Supporting Information

Rec. Nat. Prod. 15:2 (2021) 122-129

Isolation, Characterization and Antiproliferative Activity of Secondary Metabolites from *Tanacetum alyssifolium* (Bornm.) Grierson

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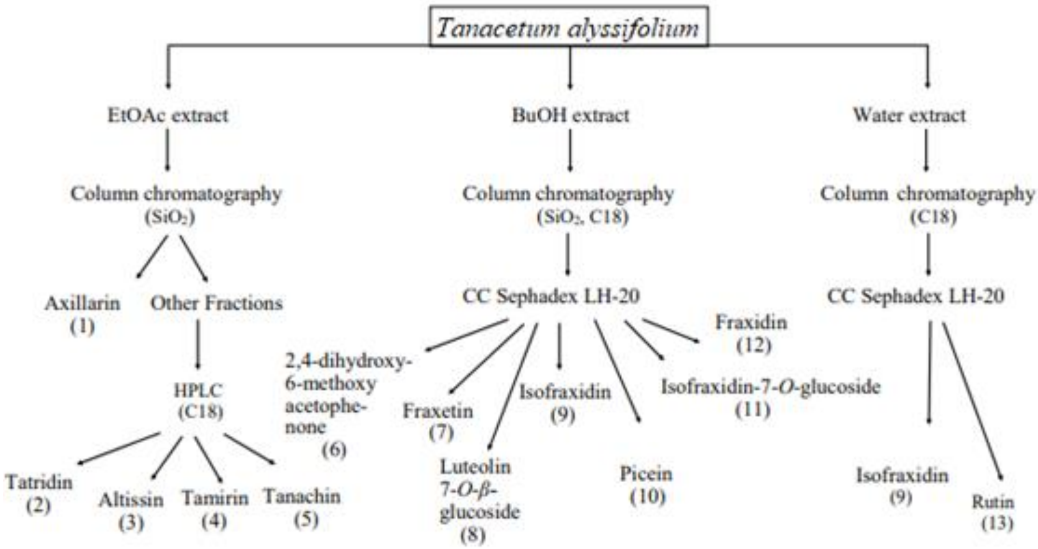

Figure S1: Scheme for isolation of secondary metabolites of *T. alyssifolium*

Axillarin (1): ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H 7,54 (brs, 1H, H-6′), 7,46 (d, *J* = 8,0 Hz, 1H, H-1′), 6,91 (d, *J* = 8,0 Hz, 1H, H-3′), 6,51 (s, 1H, H-8), 3,78 (brs, 4H, -OCH$_3$), 3,75 (s, 3H, -OCH$_3$). ^{13}C NMR (150 MHz, DMSO-d$_6$) δ_C 178.8 (C-4),158.2 (C-7), 156.1 (C-2), 152.8 (C-5), 152.0 (C-9), 149.2 (C-4’), 145.7 (C-3’), 137.8 (C-3), 111.7 (C-6), 121.3 (C-1’), 121.0 (C-6’), 116.2 (C-5’), 115.9 (C-2’), 104.9 (C-10), 94.4 (C-8), 60.4 (C-6-OCH$_3$), 60.1 (C-3-OCH$_3$).

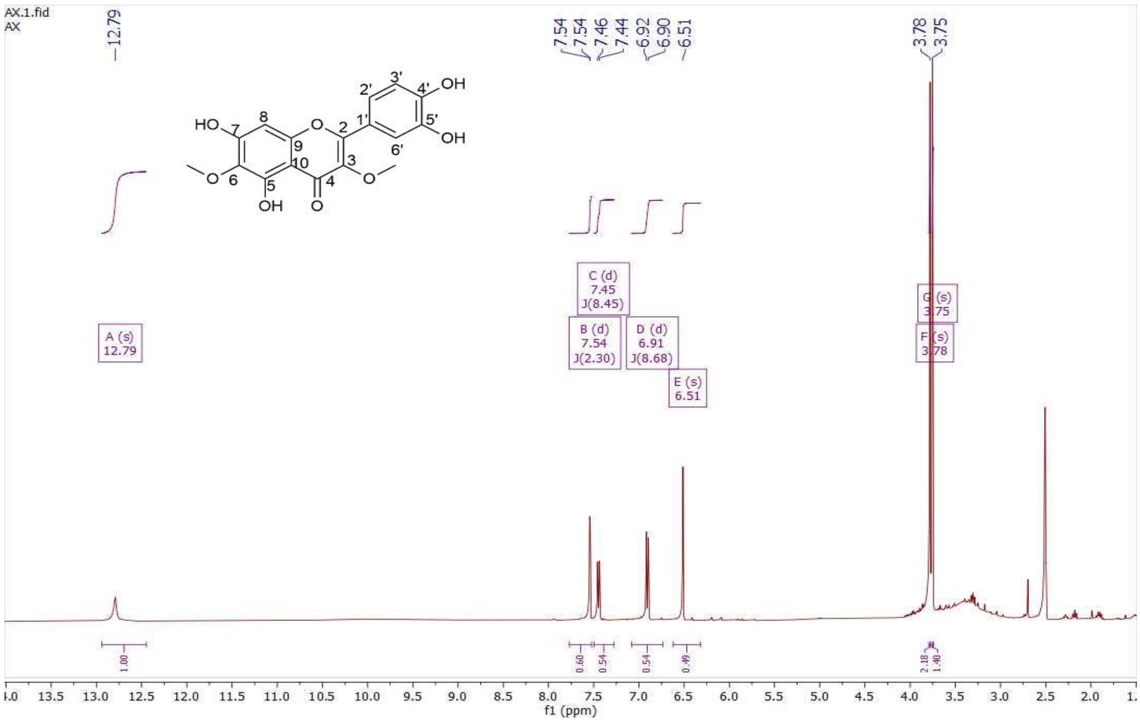

Figure S2: ^{1}H-NMR spectrum of Axillarin (1) (400 MHz, DMSO-d$_6$)

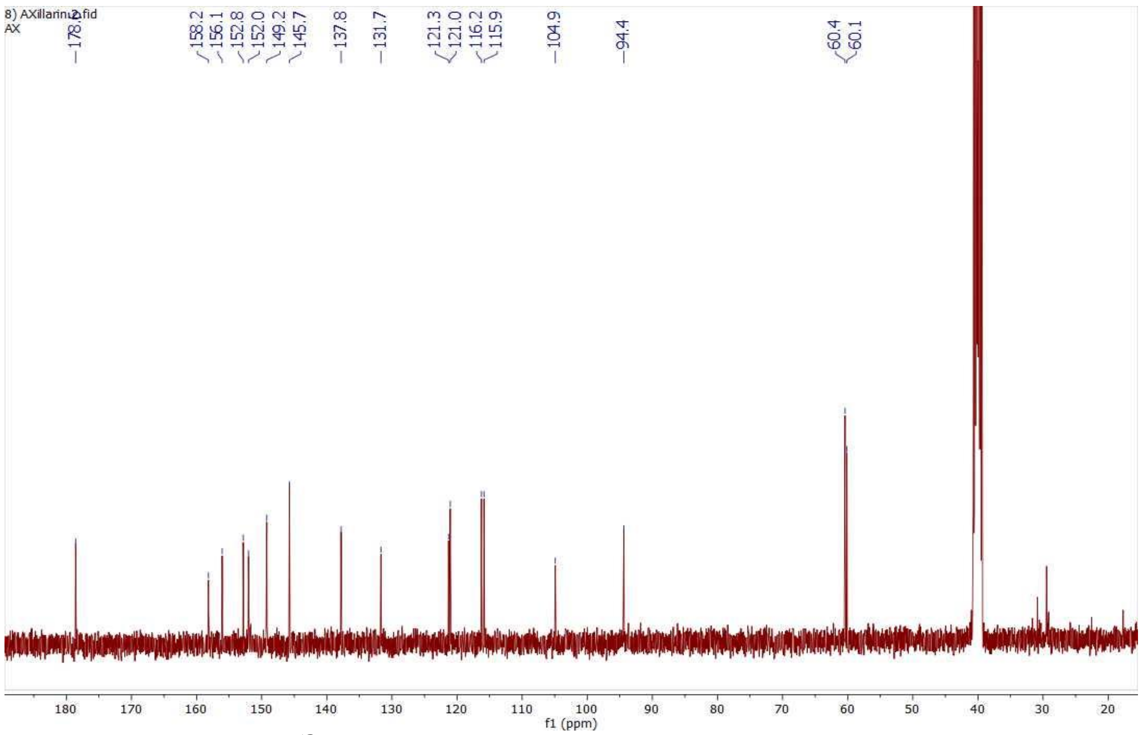

Figure S3: ^{13}C-NMR spectrum of Axillarin (1) (100 MHz, DMSO-d$_6$)

Tatridin A (2): ^{1}H NMR (400 MHz, MeOD) δ_H 6.20 (ddd, *J*=4.77, 3.23, 1.33, 2H, H-13a,b), 5.33 (d, *J*=10.2, 1H, H-9), 4.97 (d, *J*=10.55, 1H, H-5), 4.70 (t, *J*=9.55,1H, H-8), 4.47 (dd, *J*=10.55, 8.99, 1H, H-6), 4.40 (dd, *J*=10.11, 5.03, 1H, H-1), 2.81 (m, 1H, H-7), 2.29 (m, 1H, H-3a), 1.97 (m, 1H, H-2a), 1.95 (m, 1H, H-3b), 1.83 (d, *J*=1.48, 3H, H-14), 1.80 (brs, 3H, H-15), 1.75 (m, 1H, H-2b). ^{13}C NMR (100 MHz, MeOD) δ_C 171.0 (C-12), 142.4 (C-10), 138.8 (C-11), 133.4 (C-4), 130.4 (C-5), 126.4 (C-9), 121.6(C-13), 75.0 (C-8), 70.1 (C-6), 66.0 (C-1), 52.2 (C-7), 34.9 (C-3), 27.1 (C-2), 15.7 (C-14), 14.1 (C-15).

