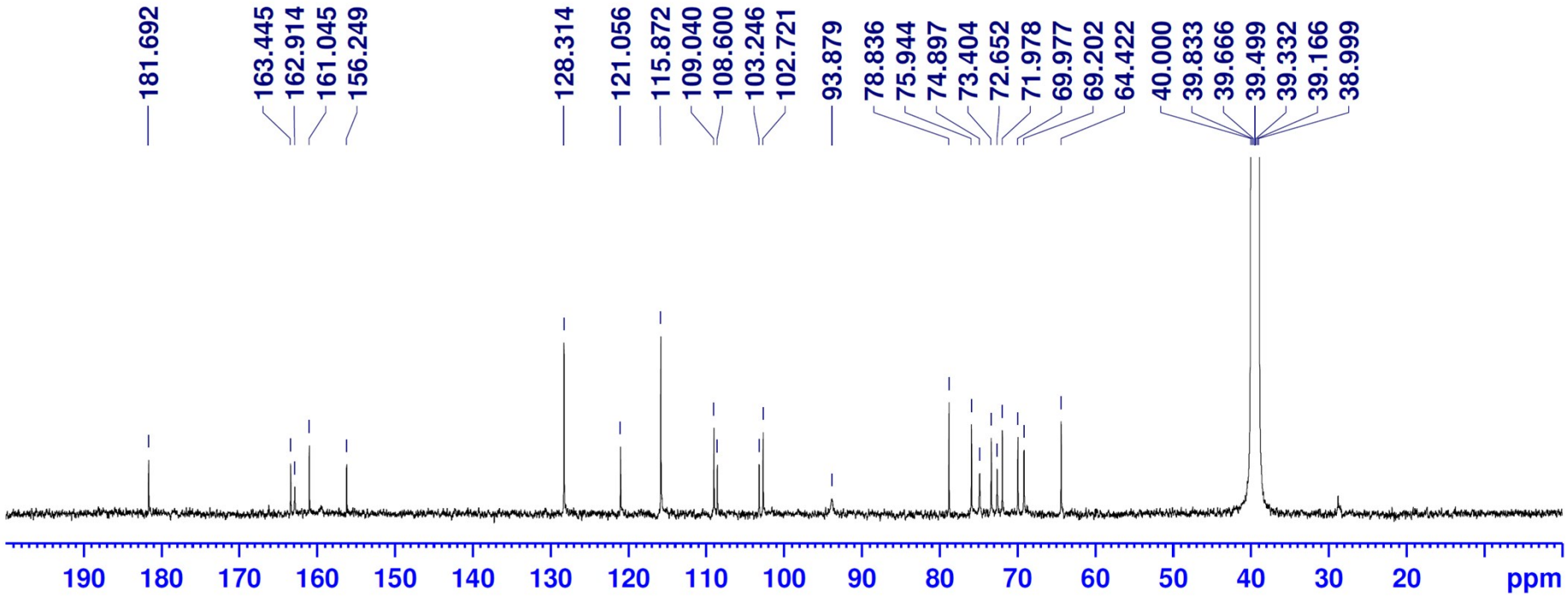
Salicylic acid (**2**). ^{1}H-NMR (CD_3OD, 500 MHz) δ_H 6.87 (1H, ddd, *J* = 8.1, 7.1, 1.1 Hz, H-5), 6.90 (1H, dd, *J* = 8.4, 1.1 Hz, H-3), 7.44 (1H, ddd, *J* = 8.4, 7.1, 1.8 Hz, H-4), 7.84 (1H, dd, *J* = 8.1, 1.8 Hz, H-6).

Figure S3

(A)


13.4882
7.9312
7.9276
7.9172
7.9135
6.9252
6.9075
6.7627
6.5165
5.1504
4.8974
4.8908
4.8203
4.8111
4.5652
4.5500
4.3657
4.2831
4.2771
4.2556
3.7708
3.7468
3.7291
3.5799
3.5729
3.5493
3.5374
3.5139
3.4968
3.3617
3.3198
3.1732
3.1521
3.0825
3.0640
2.9992
2.9773
2.4974
2.4938
2.4901
2.4864
2.4828
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.991
2.095
0.947
0.795
0.951
0.416
0.979
1.117
2.162
3.088
1.065
0.982
1.000

(B)

