Supplementary materials

Suppression of hyperglycemia and hepatic steatosis by black-soybean-leaf extract via enhanced adiponectin-receptor signaling and AMPK activation

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Chemical Structure Elucidation. ESI-MS were obtained on an Agilent 6410 triple quadrupole LC/MS system (Agilent Technologies, Palo Alto, CA, USA). UV spectra were obtained on an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Palo Alto, CA, USA). NMR spectra were recorded on a JEOL JNM-ECA600 600MHz FT-NMR spectrometer (JEOL, Tokyo, Japan) with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in δ values.

Their physico-chemical data of flavonol glycosides **1–9** were described in Table S1. The structures of flavonol glycosides **1–9** were elucidated based on ESI-MS and NMR analysis (Figure S1; Tables S2–S5) and comparison with previously published data [S1–S12].

Their physico-chemical data of isoflavones 6''-*O*-malonyldaidzin (6MD) and 6''-*O*-malonylgenistin (6MG) were also described in Table S1. The structures of isoflavones 6MD and 6MG were identified based on ESI-MS analysis (Figure S1) and HPLC diode array spectra monitoring with authentic standard compounds of 6MD and 6MG and comparison with previously published data [S13–S16]. The standard compounds 6MD and 6MG were obtained from Funakoshi (Kyoto, Japan).

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Table S1. Physico-chemical data for compounds **1**–**9**, 6MD, and 6MG.

Compounds	Physical properties	UV λ_{max} (nm)	Molecular weight	Molecular formula
1	Yellow green powder	202, 255, 353	772.66	$C_{33}H_{40}O_{21}$
2	Yellow green powder	202, 255, 353	772.66	$C_{33}H_{40}O_{21}$
3	Yellow green powder	203, 255, 352	626.52	$C_{27}H_{30}O_{17}$
4	Yellow green powder	201, 255, 353	626.52	$C_{27}H_{30}O_{17}$
5	Yellow green powder	203, 254, 354	610.52	$C_{27}H_{30}O_{16}$
6	Yellow green powder	202, 254, 354	640.55	$C_{28}H_{32}O_{17}$
7	Yellow green powder	201, 253, 354	624.55	$C_{28}H_{32}O_{16}$
8	Brown powder	196, 265, 346	740.66	$C_{33}H_{40}O_{19}$
9	Yellow powder	195, 264, 347	594.52	$C_{27}H_{30}O_{15}$
6MD	White powder	249	502.42	$C_{24}H_{22}O_{12}$
6MG	White powder	259	518.42	$C_{24}H_{22}O_{13}$