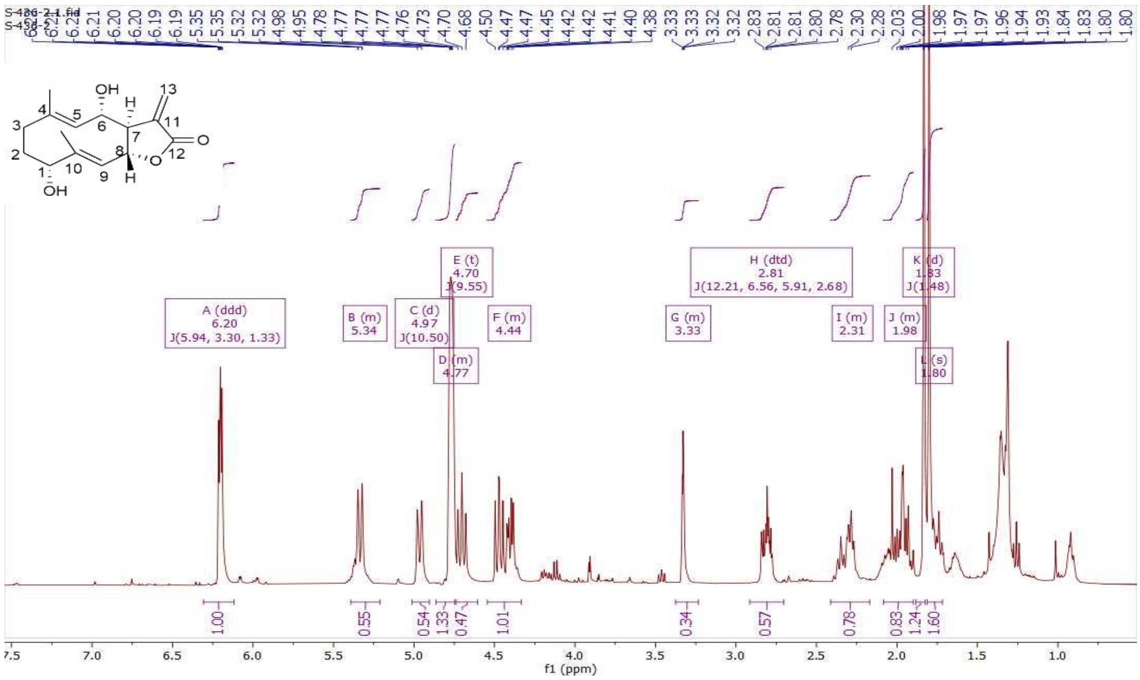

Figure S4: ^{1}H-NMR spectrum of Tatridin A **(2) (**400 MHz, MeOD)

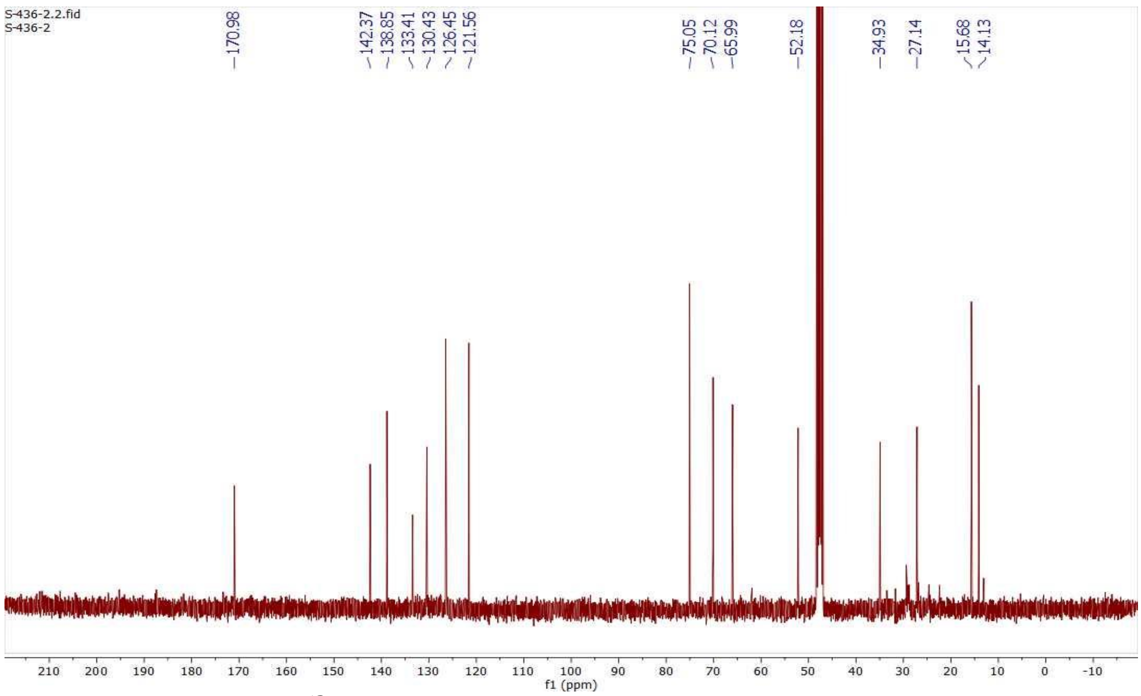

Figure S5: ^{13}C-NMR spectrum of Tatridin A **(2) (**100 MHz, MeOD)

Altissin (3): ^{1}H NMR (400 MHz, MeOD) δ_H 6.07 (d, *J*=3.19, 1H, H-13a), 5.95 (d, *J*=2.93, 1H, H-13b), 5.40 (m, 1H, H-3), 4.11 (dt, J=11.85, 3.70, 1H, H-8), 3.81 (m, 1H, H-1), 3.80 (m, 1H, H-6), 2.65 (m, 1H, H-9a), 2.61 (m, 1H, H-7), 2.34 (m, 1H, H-2a), 1.98 (m, 1H, H-2b), 1.93 (s, 3H, H-15), 1.88 (d, *J*=9.71, 1H, H-5), 1.38 (m, 1H, H-9), 0.99 (s, 3H, H-14). ^{13}C NMR (100 MHz, MeOD) δ_C 171.3 (C-12), 138.7 (C-4), 135.1 (C-11), 120.5 (C-3), 118.3 (C-13), 76.4 (C-8), 74.1 (C-6), 65.4 (C-1), 58.1 (C-5), 54.7 (C-7), 40.3 (C-10), 36.7 (C-9), 32.3 (C-2), 25.1 (C-15), 19.4 (C-14).

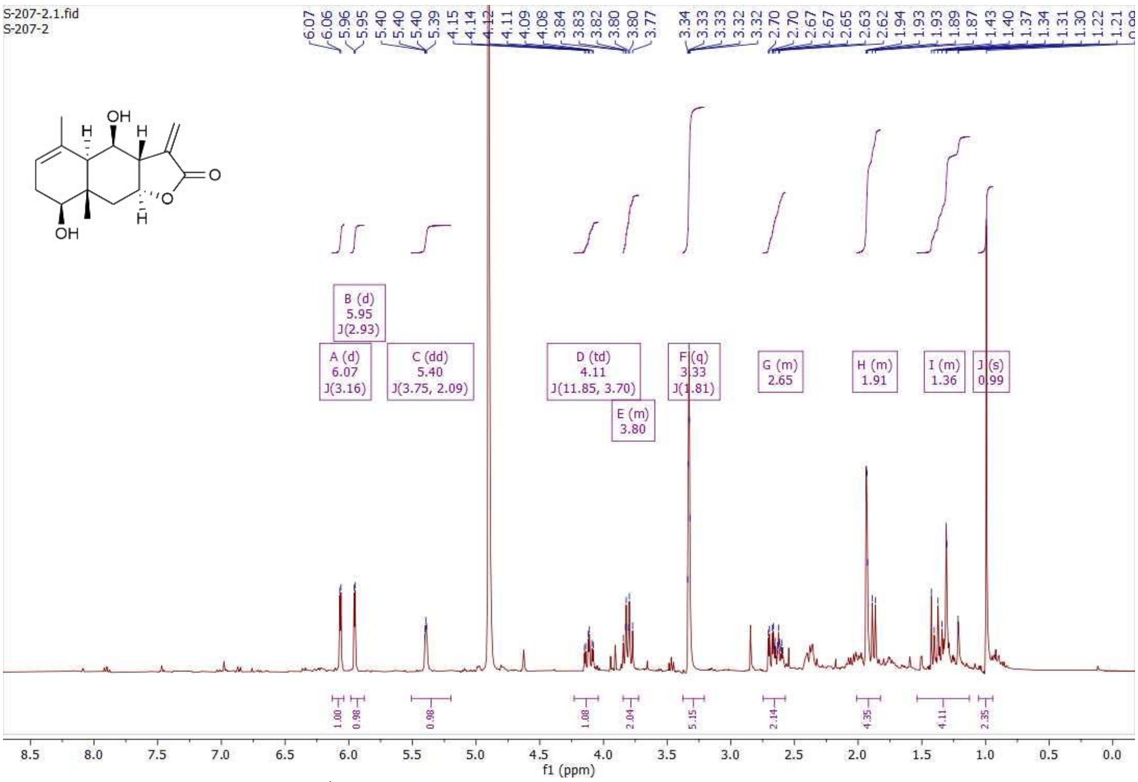

Figure S6: ^{1}H-NMR spectrum of Altissin **(3) (**400 MHz, MeOD)

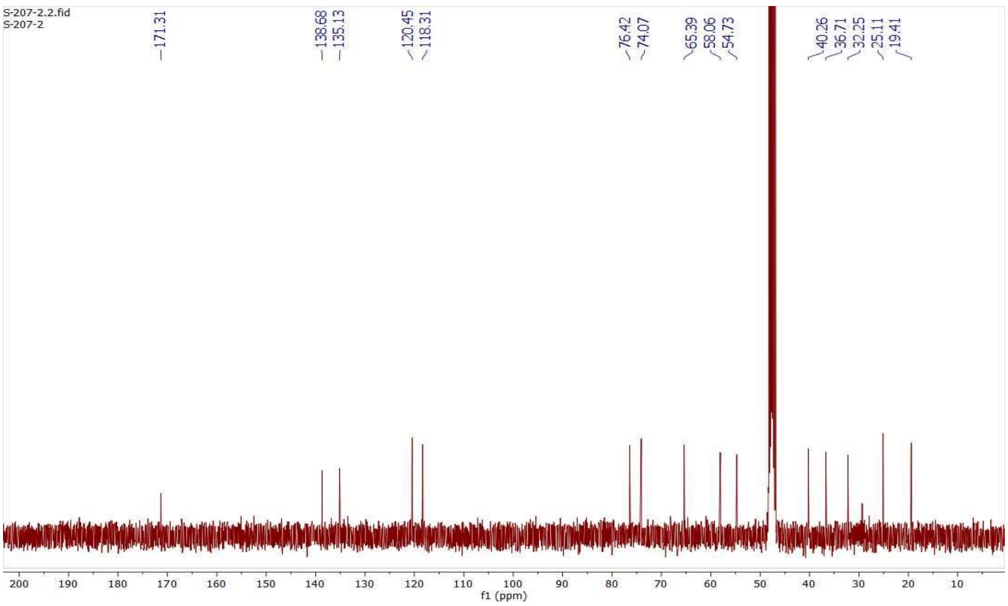

Figure S7: ^{13}C-NMR spectrum of Altissin **(3) (**100 MHz, MeOD)

