

Supporting Information

Phytochemical Profile of *Trigonella caerulea* (Blue Fenugreek) Herb and Quantification of Aroma-Determining Constituents

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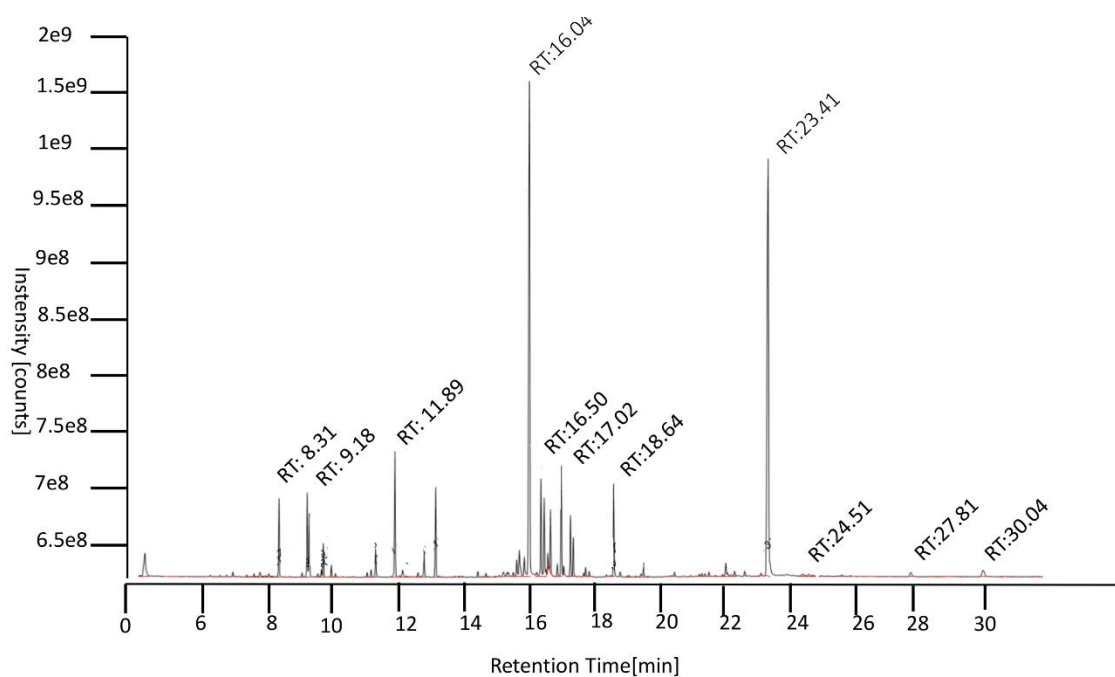


Figure S1. Total ion GC-MS chromatogram from untargeted profiling of primary metabolites. Column: ThermoFisher TG-5SilMS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m). GC conditions: 100°C hold for 5 min, 25°C/min to 160°C hold 1 min, 10°C/min to 300°C hold for 12 min. MS parameters: Fullscan 50-500 m/z. Ion source temperature: 280°C.