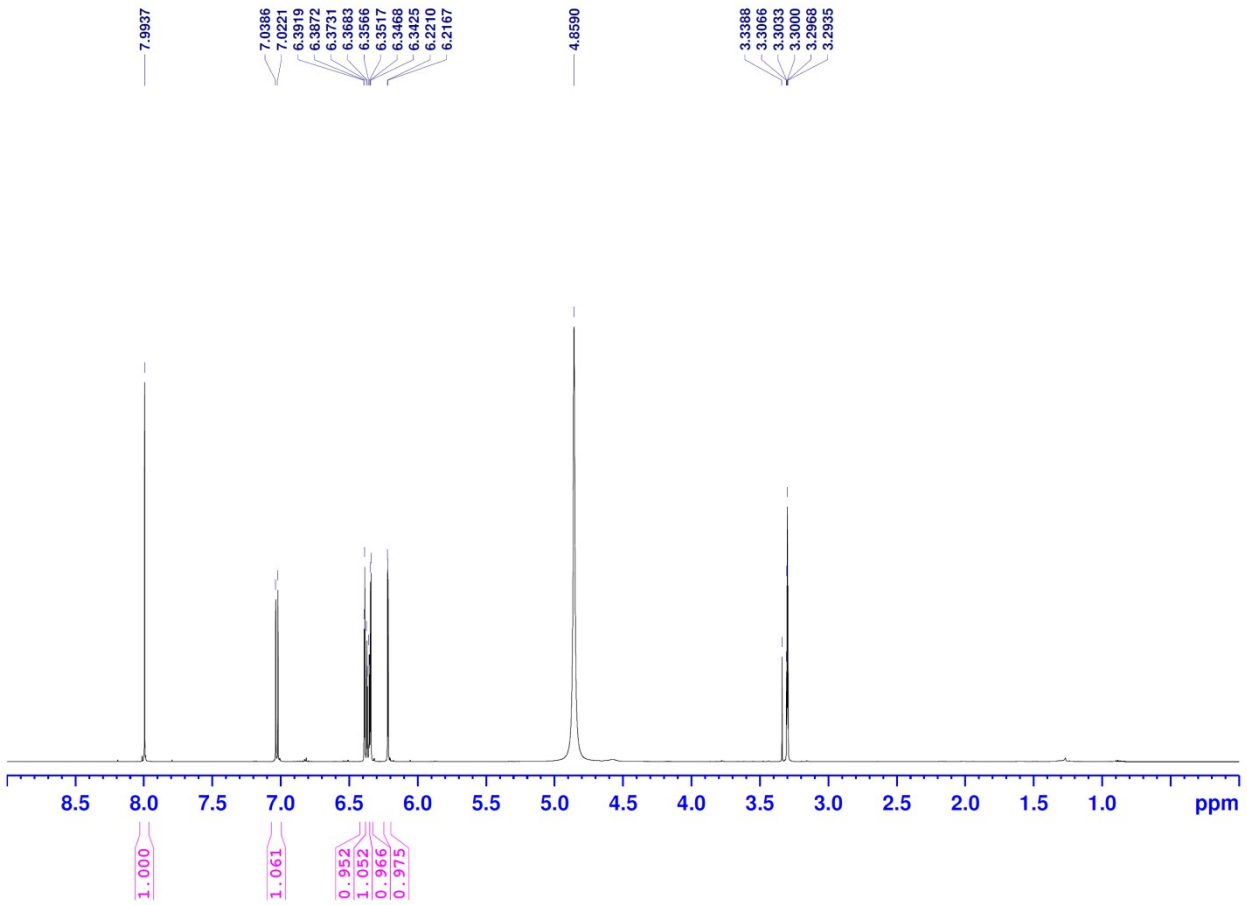

181.692
163.445
162.914
161.045
156.249
128.314
121.056
115.872
109.040
108.600
103.246
102.721
93.879
78.836
75.944
74.897
73.404
72.652
71.978
69.977
69.202
64.422
40.000
39.833
39.666
39.499
39.332
39.166
38.999
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

(C)

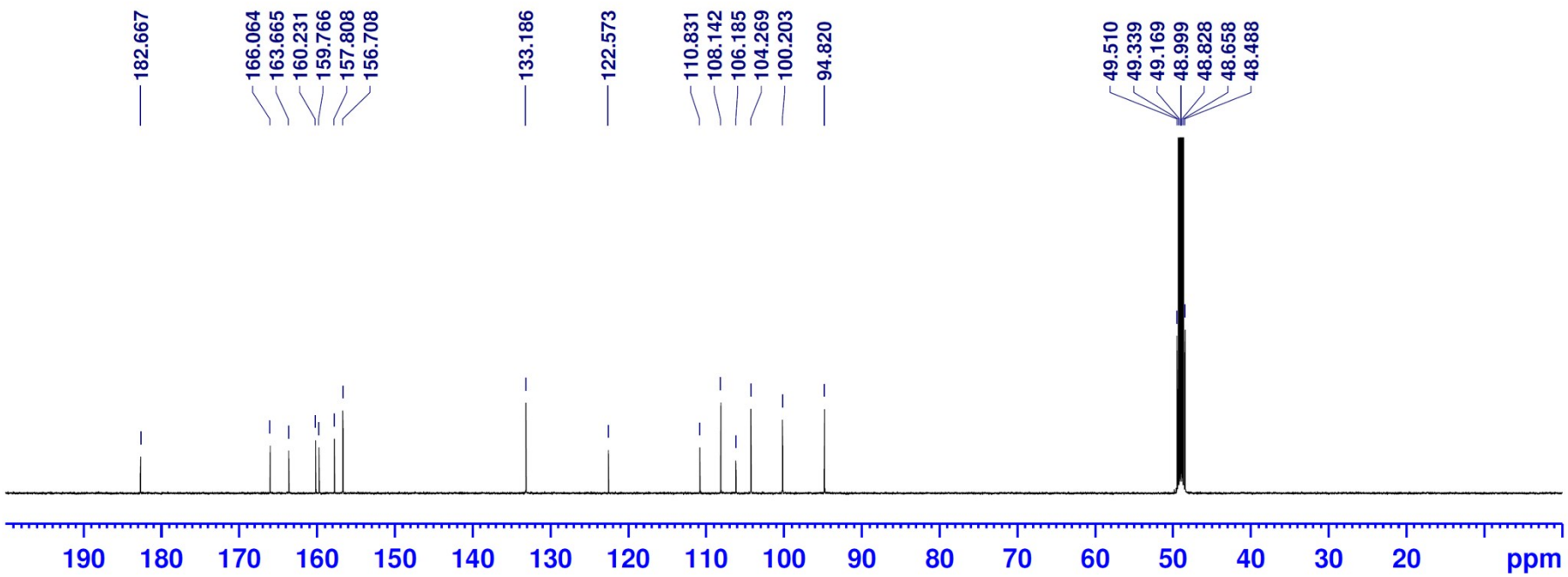
Apigenin 6-*C*-*β*-D-apiofuranosyl(1→2)-*α*-D-xylopyranoside (**3**). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H 2.52 (1H, m, H-4‴a), 2.99 (1H, d, *J* = 11.4 Hz, H-5‴a), 3.08 (1H, d, *J* = 9.3 Hz, H-4‴b), 3.17 (1H, d, *J* = 11.4 Hz, H-5‴b), 3.51 (1H, m, H-5″a), 3.54 (1H, m, H-3"), 3.58 (1H, m, H-2‴), 3.74 (1H, m, H-4"), 3.79 (1H, m, H-5″b), 4.28 (1H, brs, H-2"), 4.56 (1H, brs, H-1"), 6.53 (1H, s, H-8), 6.78 (1H, s, H-3), 6.91 (2H, d, *J* = 8.9 Hz, H-3′, 5′), 7.93 (2H, d, *J* = 8.9 Hz, H-2′, 6′), 13.50 (5-OH). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ_C 64.4 (C-5‴), 69.2 (C-4″), 70.0 (C-5″), 72.0 (C-1″), 72.7 (C-2″), 73.4 (C-4‴), 74.9 (C-3″), 75.9 (C-2‴), 78.8 (C-3‴), 93.9 (C-8), 102.7 (C-3), 103.2 (C-10), 108.6 (C-6), 109.0 (C-1‴), 115.9 (C-3′, 5′), 121.1 (C-1′), 128.3 (C-2′, 6′), 156.2 (C-9), 161.0 (C-5, 4′), 162.9 (C-2), 163.4 (C-7), 181.7 (C-4).