Tamirin (4): ^{1}H NMR (400 MHz, MeOD) δ 6.28 (dd, *J*=3.03, 1.35, 1H, H-13a), 6.19 (dd, *J*=2.66, 1.35, 1H, H-13b), 6.01 (d, J=1.53, 1H, H-14a), 5.93 (d, J=2.01, 1H, H-14b), 5.06 (m, 1H, H-5), 4.11 (m, 1H, H-6), 3.93 (m, 1H, H-8), 3.42 (ddd, *J*=9.94, 5.74, 2.88, 1H, H-2a), 3.30 (m, 1H, H-9a), 2.77 (m, 1H, H-7) 2.61 (m, 1H, H-3a), 2.52 (m, 1H, H-2b), 2.40 (m, 1H, H-3b), 2.28 (m, 1H, H-9b), 1.65 (s, 3H, H-15). ^{13}C NMR (100 MHz, MeOD) δ 204.2 (C-1), 170.6 (C-12), 145.7 (C-10), 136.8 (C-11), 134.3 (C-4), 132.8 (C-5), 124.8 (C-13), 124.6 (C-14), 77.24 (C-8), 69.4 (C-6), 50.1 (C-7), 39.4 (C-9), 36.3 (C-2), 35.2 (C-3), 15.3 (C-15).

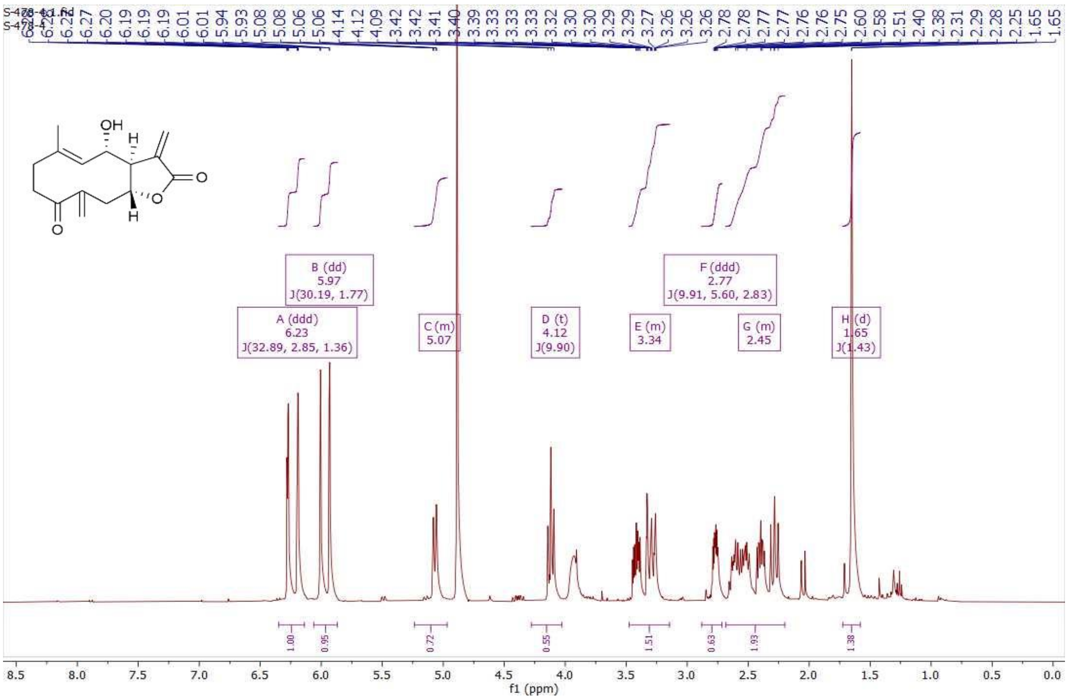

Figure S8: ^{1}H-NMR spectrum of Tamirin **(4)** **(**400 MHz, MeOD)

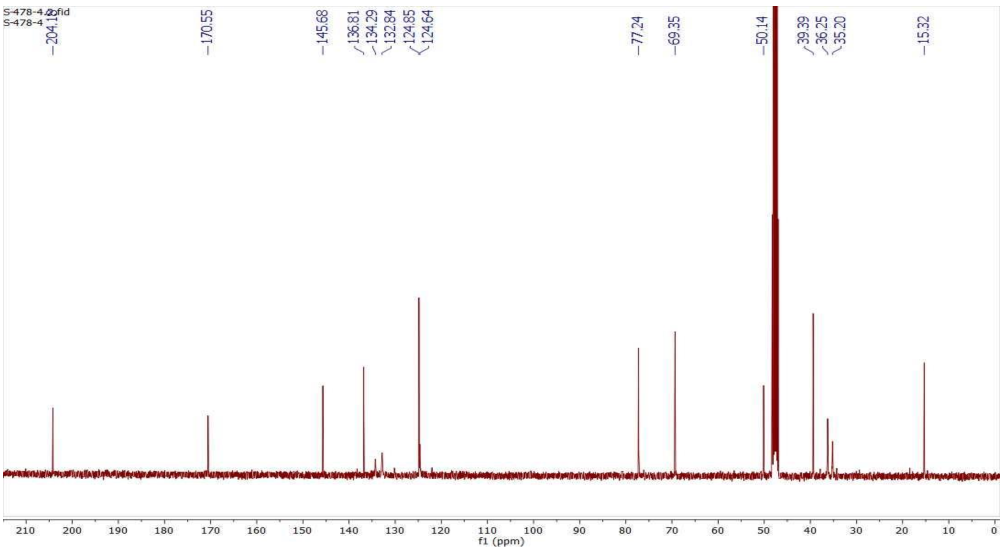

Figure S9: ^{13}C-NMR spectrum of Tamirin **(4)** **(**100 MHz, MeOD)

Tanachin (5): ^{1}H NMR (400 MHz, MeOD) δ 6.26 (dd, *J*=3.14, 1.37, 1H, H-13a), 6.21 (dd, *J*=2.79, 1.37, 1H, H-13b), 5.18 (m, 1H, H-14a), 5.14 (m, 1H, H-14b), 5.05 (d, *J*=10.16, 1H, H-5), 4.25 (m, 1H, H-6), 4.08 (m, 1H, H-8), 3.86 (dd, *J*=9.60, 5.66, 1H, H-1), 2.85 (m, 1H, H-9a), 2.83 (m, 1H, H-7), 2.44 (m, 1H, H-9b), 2.28 (m, 1H, H-3a), 2.08 (m, 2H, H-2a,b), 2.06 (m, 1H, H-3b), 1.73 (s, 3H, 15). ^{13}C NMR (100 MHz, MeOD) δ 170.8 (C-12), 146.2 (C-10), 137.6 (C-11), 134.0 (C-4), 131.5 (C-5), 124.0 (C-13), 113.7 (C-14), 79.4 (C-8), 69.9 (C-6), 69.4 (C-1), 51.7 (C-7), 41.4 (C-9), 34.0 (C-3), 30.1 (C-2), 15.9 (C-15).

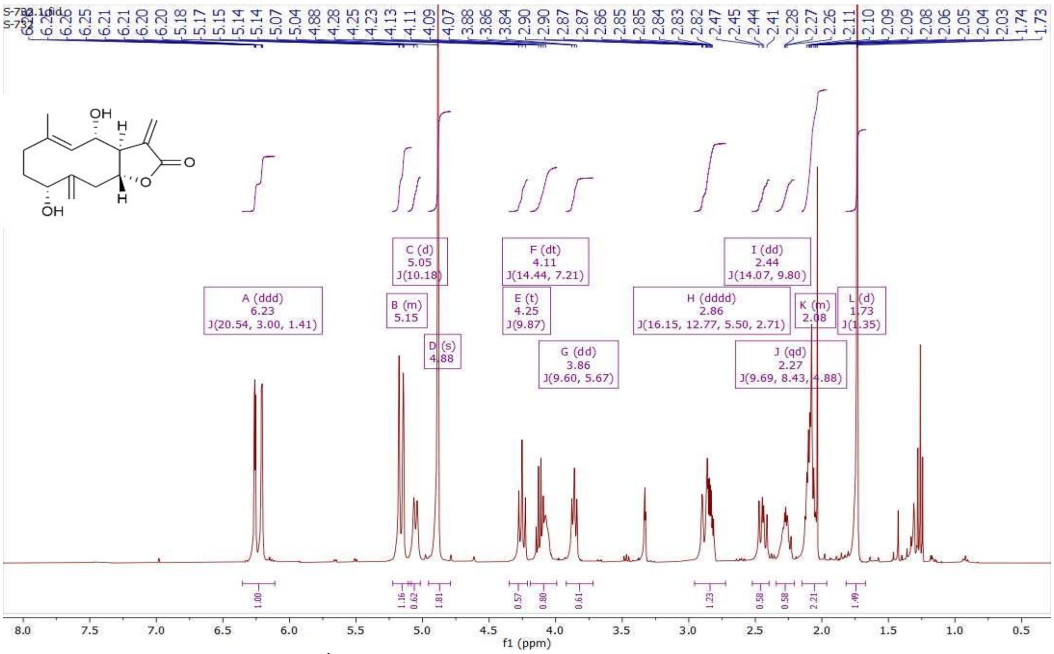

Figure S10: ^{1}H-NMR spectrum of Tanachin **(5)** **(**400 MHz, MeOD)