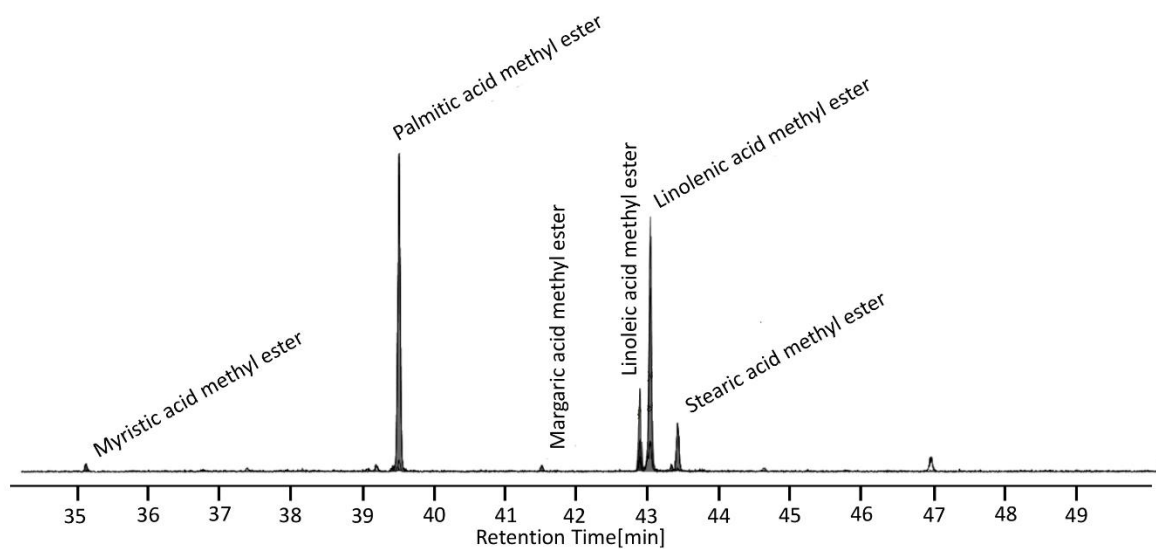


Figure S2. GC-MS chromatogram of fatty acid analysis. Column: ThermoFisher TG-5SilMS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m). GC conditions: 50°C for 5 min and heated with 5°C/min to 160°C hold 1 min, 5°C/min to 300°C and hold for 5 min. MS parameters: Full Scan from 50-500 m/z, ion source temperature: 280°C.