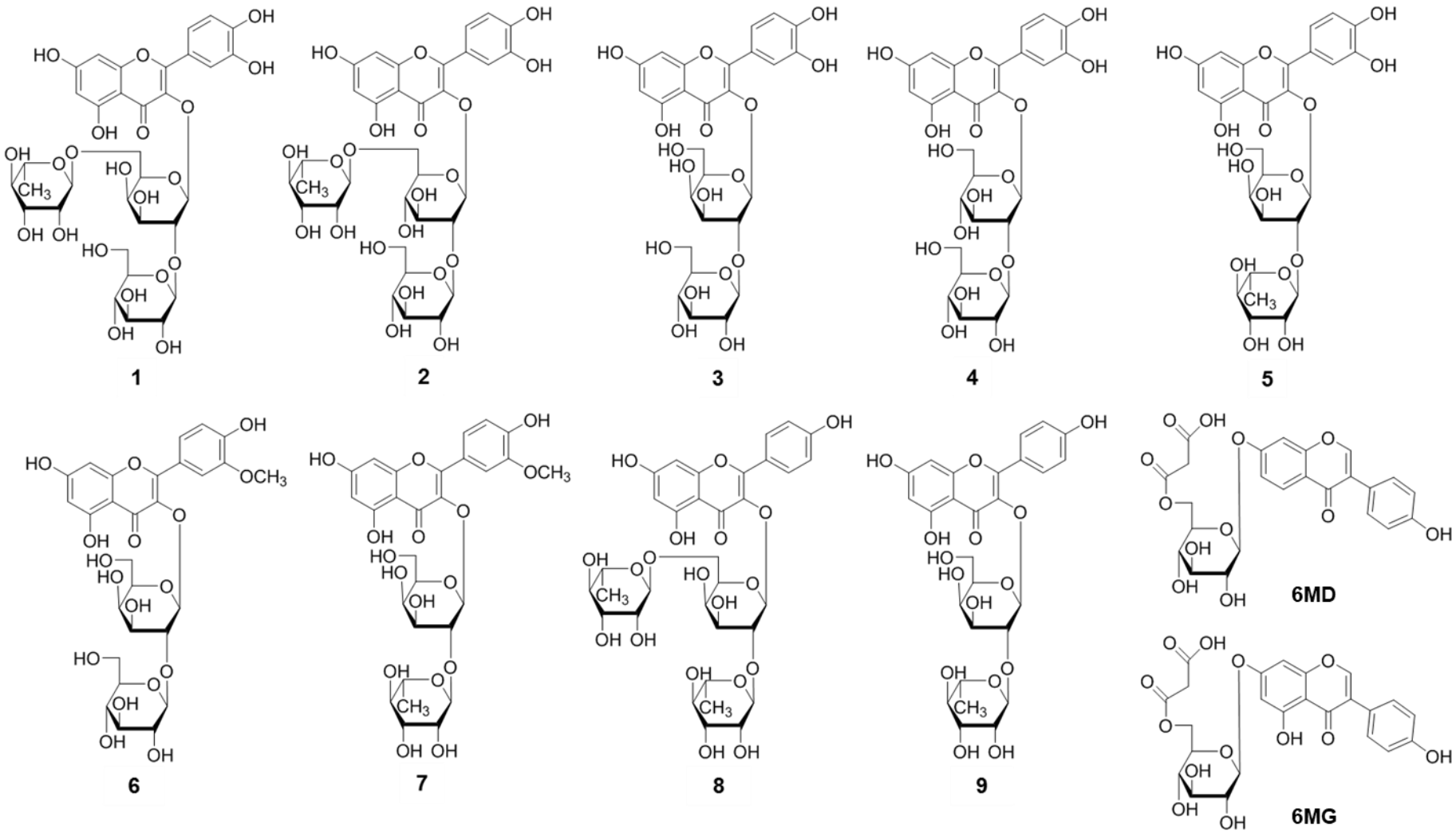

Figure S1. Chemical structures of compounds **1**–**9**, **6**MD, and 6MG isolated from EBL.

Table S2. ^{1}H-NMR spectral data for compounds **1**–**5**[a] (δ in ppm, *J* in Hz)

Position	1[b]	2[b]	3[c]	4[c]	5[c]
Aglycone					δ_H (*J* in Hz)
6	6.10 (1H, br s)	6.17 (1H, br s)	6.19 (1H, br s)	6.19 (1H, br s)	6.16 (1H, br s)
8	6.30 (1H, br s)	6.36 (1H, br s)	6.38 (1H, br s)	6.38 (1H, br s)	6.36 (1H, br s)
2'	7.51 (1H, d, 2.1)	7.51 (1H, d, 2.1)	7.73 (1H, br s)	7.73 (1H, br s)	7.69 (1H, d, 1.9)
5'	6.81 (1H, d, 8.3)	6.85 (1H, d, 8.9)	6.87 (1H, d, 7.3)	6.88 (1H, d, 7.3)	6.86 (1H, d, 8.9)
6'	7.64 (1H, dd, 8.3, 2.1)	7.54 (1H, dd, 8.3, 2.0)	7.54 (1H, d, 7.3)	7.53 (1H, d, 7.3)	7.57 (1H, dd, 8.3, 2.0)
Sugar moiety					
	Galactosyl	Glucosyl	Galactosyl	Glucosyl	Galactosyl
1"	5.58 (1H, d, 7.6)	5.54 (1H, d, 7.6)	5.24 (1H, d, 7.3)	5.34 (1H, d, 7.6)	5.75 (1H, d, 7.4)
2"	3.78 (1H, t, 17.2)	3.51 (1H, t, 15.8)	4.05 (1H, t, 16.5)	3.77 (1H, t, 15.8)	3.96 (1H, t, 16.5)
3"	3.64 (1H, m)	3.44 (1H, m)	3.70 (1H, m)	3.58 (1H, m)	3.72 (1H, m)
4"	3.63 (1H, m)	3.11 (1H, m)	3.84 (1H, m)	3.38 (1H, m)	3.85 (1H, m)
5"	3.54 (1H, m)	3.22 (1H, m)	3.43 (1H, m)	3.31 (1H, m)	3.50 (1H, m)
6"	3.54 (1H, m)	3.63 (1H, m)	3.60 (1H, m)	3.79 (1H, m)	3.64 (1H, m)
	3.15 (1H, m)	3.21 (1H, m)	3.53 (1H, m)	3.71 (1H, m)	3.61 (1H, m)
	Glucosyl	Glucosyl	Glucosyl	Glucosyl	Rhamnosyl
1'''	4.56 (1H, d, 7.6)	4.57 (1H, d, 7.6)	4.75 (1H, d, 5.5)	4.75 (1H, d, 7.6)	5.21 (1H, s)
2'''	3.08 (1H, m)	3.05 (1H, m)	3.40 (1H, m)	3.37 (1H, m)	3.99 (1H, m)
3'''	3.14 (1H, m)	3.11 (1H, m)	3.43 (1H, m)	3.40 (1H, m)	3.77 (1H, m)
4'''	3.17 (1H, m)	3.16 (1H, m)	3.41 (1H, m)	3.39 (1H, m)	3.32 (1H, m)
5'''	3.18 (1H, m)	3.16 (1H, m)	3.33 (1H, m)	3.18 (1H, m)	4.01 (1H, m)
6'''	3.48 (1H, m)	3.45 (1H, m)	3.80 (1H, m)	3.69 (1H, m)	0.92 (3H, d, 6.4)
	3.57 (1H, dd, 7.3, 4.6)	3.54 (1H, dd, 7.3, 4.6)	3.72 (1H, m)	3.52 (1H, dd, 7.3, 4.6)	
	Rhamnosyl	Rhamnosyl			
1''''	4.37 (1H, s)	4.31 (1H, s)			
2''''	3.36 (1H, m)	3.32 (1H, m)			
3''''	3.29 (1H, m)	3.22 (1H, m)			
4''''	3.07 (1H, m)	3.04 (1H, m)			
5''''	3.34 (1H, m)	3.17 (1H, m)			
6''''	1.04 (3H, d, 6.2)	0.94 (3H, d, 6.2)			