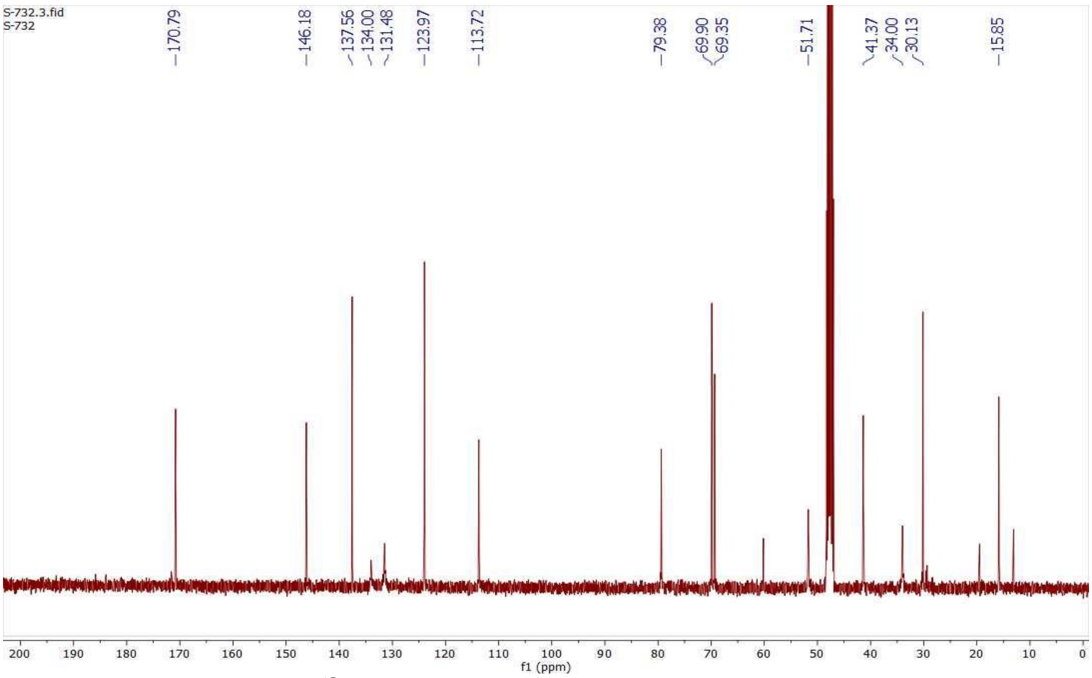

Figure S11: ^{13}C-NMR spectrum of Tanachin **(5)** (100 MHz, MeOD)

2,4-dihydroxy-6-methoxy acetophenone (6): ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.82 (brs, 1H, OH), 5.98 (d, *J*=2.21, 1H, H-5), 5.87 (d, *J*=2.21, 1H, H-3), 3.82 (s, 3H, OCH_3), 2.52 (s, 3H, H-α). ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.7 (C=O), 166.7 (C-2), 165.6 (C-4), 163.8 (C-6), 105.0 (C-1), 96.0 (C-3), 91.8 (C-5), 56.3 (OCH_3), 33.0 (C–α).

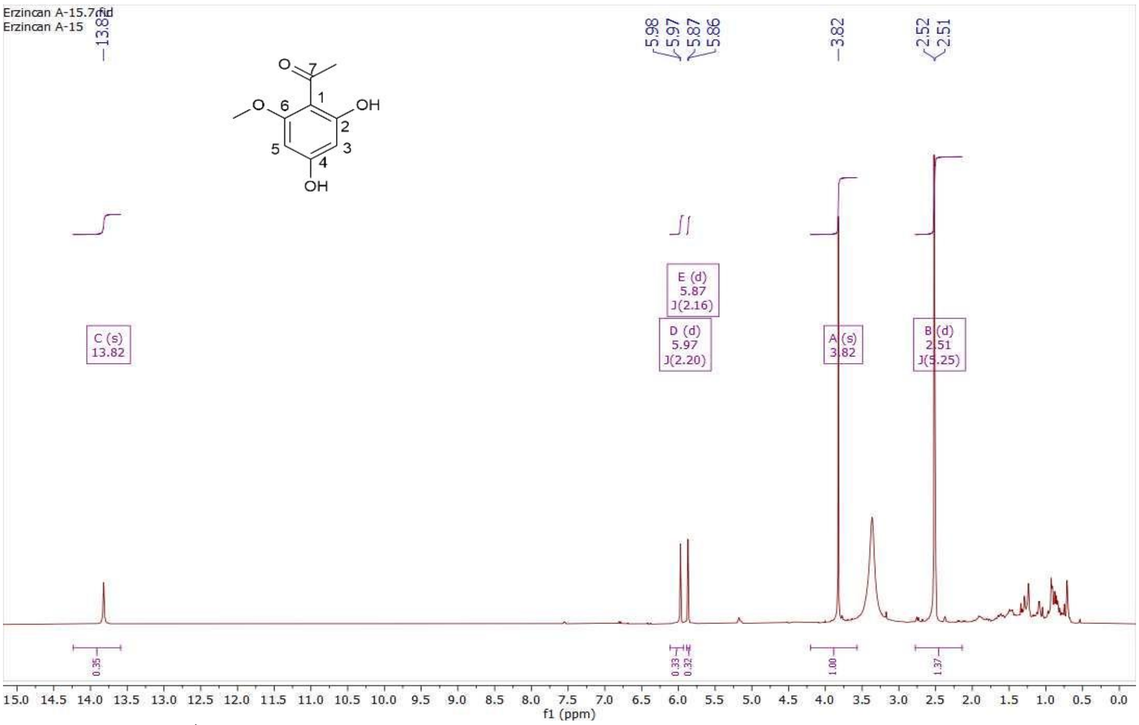

Figure S12: ^{1}H-NMR spectrum of 2,4-dihydroxy-6-methoxy acetophenone **(6)** **(**400 MHz, DMSO-d_6)

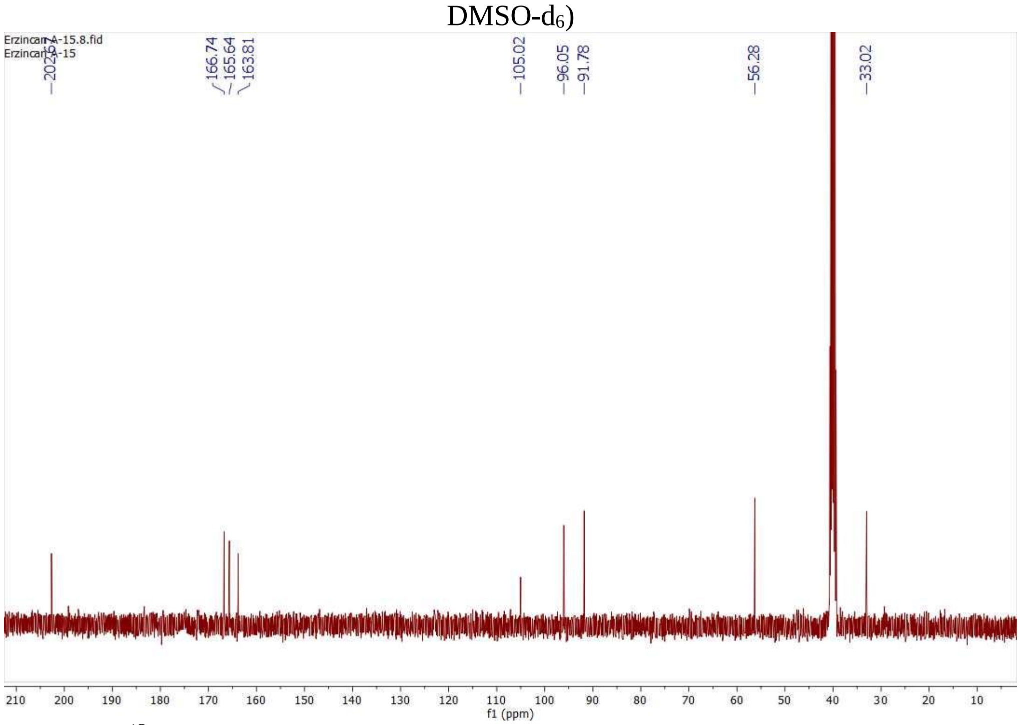

Figure S13: ^{13}C-NMR spectrum of 2,4-dihydroxy-6-methoxy acetophenone **(6)** **(**100 MHz, DMSO-d_6)

Fraxetin (7): ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (brs, 1H, OH), 7.94 (d, *J*=9.47, 1H, H-4), 7.10 (s, 1H, H-5), 6.28 (d, *J*=9.47, 1H, H-3), 3.81 (s, 3H, OCH_3). ^{13}C NMR (10 MHz, DMSO-d_6) δ 160.7 (C-2), 146.5 (C-6), 145.1 (C-4), 144.8 (C-7), 143.4 (C-8), 128.8 (C-9), 113.0 (C-3), 111.1 (C-10), 106.4 (C-5), 56.6 (-OCH_3).

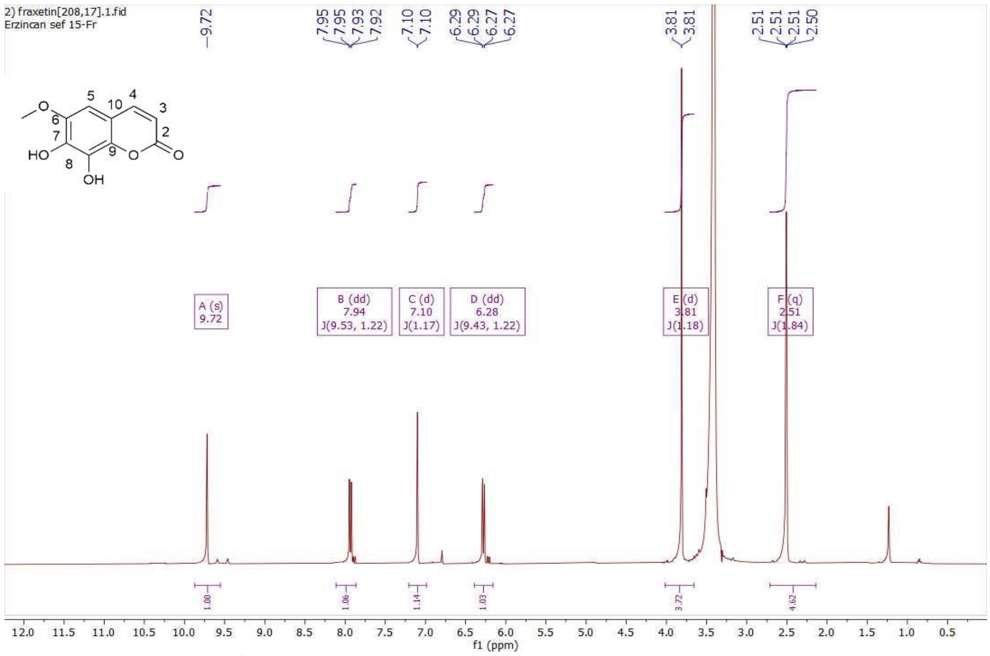

Figure S14: ^{1}H-NMR spectrum of Fraxetin **(7) (**400 MHz, DMSO-d_6)