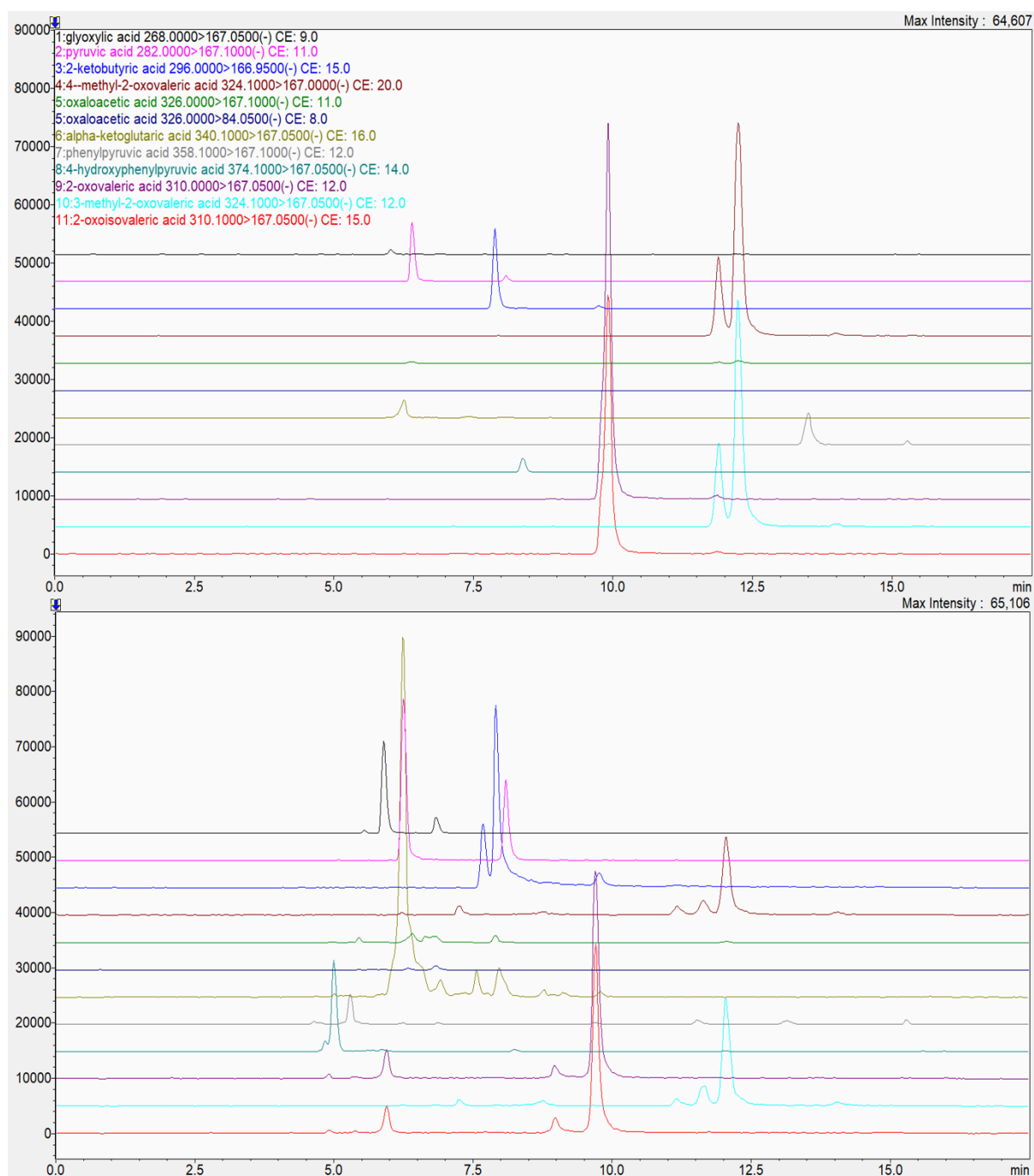


Figure S3. UHPLC-MS/MS chromatogram of standard solution (top) and sample (bottom).

Column: Phenomenex Kinetex Biphenyl (100 × 2.1 mm, 1.7 μM). LC conditions: 50 mM formic acid in water (A) and acetonitrile (B). Gradient: 0 min (10% B), 2 min (10% B), 2.5 min (35% B), 13.5 min (40% B), 13.6 min (95% B), 17.5 min (95% B). Post run: 4 min. Column temperature: 40°C. Flow rate: 0.3 mL/min. Injection: 5 μL. MS conditions: Nebulizing gas flow: 3 L/min, DL temperature 250°C, heat block temperature: 400°C, drying gas flow: 15 L/min. Collision energies and transitions used for quantification are depicted in the figures.

