Supplementary Information

Ameliorative effect of bofutsushosan (fangfengtongshengsan) extract on the progression of aging-induced obesity

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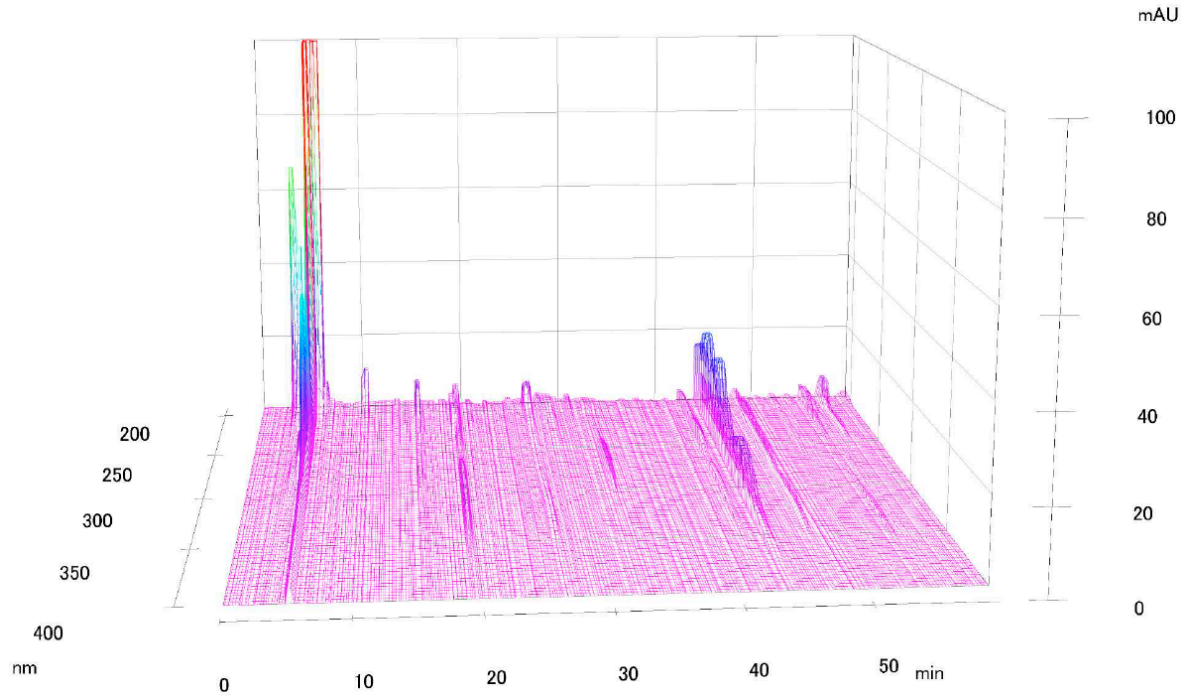