[a]Assignments were aided by ^{1}H-^{1}H COSY, HMQC, and HMBC experiments. [b]DMSO-d_6 (600 MHz). [c]D_2O/MeOD-d_4 (600 MHz).

Table S3. ^{1}H-NMR spectral data for compounds **6**–**9**[a] (δ in ppm, *J* in Hz)

Position	6[b]	7[b]	8[b]	9[b]
Aglycone				
6	6.18 (1H, br s)	6.10 (1H, br s)	6.17 (1H, br s)	6.16 (1H, br s)
8	6.39 (1H, br s)	6.29 (1H, br s)	6.37 (1H, br s)	6.36 (1H, br s)
2'	7.93 (1H, br s)	8.08 (1H, d, 2.1)	8.05 (1H, d, 8.3)	8.07 (1H, d, 8.9)
5'			6.89 (1H, d, 9.2)	6.88 (1H, d, 8.9)
6'	6.90 (1H, d, 8.3)	6.89 (1H, d, 8.3)	6.89 (1H, d, 9.2)	6.88 (1H, d, 8.9)
	7.61 (1H, dd, 8.3,	7.50 (1H, d, 8.2)	8.05 (1H, d, 8.3)	8.07 (1H, d, 8.9)
3'-OCH_3	3.96 (3H, s)	3.98 (3H, s)		
Sugar moiety				
	Galactosyl	Galactosyl	Galactosyl	Galactosyl
1"	5.54 (1H, d, 7.6)	5.86 (1H, d, 8.3)	5.6 (1H, d, 8.2)	5.70 (1H, d, 7.6)
2"	4.08 (1H, t, 16.5)	3.96 (1H, t, 16.5)	3.92 (1H, m)	3.93 (1H, t, 17.2)
3"	3.76 (1H, m)	3.72 (1H, m)	3.69 (1H, m)	3.70 (1H, m)
4"	3.86 (1H, m)	3.84 (1H, m)	3.76 (1H, m)	3.82 (1H, m)
5"	3.48 (1H, m)	3.52 (1H, m)	3.63 (1H, m)	3.49 (1H, m)
6"	3.62 (1H, m)	3.65 (1H, m)	3.71 (1H, m)	3.63 (1H, m)
	3.56 (1H, m)	3.65 (1H, m)	3.44 (1H, m)	3.58 (1H, m)
	Glucosyl	Rhamnosyl	Rhamnosyl	Rhamnosyl
1'''	4.76 (1H, d, 7.6)	5.15 (1H, s)	5.21 (1H, s)	5.20 (1H, s)
2'''	3.37 (1H, m)	3.98 (1H, m)	3.99 (1H, m)	3.98 (1H, m)
3'''	3.36 (1H, m)	3.75 (1H, m)	3.79 (1H, m)	3.77 (1H, m)
4'''	3.34 (1H, m)	3.29 (1H, m)	3.33 (1H, m)	3.32 (1H, m)
5'''	3.26 (1H, m)	4.01 (1H, m)	4.05 (1H, m)	4.02 (1H, m)
6'''	3.74 (1H, m)	0.85 (3H, d, 6.2)	0.97 (3H, d, 6.4)	0.92 (3H, d, 6.2)
	3.65 (1H, m)			
			Rhamnosyl	
1''''			4.51 (1H, s)	
2''''			3.6 (1H, m)	
3''''			3.5 (1H, m)	
4''''			3.26 (1H, m)	
5''''			3.52 (1H, m)	
6''''			1.17 (3H, d, 6.4)	