Figure S4

(A)


7.9937
7.0386
7.0221
6.3919
6.3872
6.3731
6.3683
6.3566
6.3517
6.3468
6.3425
6.2210
6.2167
4.8590
3.3388
3.3066
3.3033
3.3000
3.2968
3.2935
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.000
1.061
0.952
1.052
0.966
0.975

(B)

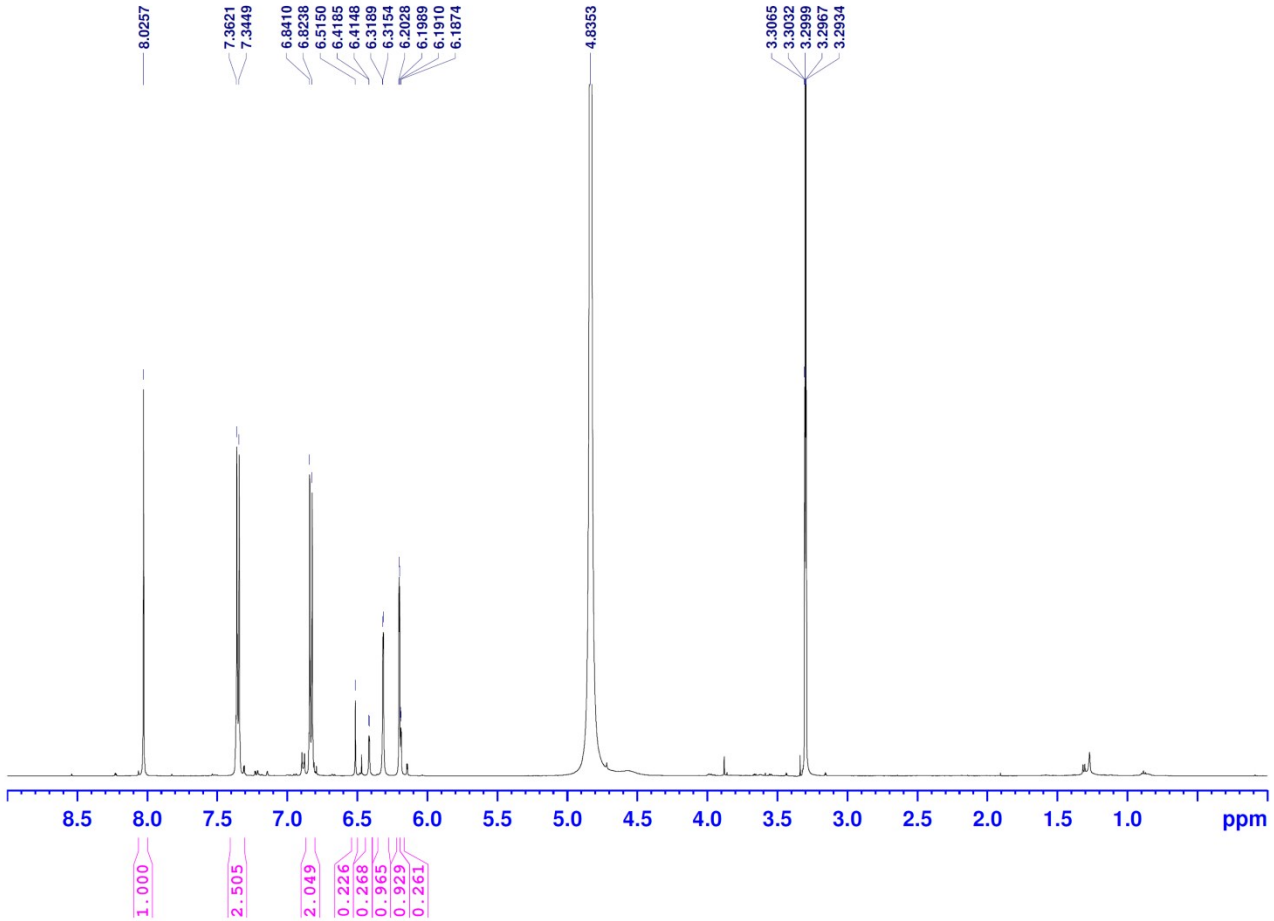

182.667
166.064
163.665
160.231
159.766
157.808
156.708
133.186
122.573
110.831
108.142
106.185
104.269
100.203
94.820
49.510
49.339
49.169
48.999
48.828
48.658
48.488
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

(C)