Figure S1. HPLC fingerprint of the methanol extract of Japanese Angelica Root

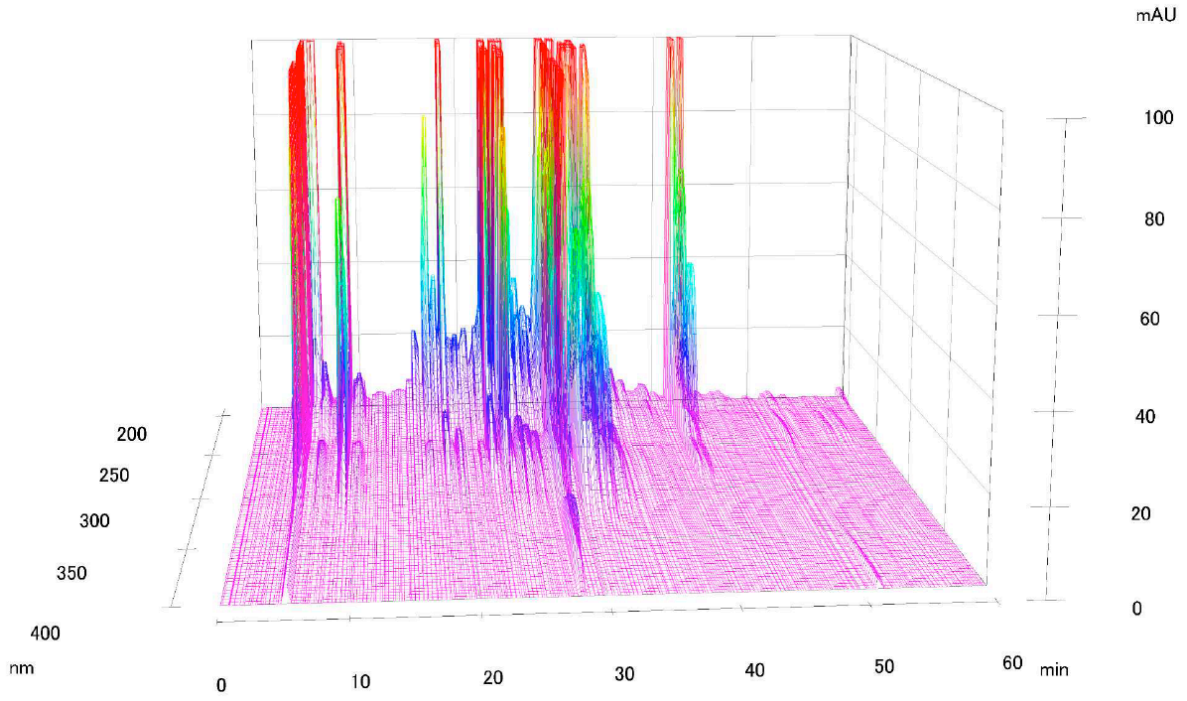

Figure S2. HPLC fingerprint of the methanol extract of Peony Root

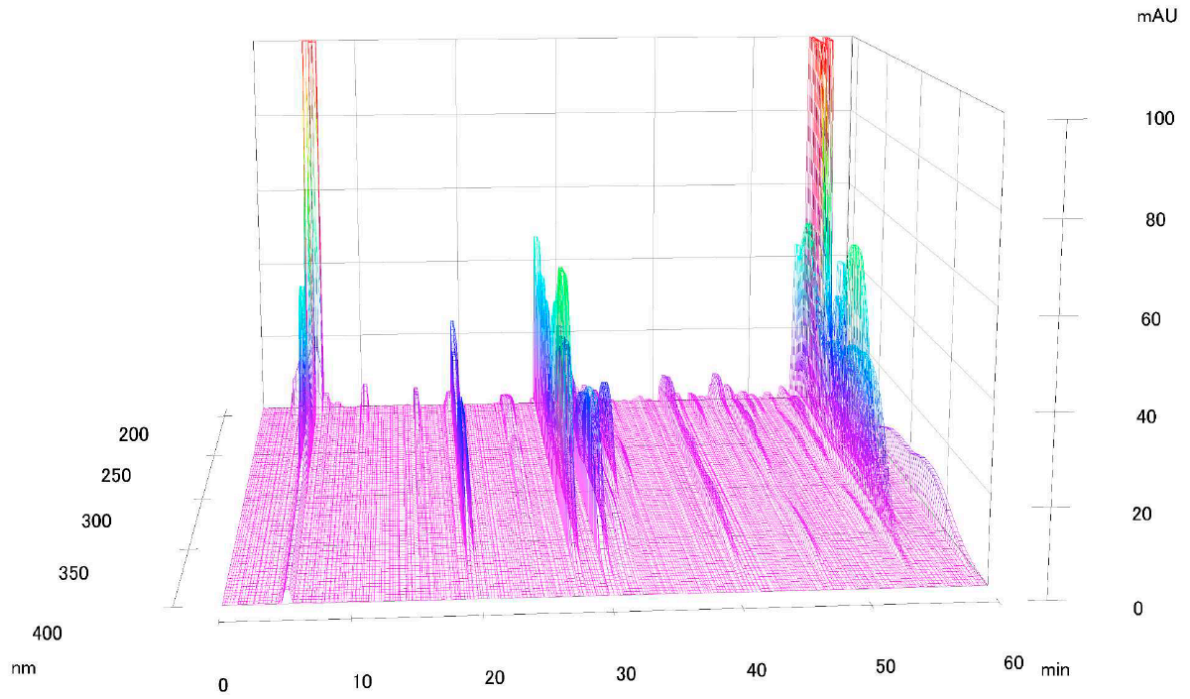