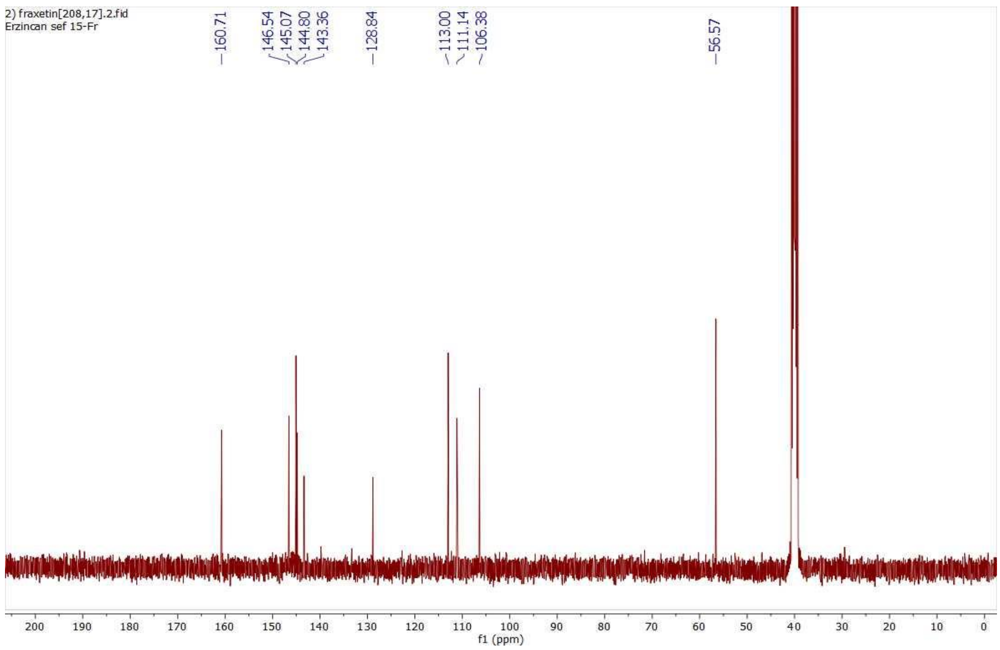

Figure S15: ^{13}C-NMR spectrum of Fraxetin **(7) (**100 MHz, DMSO-d_6)

Luteolin-7-*O*-*β*-glucoside (8): ^{1}H NMR (600 MHz, DMSO-d$_6$) δ 7.42 (dd, *J*=8.34, 2.30, 1H, H-6’), 7.39 (d, *J*=2.30, 1H, H-2’), 6.87 (d, *J*=8.34, 1H), 6.71 (s, 1H, H-3), 6.75 (d, *J*=2.18, 1H, H-8), 6.41 (d, *J*=2.18, 1H, H-6), 5.04 (d, *J*=7.48, 1H, H-1’’), 3.69 (dd, *J*=11.70, 1.90, 1H, H-6’’a), 3.46 (m, 1H, H-6’’b), 3.42 (m, 1H, H-5’’), 3.27 (m, 1H, H-3’’), 3.24 (m, 1H, H-2’’), 3.15 (d, *J*=9.14, 1H, H-4’’). ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 182.3 (C-4), 164.8 (C-2), 163.4 (C-7), 161.6 (C-5), 157.4 (C-9), 150.4 (C-4’), 146.2 (C-3’), 121.8 (C-1’), 119.6 (C-6’), 116.4 (C-5’), 114.0 (C-2’), 105.8 (C-10), 103.5 (C-3), 100.3 (C-1’’), 99.9 (C-6), 95.1 (C-8), 77.6 (C-5’’), 77.1 (C-3’’), 73.7 (C-2’’), 69.9 (C-4’’), 61.0 (C-6’’).

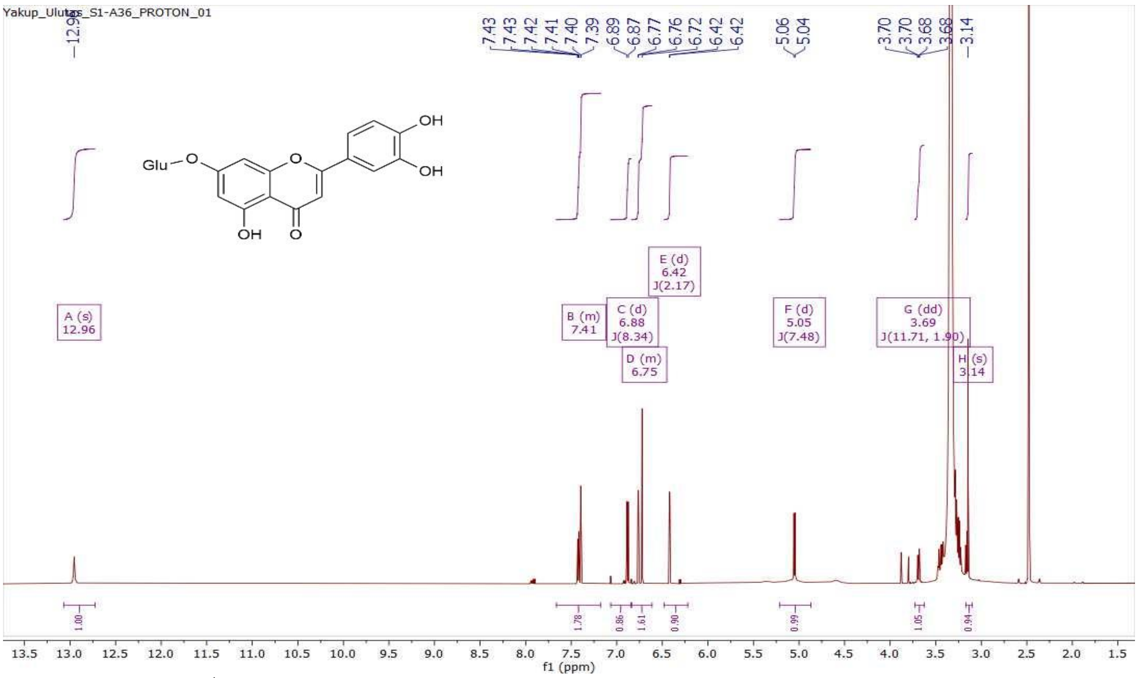

Figure S16: ^{1}H-NMR spectrum of Luteolin-7-*O*-*β*-glucoside **(8)** **(**600 MHz, DMSO-d$_6$)

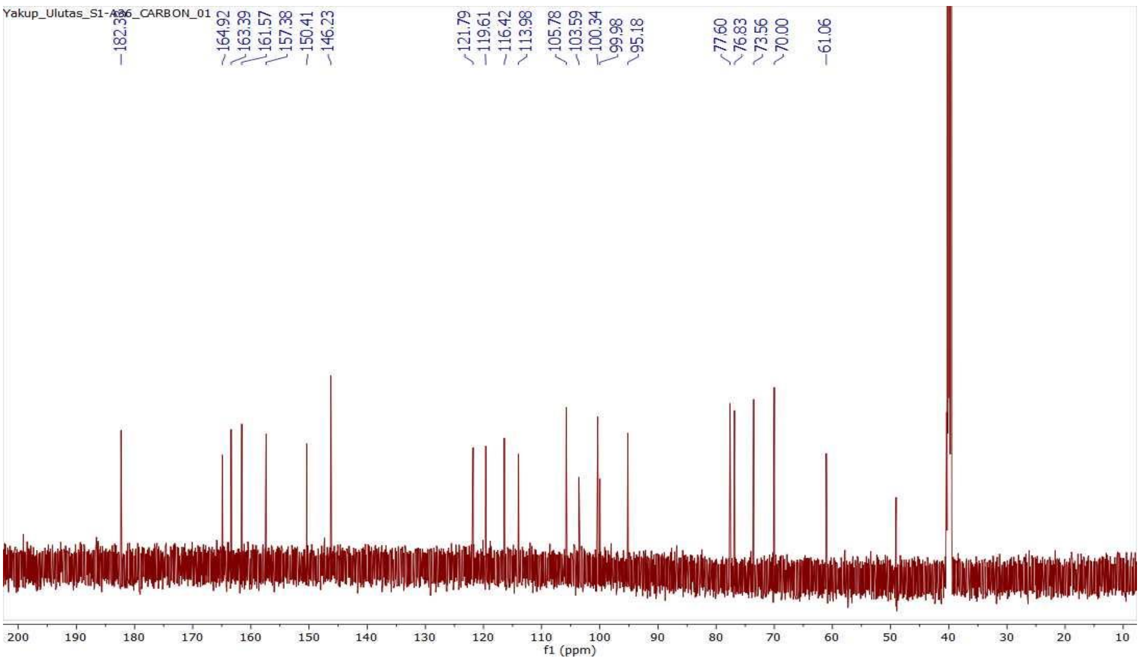

Figure S17: ^{13}C-NMR spectrum of Luteolin-7-*O*-*β*-glucoside **(8)** **(**150 MHz, DMSO-d$_6$)

Isofraxidin (9): ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91 (d, *J*=9.42, 1H, H-4), 7.08 (s, 1H, H-5), 6.30 (d, *J*=9.42, 1H, H-3), 3.88 (s, 3H, C-8-OCH_3), 3.70 82 (s, 3H, C-6-OCH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.6 (C-2), 149.8 (C-6), 147.0 (C-7), 144.7 (C-4), 144.0 (C-8), 134.1 (C-9), 114.6 (C-3), 113.8 (C-10), 105.8 (C-5), 60.9 (C-8-OCH_3), 56.5 (C-6-OCH_3).