2′-Hydroxygenistein (**4**). ^{1}H-NMR (CD_3OD, 500 MHz) δ_H 6.22 (1H, d, J = 2.2 Hz, H-6), 6.34 (1H, d, J = 2.2 Hz, H-8), 6.36 (1H, dd, J = 8.3, 2.4 Hz, H-5'), 6.39 (1H, d, J = 2.4 Hz, H-3'), 7.03 (1H, d, J = 8.3 Hz, H-6'), 7.99 (1H, s, H-2). ^{13}C-NMR (CD_3OD, 125 MHz). δ_C 94.8 (C-8), 100.2 (C-6), 104.3 (C-3'), 106.2 (C-10), 108.1 (C-5'), 110.8 (C-1'), 122.6 (C-3), 133.2 (C-6'), 156.7 (C-2), 157.8 (C-2'), 159.8 (C-9), 160.2 (C-4'), 163.7 (C-5), 166.1 (C-7), 182.7 (C-4).

Figure S5

(A)

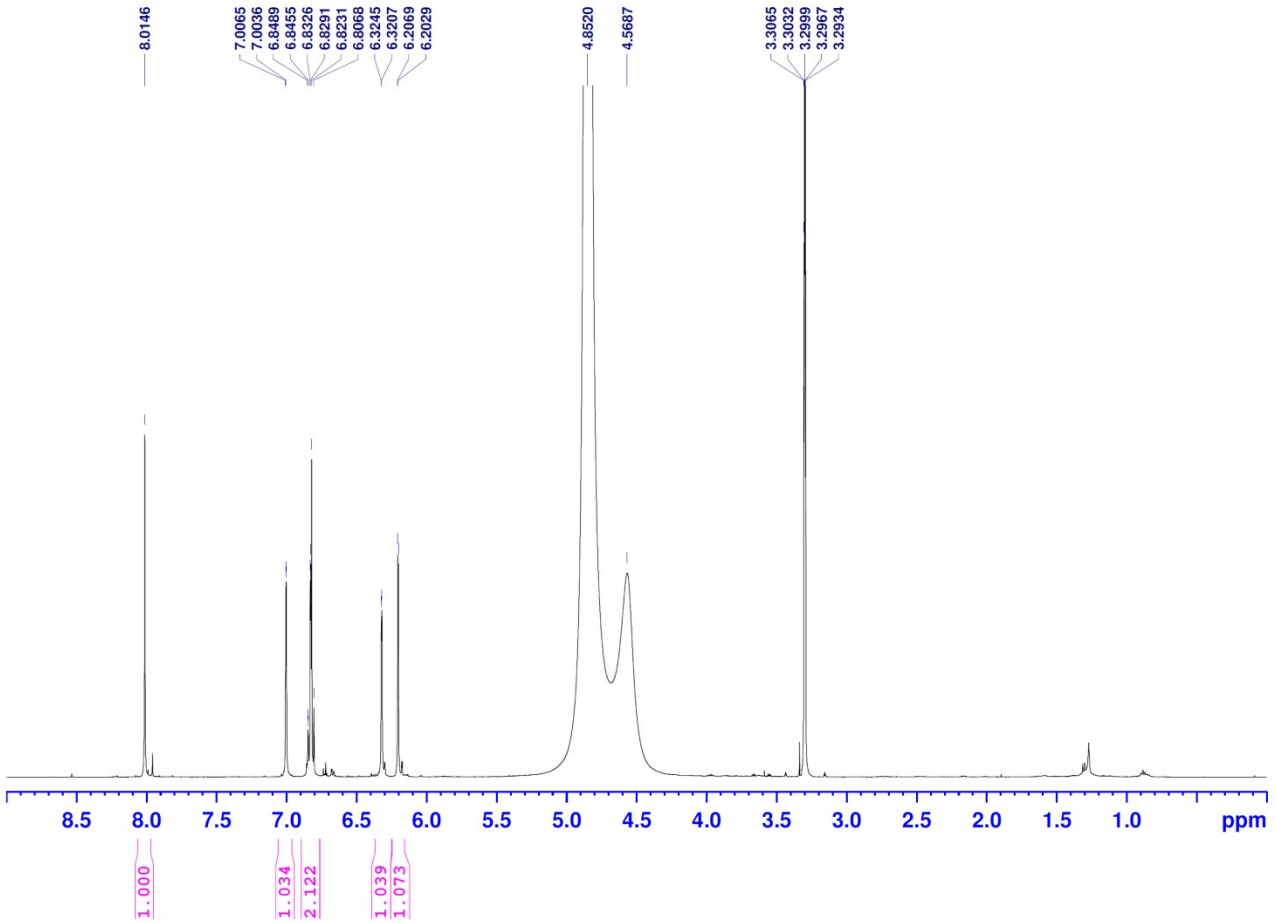

8.0257
7.3621
7.3449
6.8410
6.8238
6.5150
6.4185
6.4148
6.3189
6.3154
6.2028
6.1989
6.1910
6.1874
4.8353
3.3065
3.3032
3.2999
3.2967
3.2934
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.000
2.505
2.049
0.226
0.268
0.965
0.929
0.261

(B)

Genistein (**5**). ^{1}H-NMR (CD_3OD, 500 MHz) δ_H 6.22 (1H, d, J = 2.2 Hz, H-6), 6.34 (1H, d, J = 2.2 Hz, H-8), 6.86 (2H, d, J = 8.7 Hz, H-3', 5'), 7.37 (2H, d, J = 8.7 Hz, H-2', 6'), 8.05 (1H, s, H-2).