Figure S3. HPLC fingerprint of the methanol extract of Cnidium Rhizome

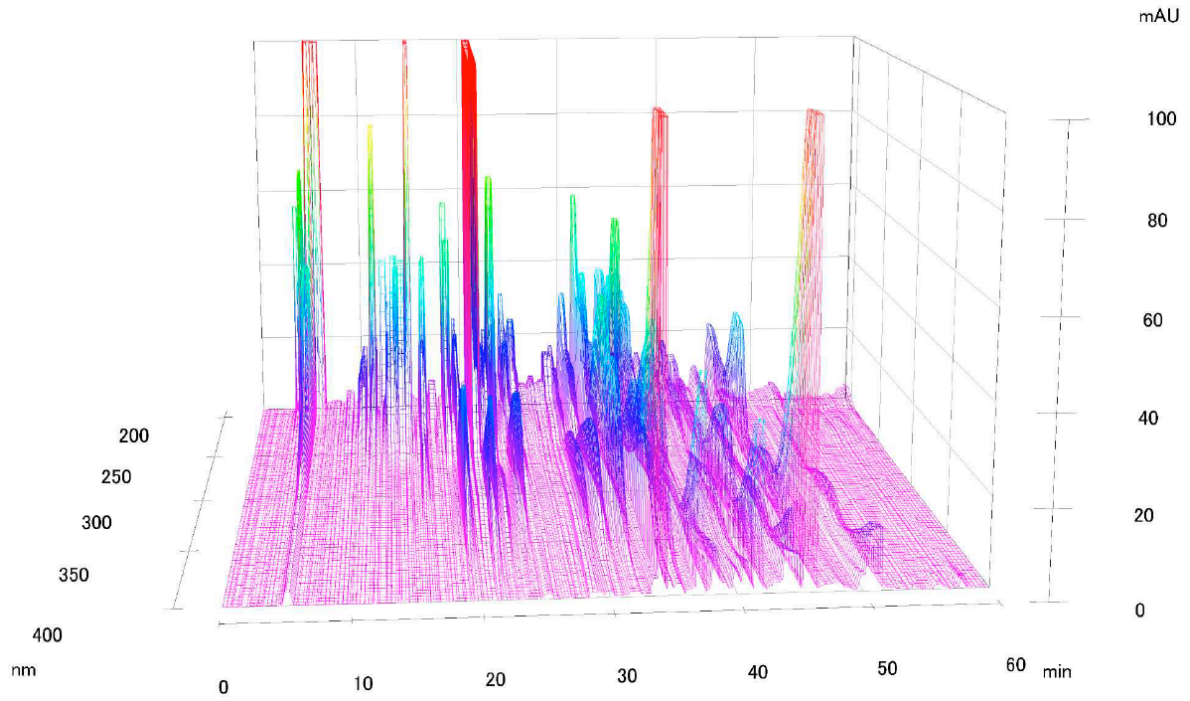

Figure S4. HPLC fingerprint of the methanol extract of Gardenia Fruit