[a]Assignments were aided by ^{1}H-^{1}H COSY, HMQC, and HMBC experiments. [b]D_2O/MeOD-*d*$_4$ (600 MHz).

Table S4. ^{13}C-NMR spectral data for compounds **1**–**5**[a] (δ in ppm)

Position	1[b]	2[b]	3[c]	4[c]	5[c]
Aglycone					δ_H (*J* in Hz)
2	155.4	156.0	158.8	158.9	158.2
3	132.8	132.9	135.1	135.1	134.6
4'	177.1	177.4	179.9	179.8	179.4
5	161.2	161.2	163.1	163.1	163.2
6	99.2	98.6	99.8	99.8	99.6
7	165.9	164.0	165.9	165.9	165.6
8	93.7	93.7	94.6	94.6	94.4
9	156.4	156.3	158.5	158.5	158.3
10	103.1	103.9	105.7	105.8	105.9
1'	120.9	121.1	122.8	123	123.3
2'	115.8	116.2	117.8	117.7	117.3
3'	145	144.7	145.9	145.9	145.9
4'	149.6	148.4	149.9	149.8	149.6
5'	115.4	115.4	116.2	116.1	116.1
6'	122	121.8	123	123	123
Sugar moiety					
	Galactosyl	Glucosyl	Galactosyl	Glucosyl	Galactosyl
1"	98.6	98.3	101.8	101.2	100.8
2"	80.5	82.6	80.6	82.9	77.5
3"	73.1	76.4	94.6	77.9	75.7
4"	67.6	69.5	70.0	70.9	70.9
5"	73.4	75.8	77.9	78.1	77.1
6"	64.6	66.2	61.9	62.3	62.1
	98.6	98.3	101.8	101.2	100.8
	Glucosyl	Glucosyl	Glucosyl	Glucosyl	Rhamnosyl
1'''	104.2	104.1	105.0	105	102.6
2'''	74.3	74.3	75.4	75.5	72.4
3'''	76.8	76.8	76.9	77.9	72.3
4'''	69.6	69.5	71.0	71.1	74
5'''	76.6	76.5	78.1	78.3	69.8
6'''	60.7	60.7	62.2	62.4	17.3
	Rhamnosyl	Rhamnosyl			
1''''	99.8	100.1			
2''''	70.4	70.3			
3''''	70.6	70.5			
4''''	71.9	71.8			
5''''	68.2	68.2			
6''''	17.9	17.7			

[a]Assignments were aided by ^{1}H-^{1}H COSY, HMQC, and HMBC experiments [b]DMSO-d_6 (150 MHz).

[c]D_2O/MeOD-d_4 (150 MHz).