Figure S6

(A)

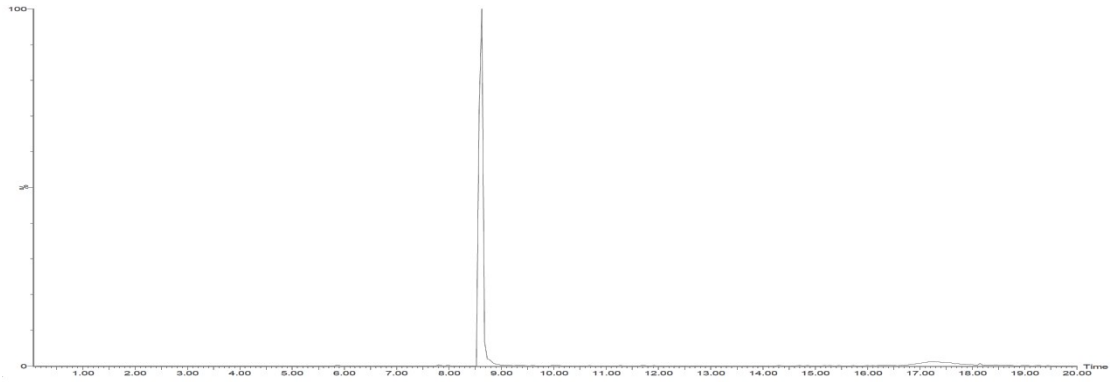

8.0146
7.0065
7.0036
6.8489
6.8455
6.8326
6.8291
6.8231
6.8068
6.3245
6.3207
6.2069
6.2029
4.8520
4.5687
3.3065
3.3032
3.2999
3.2967
3.2934
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.000
1.034
2.122
1.039
1.073

(B)

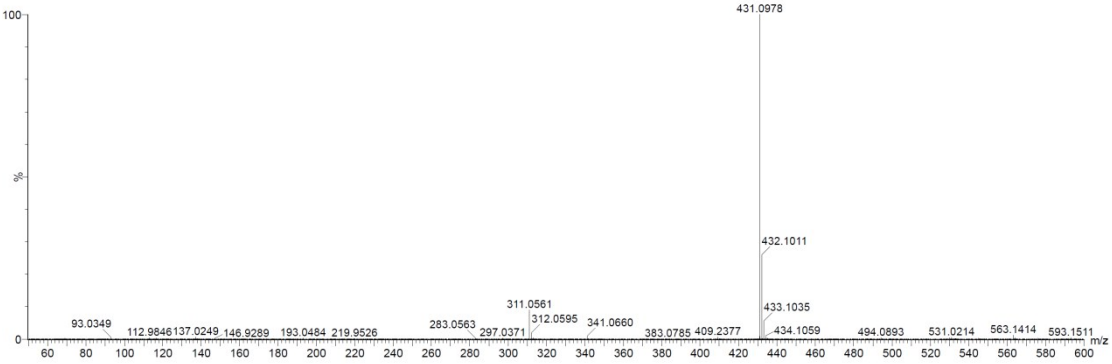
5,7-Dihydroxy-3',5'-dihydroxyisoflavone (**6**). ^{1}H-NMR (CD_3OD, 500 MHz) δ_H 6.20 (1H, d, *J* = 2.0 Hz, H-6), 6.32 (1H, d, *J* = 2.0 Hz, H-8), 6.83 (2H, d, *J* = 1.8 Hz, H-2', 6'), 7.00 (1H, d, *J* = 1.5 Hz, H-4'), 8.01 (1H, s, H-2).

Figure S7

(A)



(B)



(C)

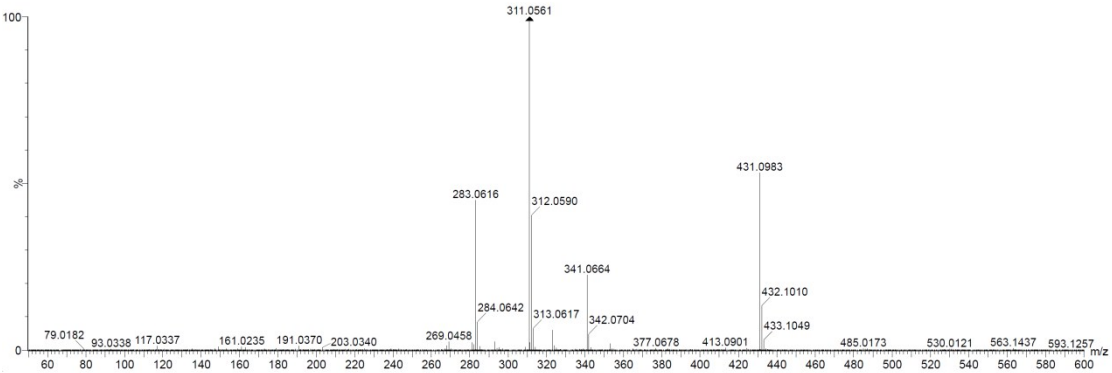
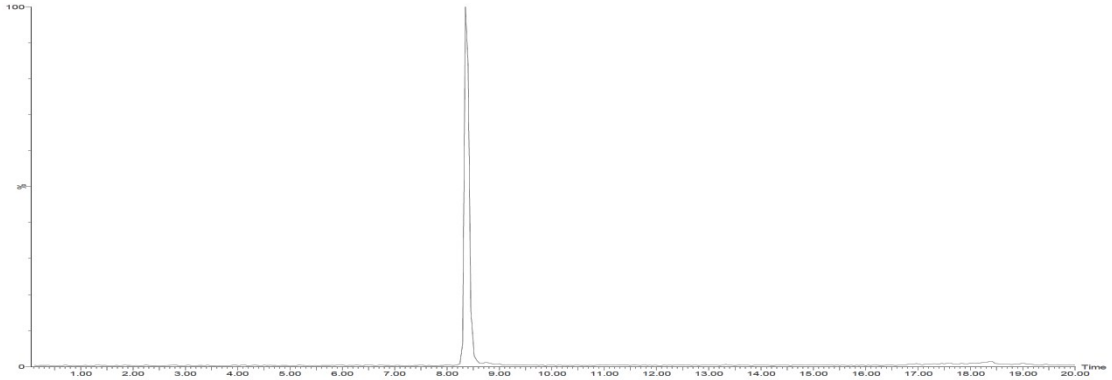
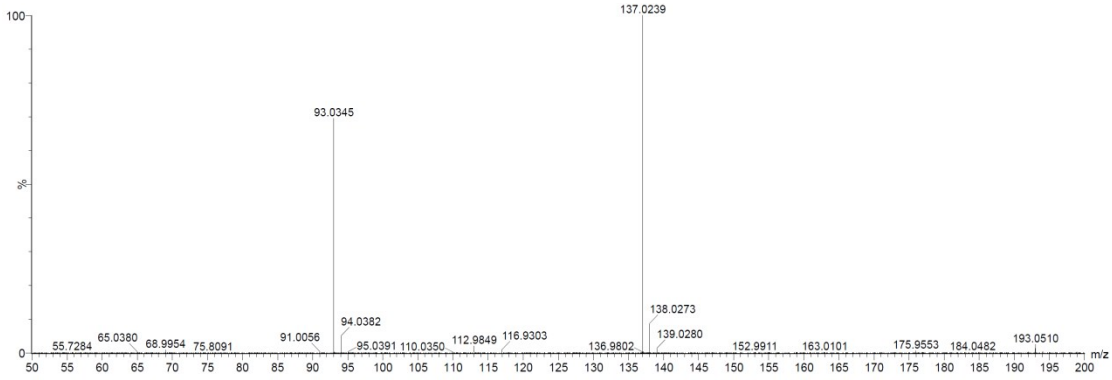