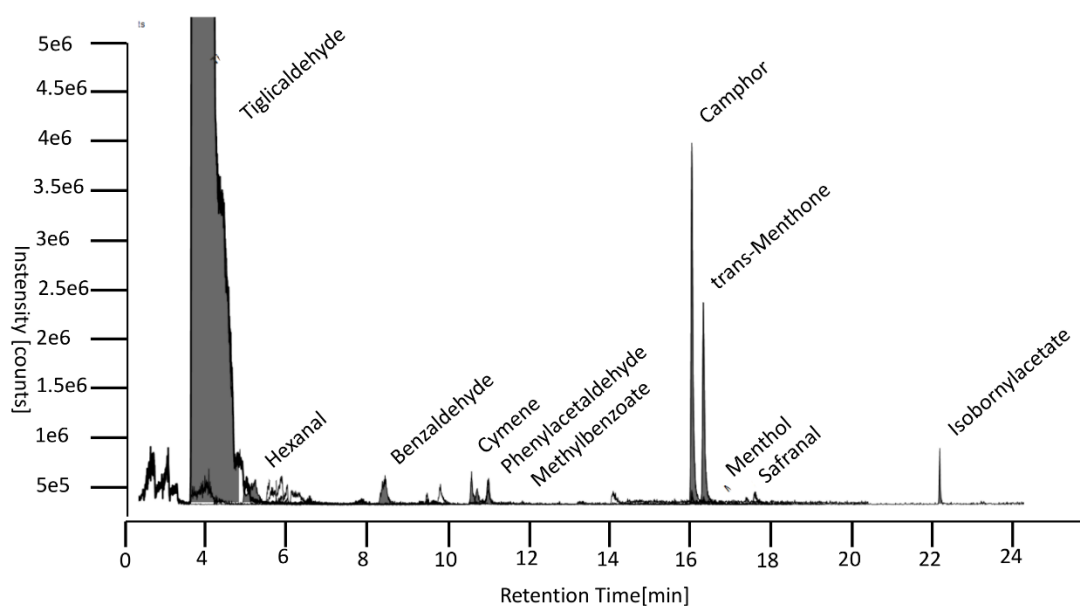


Figure S4. Total ion chromatogram of headspace GC-MS analysis. Column: ThermoFisher TG-5SilMS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m). GC conditions: 35°C hold 1 min, 5°C/min to 120°C and hold 1 min, 30°C/min to 300°C and hold 1 min. The MS parameters were as follows: 43-300 m/z scan with MS Source at 280°C.