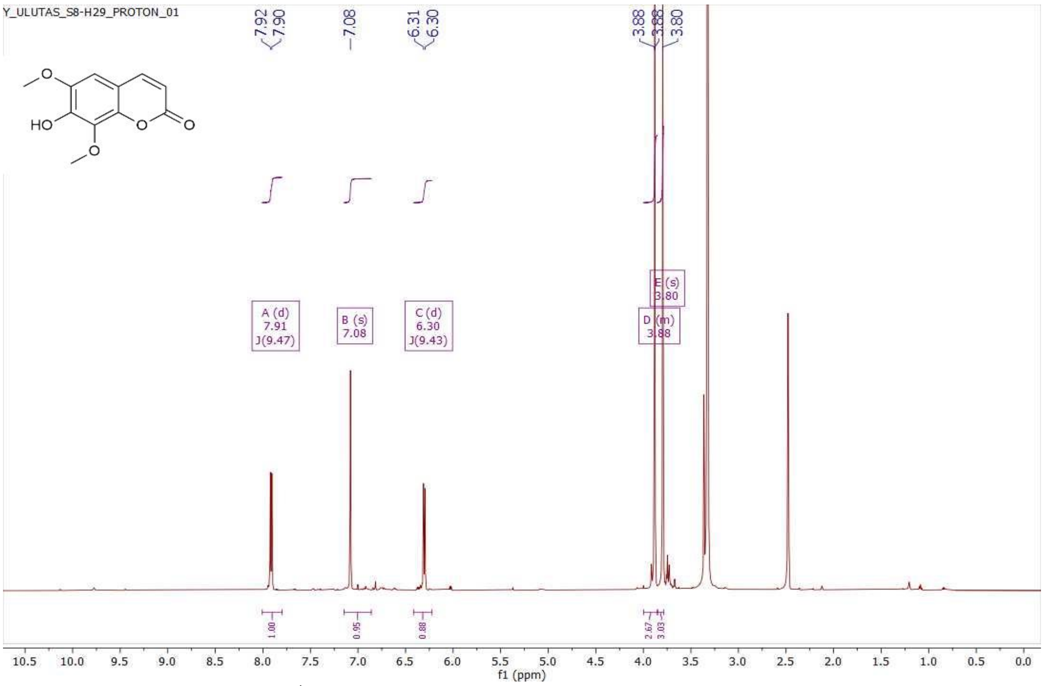

Figure S18: ^{1}H-NMR spectrum of Isofraxidin **(9) (**600 MHz, DMSO-d_6)

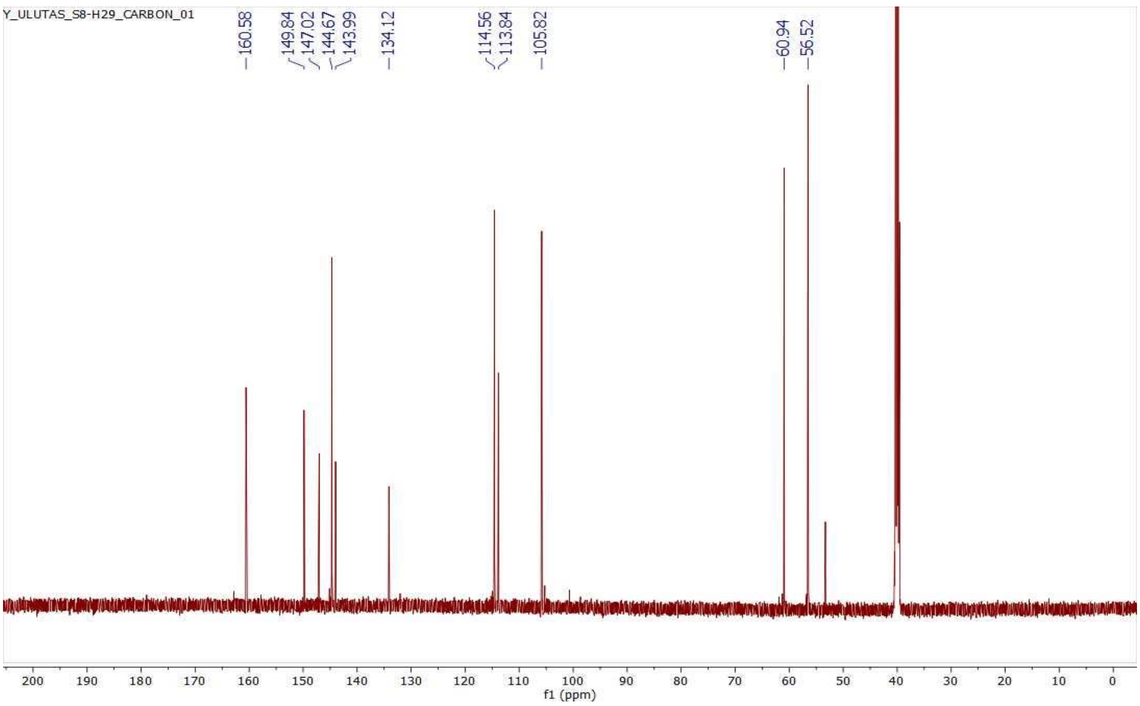

Figure S19: ^{13}C-NMR spectrum of Isofraxidin **(9) (**150 MHz, DMSO-d_6)

Picein (10): ^{1}H NMR (400 MHz, DMSO) δ 7.92 (d, *J*=8.89, 2H, H-2, H-6), 7.12 (d, *J*=8.89, 2H, H-3, H-5), 5.01 (d, *J*=7.42, 1H, H-1'), 4.07 (dd, *J*=11.17, 1.89, 1H, H-6'a), 3.75 (dd, *J*=11.17, 6.44, 1H, H-6'b), 3.60 (ddd, *J*=8.52, 6.44, 1.89, 1H, H-5'), 3.33 (m, 1H, H-3'), 3.28 (m, 1H, H-2'), 3.15 (dd, *J*=8.52, 4.79, 1H, H-4'), 2.53 (s, 3H, Hα). ^{13}C NMR (100 MHz, MeOD) δ 196.9 (C=O), 161.5 (C-4), 131.3 (C-1), 130.7 (2C, C-2, C-6), 116.3 (2C, C-3, C-5), 100.2 (C-1'), 76.8 (C-3'), 75.2 (C-5'), 73.6 (C-2'), 70.3 (C-4'), 66.1 (C-6'), 26.9 (C-α).

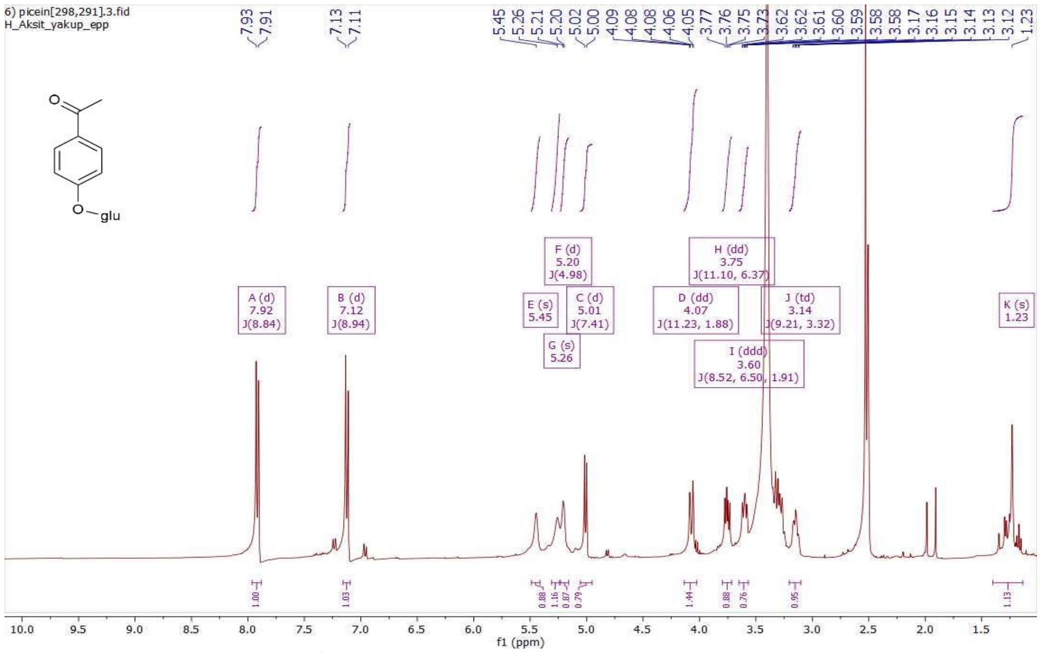

Figure S20: ^{1}H-NMR spectrum of Picein **(10)** **(**400 MHz, DMSO-d_6)

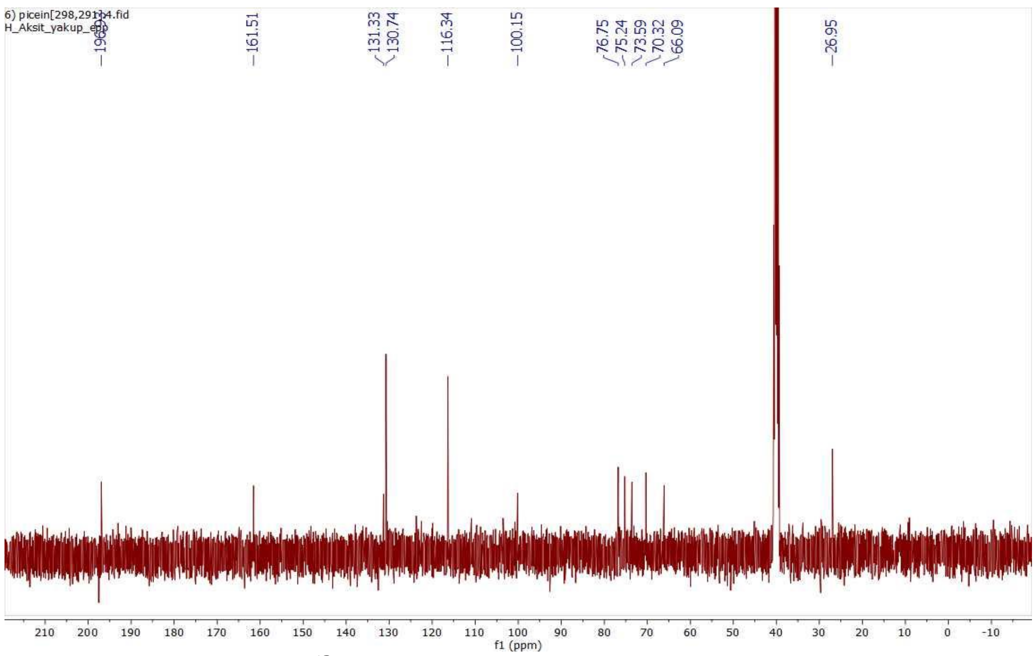

Figure S21: ^{13}C-NMR spectrum of Picein **(10)** (100 MHz, DMSO-d_6)

Isofraxidin-7-*O*-glucoside (11): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (d, *J*=9.55, 1H, H-4), 7.14 (s, 1H, H-5), 6.41 (d, *J*=9.55, 1H, H-3), 5.18 (d, *J*=5.79, 1H, H-1'), 3.93 (s, 3H, C-8-OCH_3), 3.83 (s, 3H, C-6-OCH_3), 3.62 (d, *J*=11.61, 1H, H-6'a), 3.37 (m, 1H, H-6'b), 3.26 (m, 1H, H-2'), 3.25 (m, 1H, H-5'), 3.14 (m, 1H, H-4'), 3.11 (m, 1H, H-3'). ^{13}C NMR (100 MHz, , DMSO-d_6) δ 160.2 (C-2), 149.9 (C-6), 144.8 (C-4), 142.9 (C-7), 142.1 (C-8), 140.7 (C-9), 115.2 (C-3), 115.0 (C-10), 106.0 (C-5), 102.6 (C-1'), 74.6 (C-2'), 78.0 (C-3'), 70.4 (C-4'), 77.0 (C-5'), 61.3 (C-6'), 61.7 (C-8-OCH_3), 57.0 (C-6-OCH_3).