Figure S8

(A)



(B)



(C)

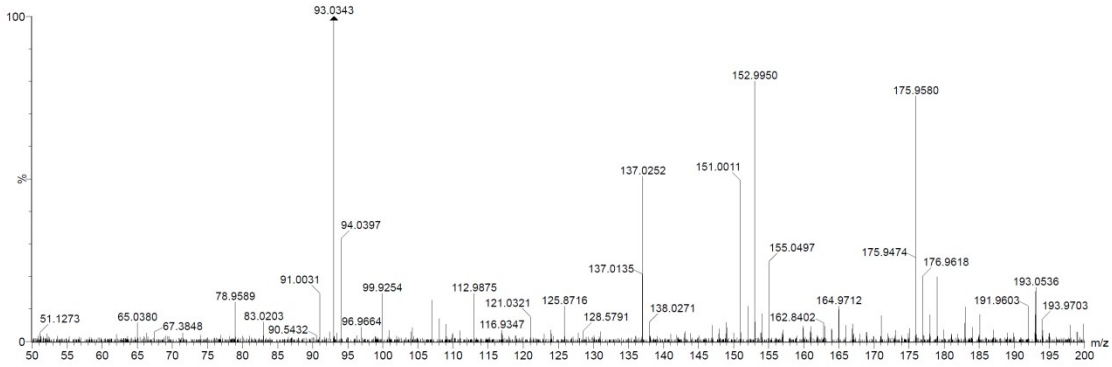
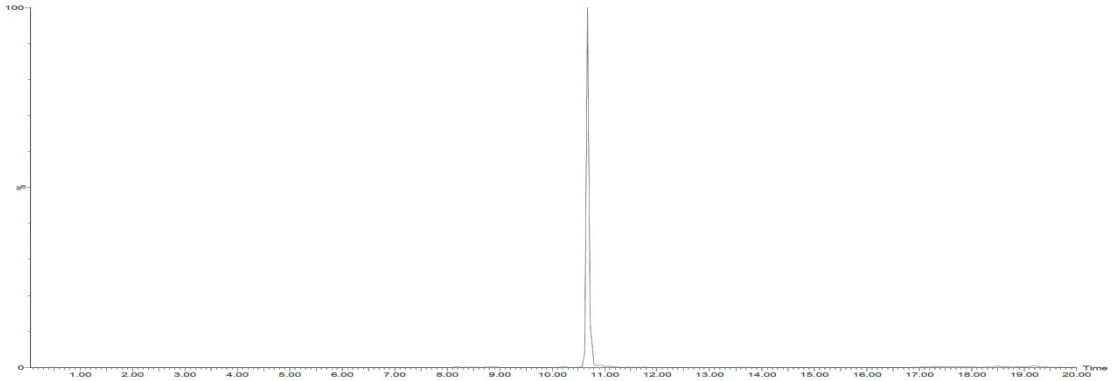
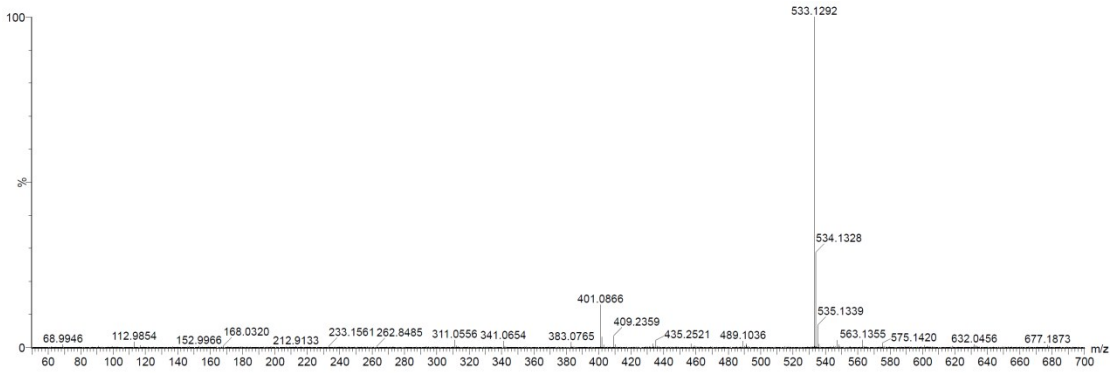