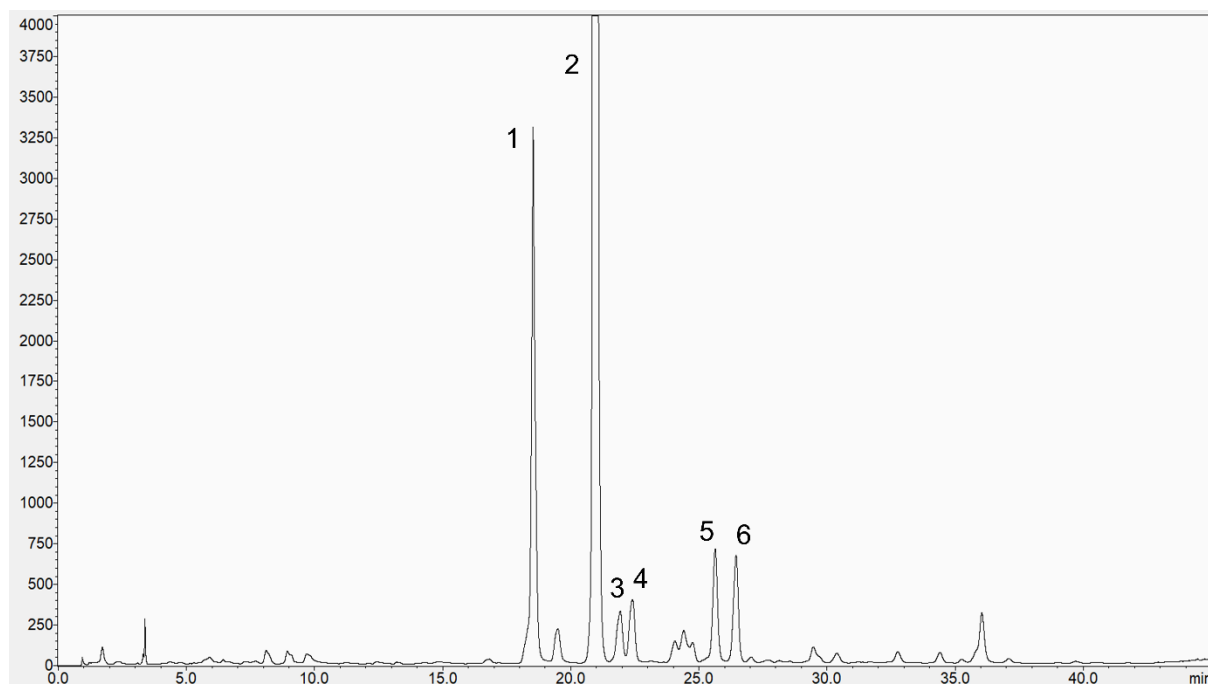


Figure S5. UHPLC-PDA chromatogram of blue fenugreek methanol extract at a wavelength of 350 nm. Column: Phenomenex Luna Omega Polar (100 × 2.1 mm, 1.6 μm particle size). Solvent system: 1% formic acid in water (A) and 1% formic acid in acetonitrile (B). Gradient: 0 min (5% B), 10 min (10% B), 40 min (20% B), 45 min (32% B), 60 min (95% B), 65 min (95% B). Post run: 10 min. Column temperature: 35°C. Flow rate: 0.3 mL/min. Injection: 2 μL. Compounds: Quercetin 3-O-(2''-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside 7-O-β-D-rhamnopyranoside (**1**), kaempferol 3-O-(2''-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside 7-O-β-D-rhamnopyranoside (**2**), quercetin 3-O-(2''-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**3**), quercetin 3-O-β-D-glucopyranoside 7-O-β-D-rhamnopyranoside (**4**), kaempferol 3-O-(2''-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**5**), and kaempferol 3-O-β-D-glucopyranoside 7-O-β-D-rhamnopyranoside (**6**).

Table S1

List of compounds obtained from untargeted profiling of primary metabolites. All values were obtained from the total ion chromatogram. Relative peaks areas are calculated from identified components.

Peak Name	t _{Ret} min	TC1* rel. area (%)	TC2* rel. area (%)	TC3* rel. area (%)	SI	RSI
Lactic Acid 2 TMS	6.48	0.06	0.06	0.06	835	920
Glycolic Acid 2 TMS	6.68	0.06	0.05	0.05	825	887
Valine TMS	6.88	0.18	0.20	0.22	835	871
Alanine 2 TMS	7.06	0.03	0.03	0.04	710	829
Leucine TMS	7.72	0.26	0.29	0.32	750	773
Isoleucine TMS	7.99	0.16	0.18	0.20	788	821
Malonic Acid 2 TMS	8.31	2.75	3.05	2.46	901	901
Serine 2 TMS	9.02	0.17	0.19	0.21	879	900
Glycerol 3 TMS	9.18	3.13	3.47	3.86	929	931
Phosphate 3 TMS	9.25	2.33	2.59	2.87	938	941
Threonine 2 TMS	9.51	0.16	0.18	0.20	824	876
Succinic Acid 2 TMS	9.68	1.16	1.29	1.43	930	935
Glyceric Acid 3 TMS	9.92	0.42	0.47	0.52	890	899
Fumaric Acid 2 TMS	10.05	0.12	0.13	0.15	902	936
Caproic acid 3-hydroxy, 2TMS	11.03	0.17	0.19	0.21	710	756
Malic acid 3TMS	11.89	4.86	5.39	4.77	923	928
Threitol 4 TMS	12.13	0.42	0.47	0.52	707	821
4-Hydroxybenzoic acid 2 TMS	13.59	0.02	0.02	0.02	754	795
Pinitol 5 TMS	16.04	28.25	31.36	30.66	943	944
Fructose MOX, 5 TMS Isomer 1	16.40	4.16	2.81	2.77	925	926
Fructose MOX, 5 TMS Isomer 2	16.50	3.41	3.79	3.34	932	932
Glucose MOX 5 TMS	16.69	2.38	2.64	2.93	919	919
Mannitol 6 TMS	17.02	4.62	5.13	3.87	946	946
Gluconic Acid 6 TMS	17.71	0.16	0.18	0.20	828	903
Palmitic Acid TMS	17.88	0.23	0.26	0.28	864	884
Ferulic acid 2 TMS	18.48	0.05	0.06	0.06	798	872
Myo-Inositol 6 TMS	18.64	3.91	4.34	3.33	946	964
Galactose MOX, 6 TMS	18.84	0.22	0.24	0.27	820	868
Linoleic acid TMS	19.48	0.18	0.20	0.22	842	930
Linolenic acid TMS	19.56	0.47	0.52	0.58	884	888
Stearic acid TMS	19.70	0.05	0.06	0.06	716	802
Methylhydroxycinnamate TMS	22.37	0.25	0.28	0.31	667	767
Saccharose 8 TMS	23.41	33.68	28.41	31.55	946	961
Aucubin 6 TMS	24.54	0.11	0.12	0.14	780	826
Galacitinol 9 TMS	27.81	0.47	0.52	0.58	861	884
Glycerol-Galactopyranoside 6 TMS	30.04	0.94	1.04	0.81	832	877

* TC1 means *T. caerulea* sample 1, TC2 means *T. caerulea* sample 2, TC3 means *T. caerulea* sample 3.