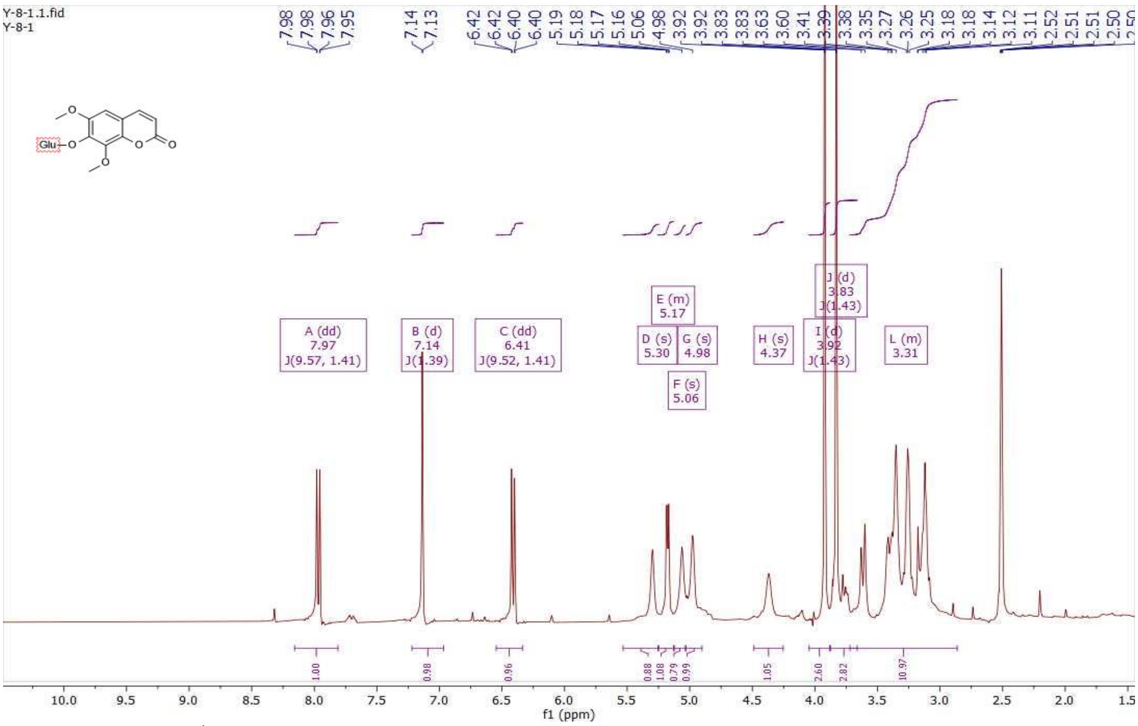

Figure S22: ^{1}H-NMR spectrum of Isofraxidin-7-*O*-glucoside **(11) (**400 MHz, DMSO-d_6)

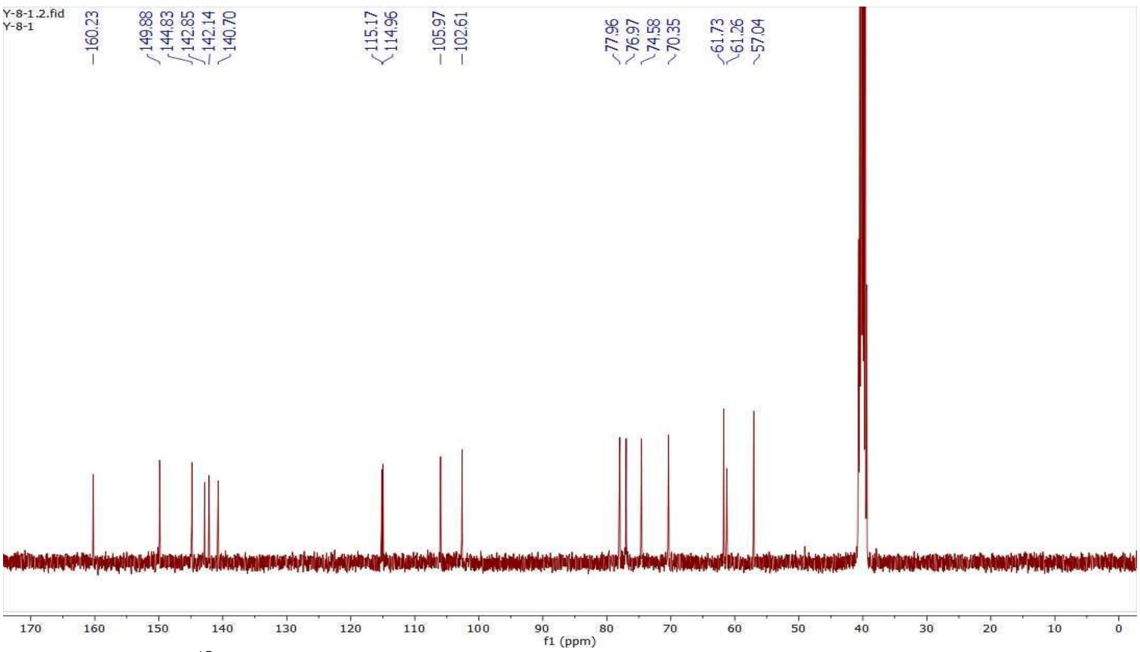

Figure S23: ^{13}C-NMR spectrum of Isofraxidin-7-*O*-glucoside **(11) (**100 MHz, DMSO-d_6)

Fraxidin (12): ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (brs, 1H, OH), 7.93 (d, *J*=9.57, 1H, H-4), 6.83 (s, 1H, H-5), 6.36 (d, *J*=9.57, 1H, H-3), 3.82 (s, 3H, C-7-OCH_3), 3.78 (s, 3H, C-8-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6 (C-2), 150.2 (C-6), 145.1 (C-4), 140.6 (C-7), 139.0 (C-8), 138.8 (C-9), 115.0 (C-3), 114.8 (C-10), 100.7 (C-5) 61.0 (C-7-OCH_3), 56.6 (C-6-OCH_3).

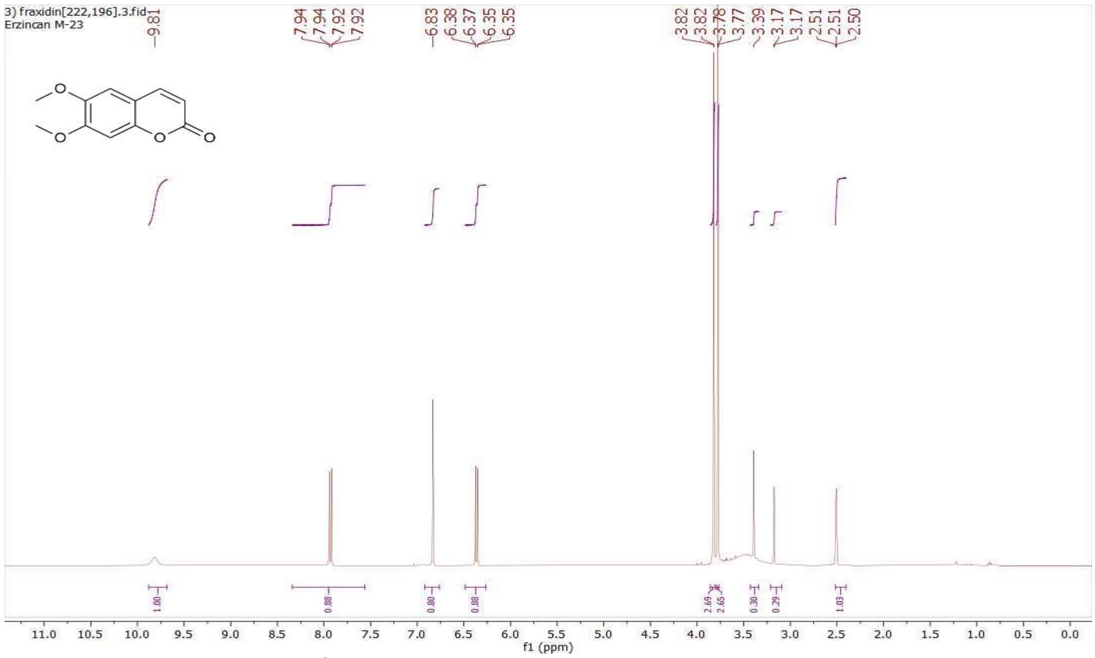

Figure S24: ^{1}H-NMR spectrum Fraxidin **(12)** **(**400 MHz, DMSO-d_6)