Table S5. ^{13}C-NMR spectral data for compounds **6**–**9**[a] (δ in ppm)

Position	6[b]	7[b]	8[b]	9[b]
Aglycone				
2	158.8	157.6	158.7	158.4
3	134.8	134.2	134.4	134.5
4'	179.6	179.1	179.4	179.5
5	163.1	162.9	163.1	163.2
6	99.8	101.4	99.7	99.7
7	165.9	168.8	165.6	165.6
8	94.7	95.2	94.6	94.5
9	158.4	158.6	158.4	158.4
10	105.8	105.1	106.0	105.9
1'	123.1	123.5	123.0	123.0
2'	114.4	114.6	132.2	132.2
3'	148.5	148.5	116.2	116.1
4'	150.9	150.5	161.3	161.3
5'	116.1	115.9	116.2	116.1
6'	124	123.1	132.2	132.2
3'-OCH_3	57.1	57.1		
Sugar moiety				
	Galactosyl	Galactosyl	Galactosyl	Galactosyl
1"	101.3	100.6	100.9	100.6
2"	79.7	77.9	77.5	77.7
3"	74.7	75.7	75.7	75.7
4"	70	70.7	70.7	70.8
5"	77.1	77.1	75.3	77.0
6"	62.1	62.3	67.1	62.1
	Glucosyl	Rhamnosyl	Rhamnosyl	Rhamnosyl
1'''	104.2	102.8	102.6	102.6
2'''	75.2	72.4	72.4	72.4
3'''	77.8	72.3	72.2	72.3
4'''	71.2	74	74.1	74.0
5'''	78.2	69.8	69.8	69.8
6'''	62.5	17.5	17.5	17.5
			Rhamnosyl	
1''''			101.8	
2''''			72.1	
3''''			72.3	
4''''			73.9	
5''''			69.7	
6''''			17.9	

[a]Assignments were aided by ^{1}H-^{1}H COSY, HMQC, and HMBC experiments. [b]D_2O/MeOD-d_4 (150 MHz).

Table S6. The composition of experimental diets

	Normal diet	High-fat diet
Ingredient (g)		
Casein	200	200
L-Cystine	3	12
Corn starch	506.2	0
Maltodextrin	125	125
Sucrose	68.8	68.8
Cellulose	50	50
Soybean oil	25	25
Lard	20	20
Mineral mix	10	10
DiCarcium phosphate	13	13
Carcium carbonate	5.5	5.5
Potassium citrate, $1H_2O$	16.5	16.5
Vitamin mix	10	10
Choline bitartrate	2	2
Total (g)	1055.05	773.85
Energy (%)		
Protein	20	20
Carbohydrate	70	20
Fat	10	60
Gross energy (kcal/g)	3.85	5.24

Table 7S. Sequences of primer used for real-time qRT-PCR

Gene name	Forward primer	Reverse primer
ACC1	AGTTTCCCAGCCAGCAGATT	ATCCATCACCACAGCCTTCA
ACC2	CCCATCACCACTCCTTCTGA	GTCCGAGTCTCCACAGCAAT
Adiponectin	CATGCCGAAGATGACGTTAC	CGATACACATAAGCGGCTTC
AdipoR1	CTCATCTACCTCTCCATCGT	GAACACTCCTGCTCTTGTCT
AdipoR2	GATTGTCATCTGTGTGCTGG	TAGGGATGATTCCACTCAGG
CPT-1α	CTGCACTCCTGGAAGAAGAA	GTTCTTCGTCTGGCTTGACA
FAS	TGTGAGTGGTTCAGAGGCAT	TTCTGTAGTGCCAGCAAGCT
Foxa2	CCTTCA ACCACCCCTTCTCTATC	GTGGCTGTGGTGATGTTGCT
FoxO1	TGGGCCCTAATTCGGTCAT	TTGGGTCAGGCGGTTCATAC
GAPDH	ACATCATCCCTGCATCCACT	AGATCCACGACGGACACATT
GLUT-2	TTTGTCATCGCCCTCTGCTT	GCAGCGATTTCCTCAAAAGACT
GLUT-4	GCCCCACAGAAGGTGATTGA	AGCGTAGTGAGGGTGCCTTGT
IR	CTGAACAAAGATGACAACGAGGAA	CTTACAGATGGTTGGGCAAACTT
IRS-1	GAGAAGAGACTGGCTCGGAAGA	GCCTATTCTGCCCAACTCAACT
IRS-2	GGCCCGAACCTCAATAACAA	CCGCGCAACACGAAAAAG
PGC-1	GTGCAGCCAAGACTCTGTAT	GGTCGCTACACCACTTCAAT
PPARα	CCTGAACATCGAGTGTCGAA	GTACTGGCATTTGTTCCGGT
PPARγ	TGGGAGATTCTCCTGTTGAC	AGGTGGAGATGCAGGTTCTA
PPARδ	GCAGCCTCAACATGGAATGT	GTTGCGGTTCTTCTTCTGGA