Table S2NMR data for compound **1** in DMSO-*d*₆ (400 MHz, δ in ppm, *J* in Hz).

Position	¹ H NMR	¹³ C NMR	HMBC	H,H COSY
aglycone				
2		156.7		
3		133.2		
4		177.5		
5		160.9		
6	6.42 (s)	99.4	C-5, C-7, C-8, C-10	
7		161.6		
8	6.79 (d, 2.0)	94.2	C-6, C-7, C-9, C-10,	
9		155.9		
10		105.6		
1'		121.0		
2'	7.58 (d, 2.2)	116.1	C-2, C-3', C-4', C-6'	
3'		145.0		
4'		148.7		
5'	6.85 (d, 8.5)	115.2	C-3', C-4', C-6'	
6'	7.64 (dd, 8.4, 2.2)	121.8	C-2, C-2', C-4'	
OH-5	12.64			
OH-3'	9.23			
OH-4'	9.88			
3-O-glucose				
1''	5.65 (d, 7.7)	98.3	C-3	H-2''
2''	3.51(m)	77.4	C-1'', C-3'', C-1''''	H-1''
3''	3.42 (m)	77.3		
4''	3.09 (m)	70.3		
5''	3.08 (m)	77.6		
6''	3.29 (m)			
6''	3.57 (dd, 12.3, 5.7)	60.9		
7-O-rhamnose				
1'''	5.55 (brs)	98.4	C-7, C-3'''	H-2'''
2'''	3.84 (m)	69.8		H-3'''
3'''	3.63 (m)	70.2		H-2''', H-4'''
4'''	3.32 (m)	71.6		H-5'''
5'''	3.44 (m)	70.1		H-6'''
6'''	1.11 (d, 6.1)	18.0	C-4''', C-5'''	H-5'''
2''-O-rhamnose				
1''''	5.07 (brs)	100.5	C-2'', C-3''', C-5''''	H-2''''
2''''	3.73 (m)	70.6		H-3''''
3''''	3.46 (m)	70.7	C-4''''	H-2''', H-4''''
4''''	3.12 (m)	71.9		H-5''''
5''''	3.71 (m)	68.3		H-3''''
6''''	0.77 (d, 6.3)	17.3	C-4''', C-5''''	H-5''''

Table S3NMR data for compound **2** in DMSO-*d*₆ (400 MHz, δ in ppm, *J* in Hz).

Position	¹ H NMR	¹³ C NMR	HMBC	H,H COSY
aglycone				
2		156.7		
3		133.0		
4		177.6		
5		160.9		
6	6.44 (d, 2.2)	99.5	C-5, C-7, C-8, C-10,	H-8
7		161.6		
8	6.83 (d, 2.0)	94.5	C-6, C-7, C-9, C-10	H-6
9		156.0		
10		105.7		
1'		120.8		
2'	8.08 (d, 9.0)	130.9	C-2, C-4'	H-3'
3'	6.89 (d, 9.0)	115.2	C-1', C-4'	H-2'
4'		160.1		
5'	6.89 (d, 9.0)	115.2	C-1', C-4'	H-6'
6'	8.08 (d, 9.0)	130.9	C-2, C-4'	H-5'
OH-5	12.62			
OH-4'	10.26			
3-O-glucose				
1''	5.66 (d, 7.0)	98.3	C-3	H-2''
2''	3.44 (m)	77.4	C-3''	H-1''
3''	3.39 (m)	77.3		
4''	3.09 (m)	70.3		
5''	3.08 (m)	77.6		
6''	3.28 (m)			
6''	3.55 (dd, 11.3, 5.6)	60.8		
7-O-rhamnose				
1'''	5.55 (d, 1.5)	98.4	C-7, C-2'''	
2'''	3.84 (m)	69.8		
3'''	3.63 (m)	70.2		
4'''	3.31 (m)	71.6		
5'''	3.42 (m)	70.1	C-4''', C-6'''	
6'''	1.11 (d, 6.2)	18.0	C-4''', C-5'''	
2''-O-rhamnose				
1''''	5.07 (d, 0.9)	100.7	C-2'', C-3''', C-5''''	H-2''''
2''''	3.73 (m)	70.6		H-3''''
3''''	3.46 (m)	70.5		H-4''''
4''''	3.13 (m)	71.8	C-6''''	H-3''', H-5''''
5''''	3.70 (m)	68.4	C-4''''	H-4''''
6''''	0.75 (d, 6.2)	17.3	C-4''', C-5''''	H-5''''