Figure S9

(A)



(B)



(C)

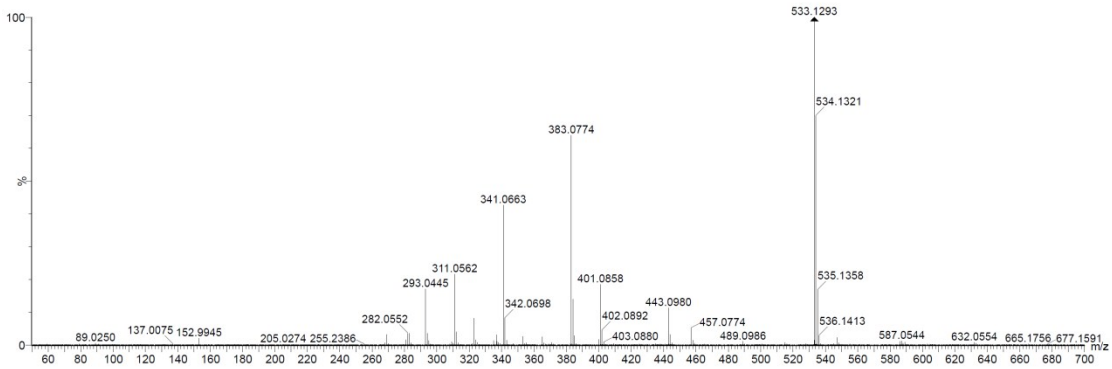
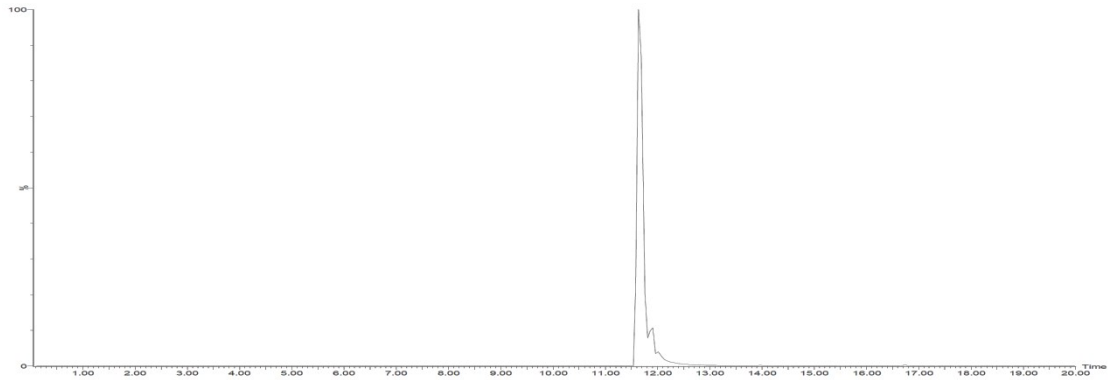
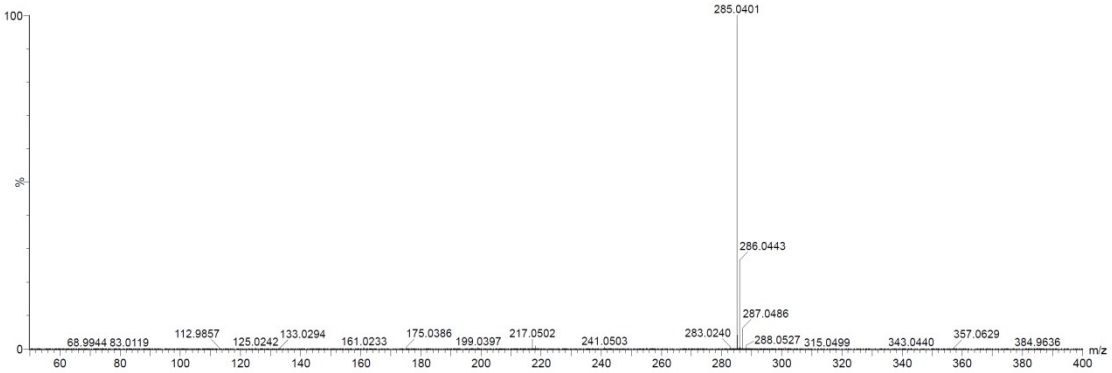


Figure S10

(A)



(B)



(C)