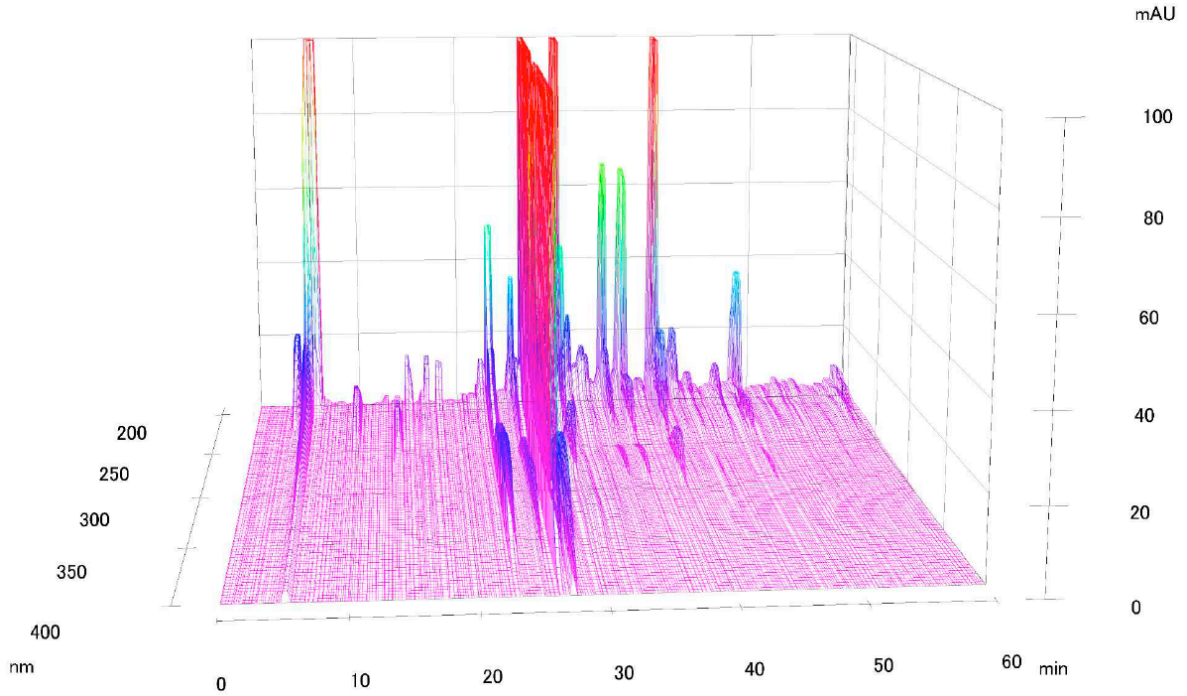

Figure S5. HPLC fingerprint of the methanol extract of Forsythia Fruit

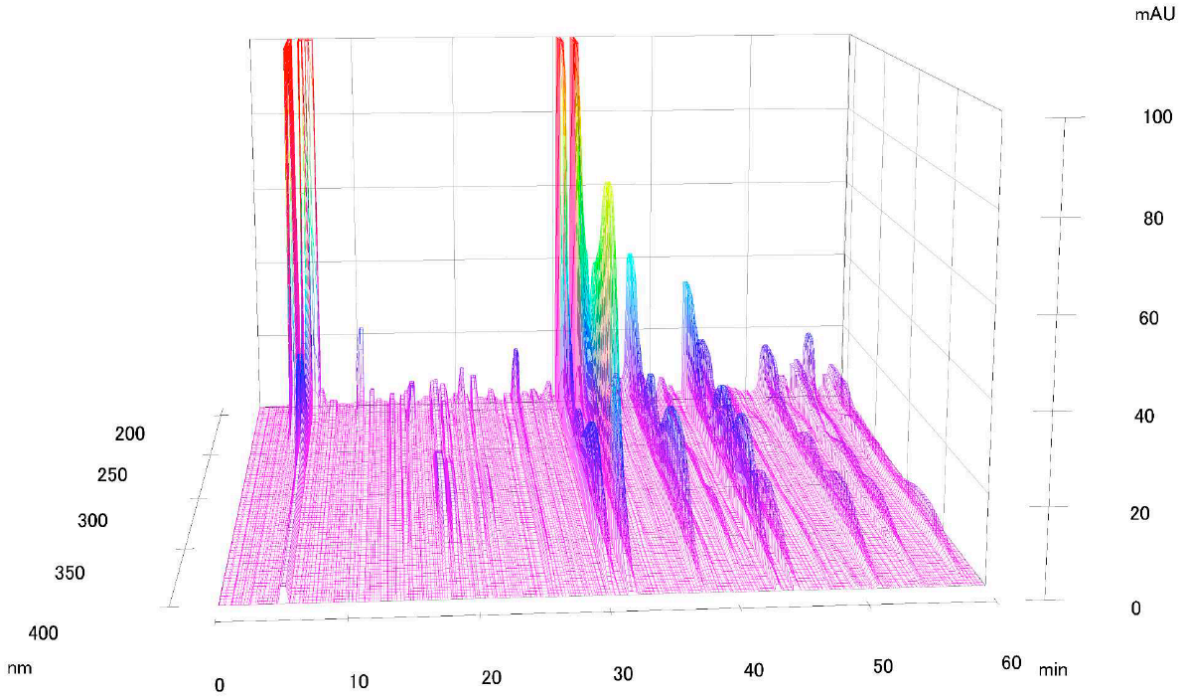

Figure S6. HPLC fingerprint of the methanol extract of Mentha Herb