Table S4NMR data for compound **3** in DMSO-*d*₆ (400 MHz, δ in ppm, *J* in Hz).

Position	¹ H NMR	¹³ C NMR	HMBC	H,H COSY
aglycone				
2		157.0		
3		133.1		
4		177.5		
5		161.6		
6	6.12 (s)	99.6		H-8
7		166.5		
8	6.33 (d, 8.1)	94.2		H-6
9		156.9		
10		103.6		
1'		121.3		
2'	7.52 (d, 2.2)	116.2	C-3'	
3'		145.5		
4'		156.1		
5'	6.87 (d, 8.6)	115.6	C-1', C-3'	H-6'
6'	7.60 (dd, 10.4, 6.2)	122.1	C-4'	H-5'
OH-5	12.66			
OH-3'	8.46			
OH-4'	8.24			
3-O-glucose				
1''	5.66 (d, 7.4)	98.8	C-3	H-2'', H-3''
2''	3.50(m)	78.0	C-1'''	H-3''
3''	3.39 (m)	77.8	C-4''	H-4''
4''	3.09 (m)	70.7		H-3''
5''	3.08 (m)	77.7		H-6''
	3.30 (m)		C-2''	
6''	3.56 (dd, 12.3, 5.8)	61.4		
2''-O-rhamnose				
1'''	5.08 (brs)	100.9	C-2'', C-3'''	H-5'''
2'''	3.74 (m)	70.8		H-3''', H-4'''
3'''	3.46 (m)	71.0	C-4'''	H-4''', H-5'''
4'''	3.13 (m)	72.3	C-2''', C-5'''	H-3''', H-5'''
5'''	3.72 (m)	68.7	C-2''', C-4'''	H-4''', H-6'''
6'''	0.78 (d, 6.1)	17.7	C-4''', C-5'''	H-2'''

Table S5NMR data for compound **4** in DMSO-*d*₆ (400 MHz, δ in ppm, *J* in Hz).

Position	¹ H NMR	¹³ C NMR	HMBC	H,H COSY
aglycone				
2		157.1		
3		134.0		
4		172.4		
5		161.3		
6	6.44 (s)	99.8	C-5, C-7, C-10	
7		162.0		
8	6.80 (d, 2.0)	94.8	C-7	H-6
9		155.9		
10		106.0		
1'		121.2		
2'	7.63 (d, 2.2)	116.7	C-2, C-1', C-3', C-6'	
3'		145.4		
4'		149.4		
5'	6.85 (d, 9.0)	115.7	C-1', C-3', C-4'	H-6'
6'	7.61 (d, 2.3)	122.2	C-2, C-4'	
OH-5	12.62			
OH-3'	8.41			
OH-4'	8.29			
7-O-rhamnose				
1''	5.56 (brs)	98.8	C-7, C-3''	H-2''
2''	3.84 (m)	70.3		
3''	3.63 (m)	70.7	C-4''	
4''	3.29 (m)	71.9	C-3''	
5''	3.43 (m)	70.5	C-4''	
6''			C-4'', C-5''	H-5''
	1.12 (d, 6.3)	18.4		
3-O-glucose				
1'''	5.50 (d, 7.7)	101.5	C-3	H-2''', H-3'''
2'''	3.24 (m)	74.5	C-3'''	
3'''	3.26 (m)	77.0		H-1'''
4'''	3.09 (m)	70.4	C-5'''	
5'''	3.08 (m)	78.1		
	3.32 (m)			
6'''	3.65 (d, 3.2)	61.4		

Table S6NMR data for compound **5** in DMSO-*d*₆ (400 MHz, δ in ppm, *J* in Hz).