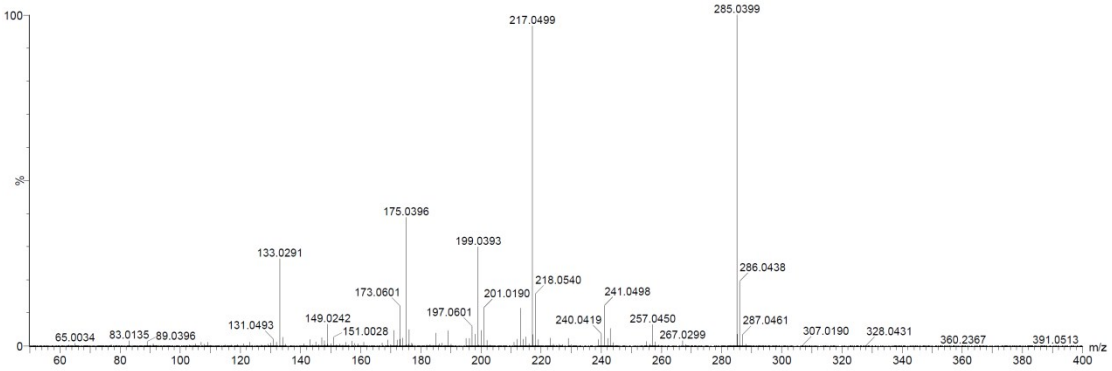
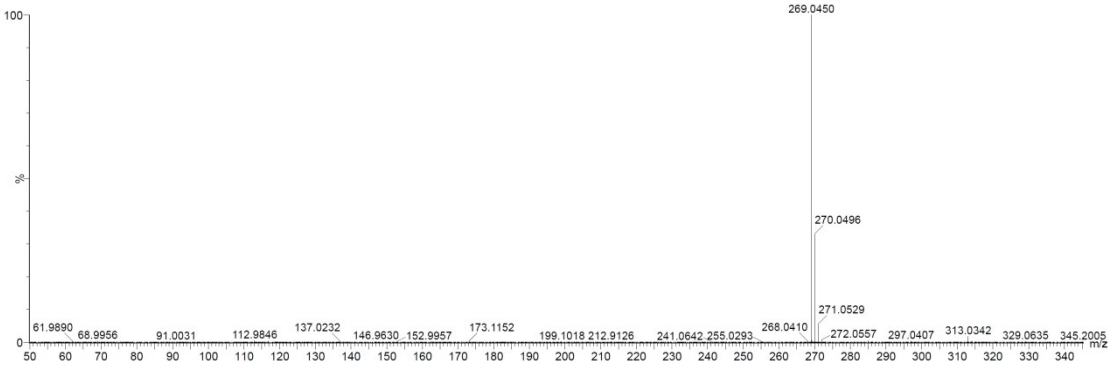


Figure S11

(A)

(B)



(C)

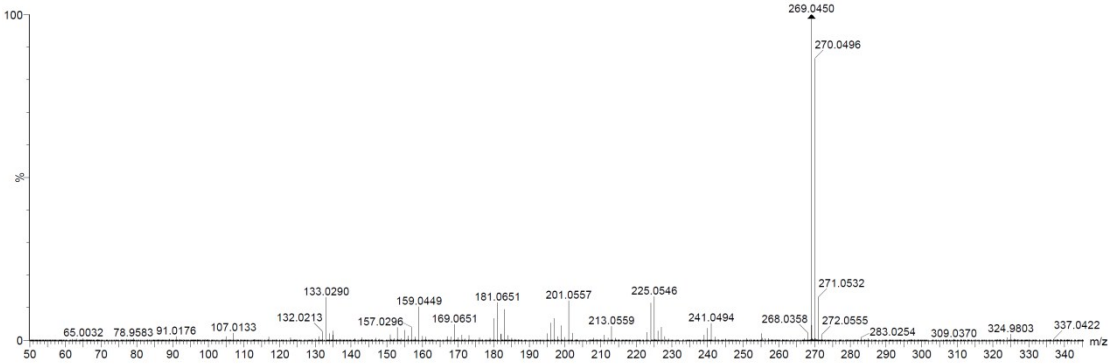
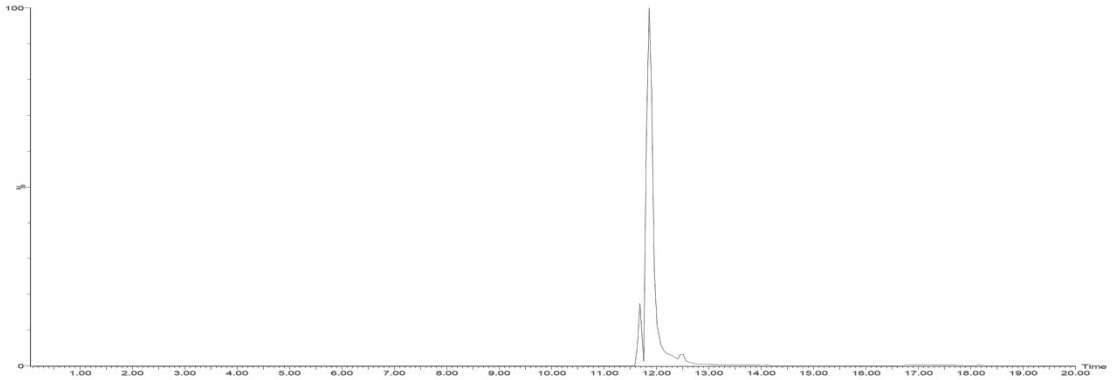
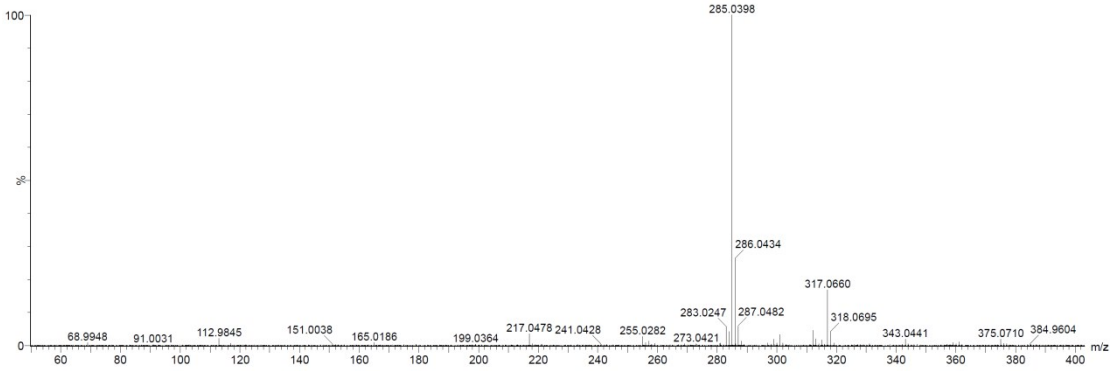


Figure S12

(A)



(B)



(C)