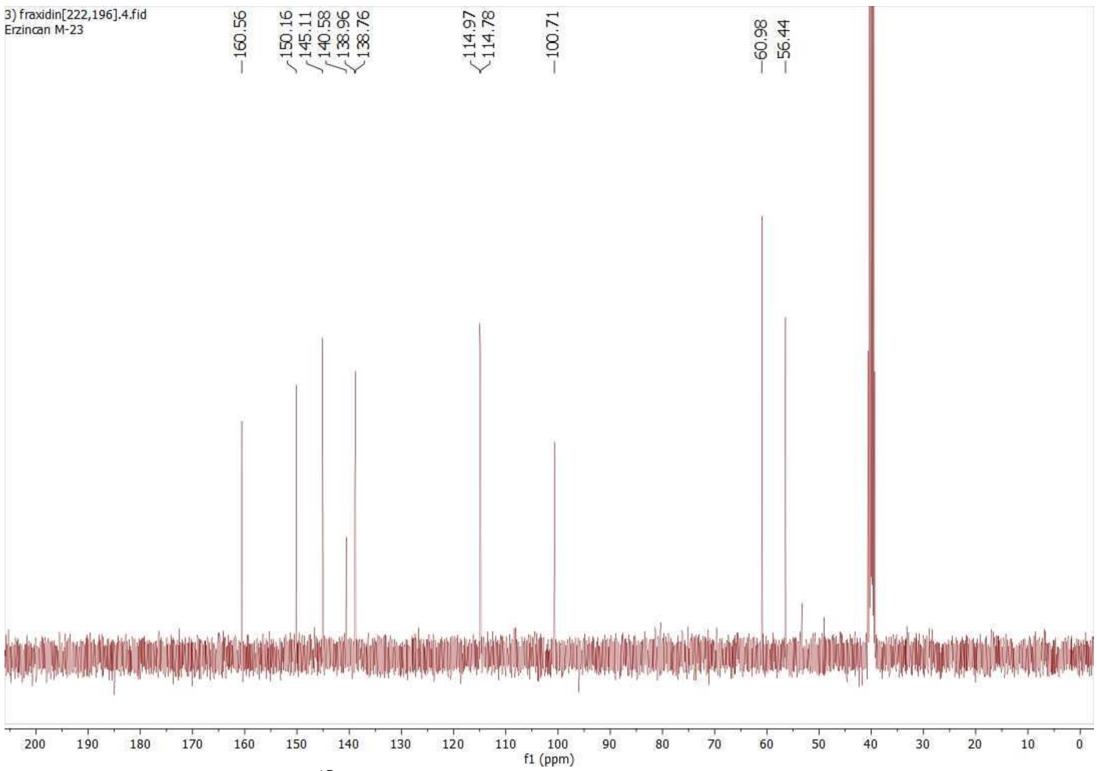

Figure S25: ^{13}C-NMR spectrum Fraxidin **(12)** **(**100 MHz, DMSO-d_6)

Rutin (13): ^{1}H NMR (600 MHz, DMSO-d$_6$) δ 7.54 (m, 1H, H-2'), 7.54 (m, 1H, H-6'), 6.38 (d, *J*=1.95, 1H, H-8), 6.19 (d, *J*=1.95, 1H, H-6), 5.34 (d, *J*=7.07, 1H, H-1''), 4.38 (brs, 1H, H-1'''), 3.71 (d, *J*=10.2, 1H, H-6''a), 3.39 (m, 1H, H-3'''), 3.31 (m, 1H, H-4''), 3.31 (m, 1H, H-5'''), 3.26 (m, 1H, H-6''b), 3.24 (m, 1H, H-2''), 3.24 (m, 1H, H-3''), 3.24 (m, 1H, H-5''), 3.06 (m, 1H, H-2'''), 3.06 (m, 1H, H-4'''), 0.99 (d, *J*=6.04, 3H, H-6'''). ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 178.0 (C-4),164.7 (C-7), 161.6 (C-5), 157.9 (C-9) 157.1 (C-2), 148.4 (C-4'), 144.4 (C-3'), 134.2 (C-3), 122.2 (C-1'), 121.2 (C-6'), 116.3 (C-2'), 114.7 (C-5'), 104.2 (C-10), 103.3 (C-1''), 101.0 (C-1'''), 98.6 (C-6), 93.5 (C-8), 76.8 (C-3''), 75.8 (C-5''), 74.3 (C-2''), 72.5 (C-4'''), 71.0 (C-4''), 70.8 (C-3'''), 70.7 (C-2'''), 68.3 (C-5'''), 67.2 (C-6''), 16.5 (C-6''').

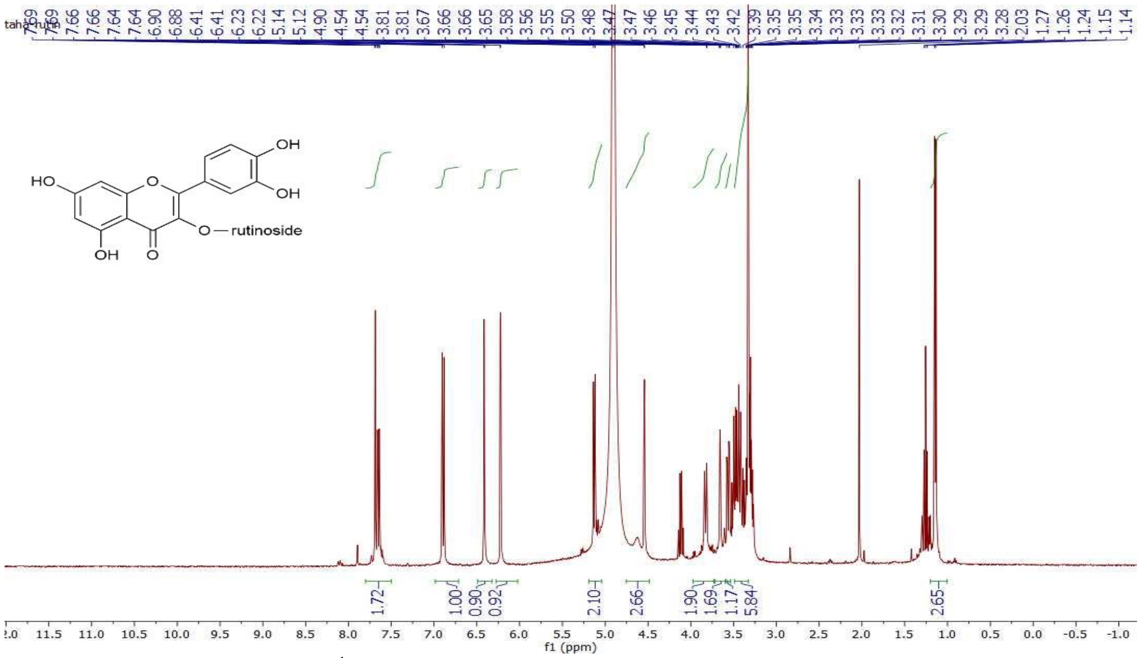

Figure S26: ^{1}H-NMR spectrum Rutin **(13)** **(**400 MHz, DMSO-d$_6$)

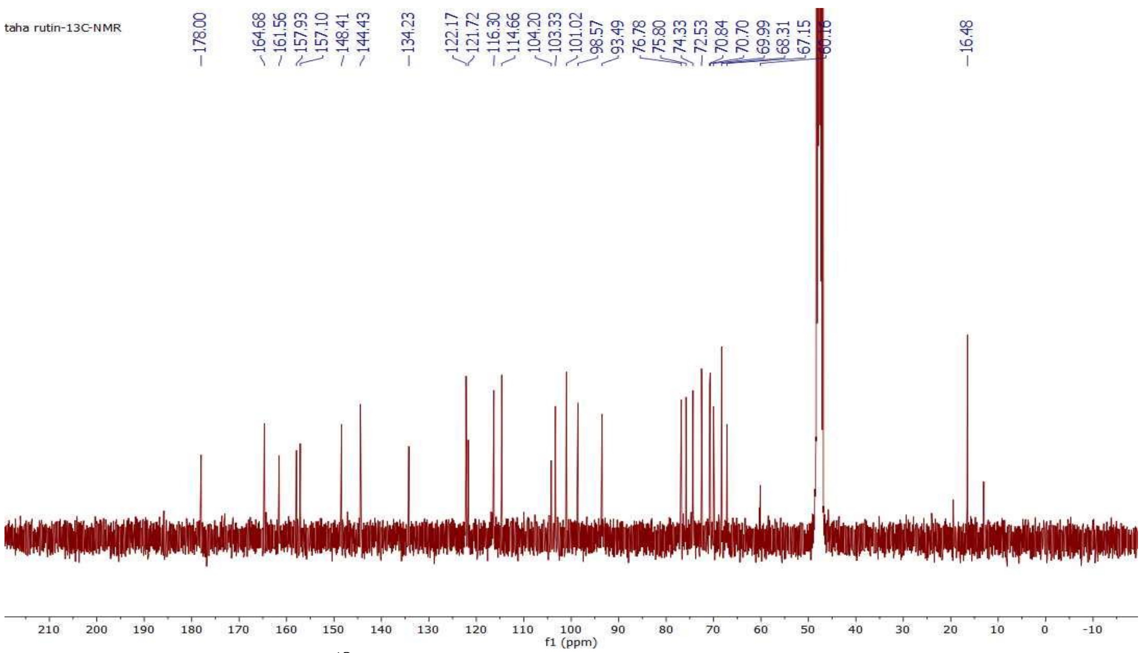

Figure S27: ^{13}C-NMR spectrum Rutin **(13)** **(**400 MHz, DMSO-d$_6$)

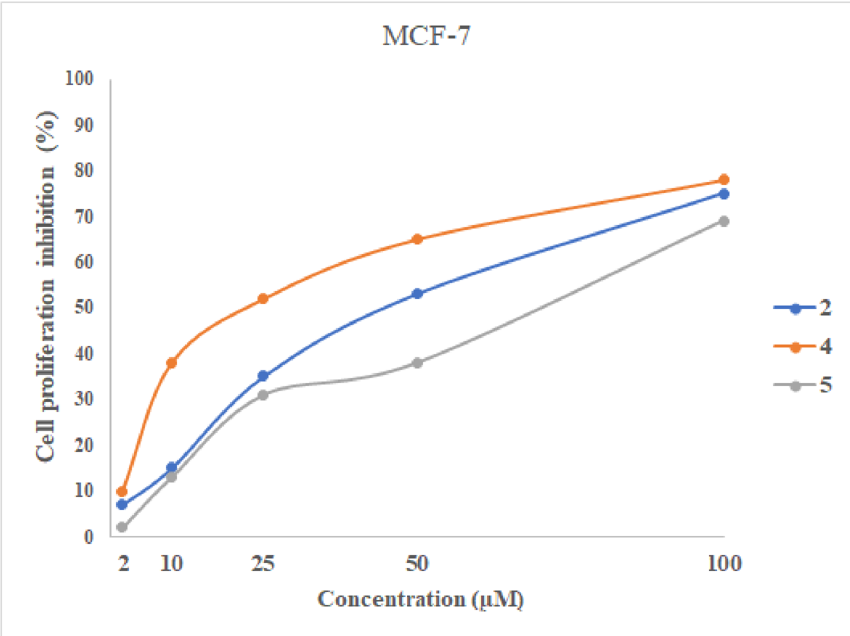

Figure S28: Antiproliferative activities of the compounds (**2**, **4**, and **5**) against MCF-7 cell line by XTT colorimetric assay. The results represent the mean of standard deviation (±SD) from three independent experiments (n≥3).