Position	¹ H NMR	¹³ C NMR	HMBC	H,H COSY
aglycone				
2		156.6		
3		132.5		
4		177.0		
5		161.1		
6	6.09 (d, 2.2)	99.4	C-5, C-7, C-8, C-10	
7		166.0		
8	6.32 (d, 2.0)	94.0	C-6, C-7, C-9, C-10	
9		160.1		
10		103.0		
1'		120.9		
2'	8.02 (d, 9.0)	130.6	C-1, C-9	
3'	6.88 (d, 2.0)	115.1	C-9	
4'		155.5		
5'	6.88 (d, 2.0)	115.1	C-9	
6'	8.02 (d, 9.0)	130.6	C-1, C-9	
OH-5	12.62			
OH-4'	8.47			
3-O-glucose				
1''	5.66 (d, 7.1)	98.3	C-3, C-2''	H-2''
2''	3.44 (m)	77.5		H-1'', H-6''
3''	3.38 (m)	77.4		H-2'', H-5''
4''	3.09 (m)	70.2		
5''	3.08 (m)	77.3		H-3''
6''	3.30 (m)			
2''-O-rhamnose	3.56 (dd, 11.8, 6.8)	61.2		
2''-O-rhamnose				
1'''	5.08 (s)	100.6	C-2'', C-2''', C-5'''	H-2'''
2'''	3.74 (m)	70.5		H-4'''
3'''	3.46 (m)	70.6		H-4''', H-5'''
4'''	3.12 (m)	71.8		H-3''', H-5'''
5'''	3.72 (m)	68.8	C-2''', C-4'''	H-1''', H-3''', H-4'''
6'''	0.76 (d, 6.1)	17.7	C-4''', C-5'''	H-5'''

Table S7NMR data for compound **6** in DMSO-*d*₆ (400 MHz, δ in ppm, *J* in Hz).

Position	¹ H NMR	¹³ C NMR	HMBC	H,H COSY
aglycone				
2		157.2		
3		133.8		
4		178.0		
5		161.4		
6	6.45 (d, 2.2)	99.8	C-5, C-7, C-8, C-10	H-8
7		162.0		
8	6.84 (d, 2.0)	94.9	C-6, C-7, C-9, C-10	H-6
9		156.4		
10		106.1		
1'		120.9		
2'	8.09 (d, 9.0)	131.5	C-2, C-3', C-4'	H-3'
3'	6.89 (d, 9.0)	115.7	C-1', C-4'	H-2'
4'		160.9		
5'	6.89 (d, 9.0)	115.7	C-2, C-4', C-6'	H-6'
6'	8.09 (d, 9.0)	131.5	C-1', C-4'	H-5'
OH-5	12.62			
OH-4'	8.47			
7-O-rhamnose				
1''	5.56 (d, 1.2)	98.8	C-7, C-5''	
2''	3.85 (m)	70.3		
3''	3.63 (m)	70.7		
4''	3.32 (m)	72.0	C-5''	
5''	3.43 (m)	70.5		
6''			C-4'', C-5''	H-5''
	1.12 (d, 6.2)	18.4		
3-O-glucose				
1'''	5.49 (d, 7.3)	101.2	C-3	H-2''', H-3'''
2'''	3.20 (m)	74.6		
3'''	3.22 (m)	76.9		
4'''	3.10 (m)	70.4		
5'''	3.09 (m)	78.0		
	3.33 (m)			
6'''	3.57 (d, 11.4)	61.3		