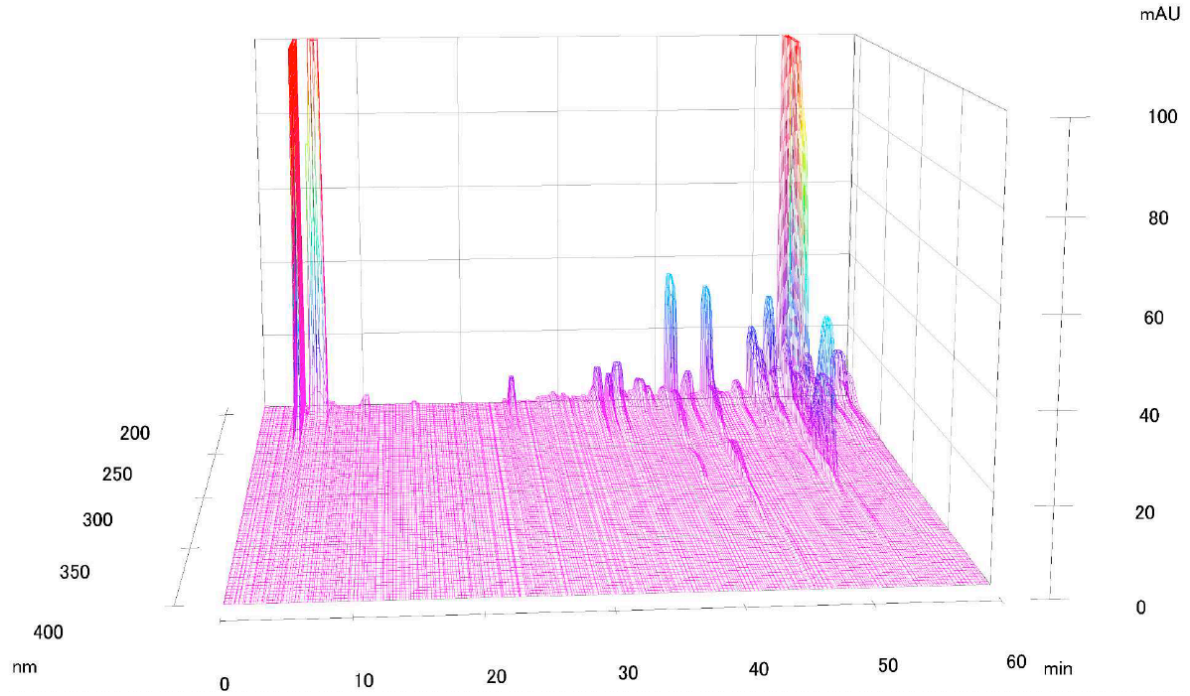

Figure S7. HPLC fingerprint of the methanol extract of Ginger

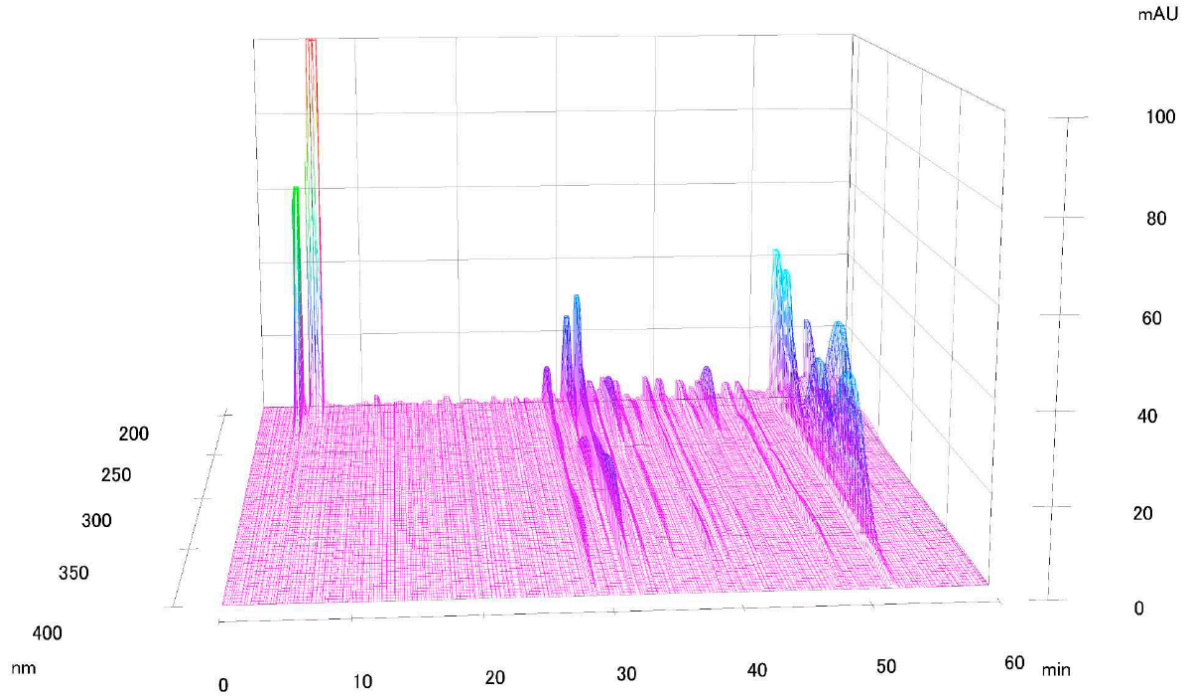

Figure S8. HPLC fingerprint of the methanol extract of Schizonepeta Spike

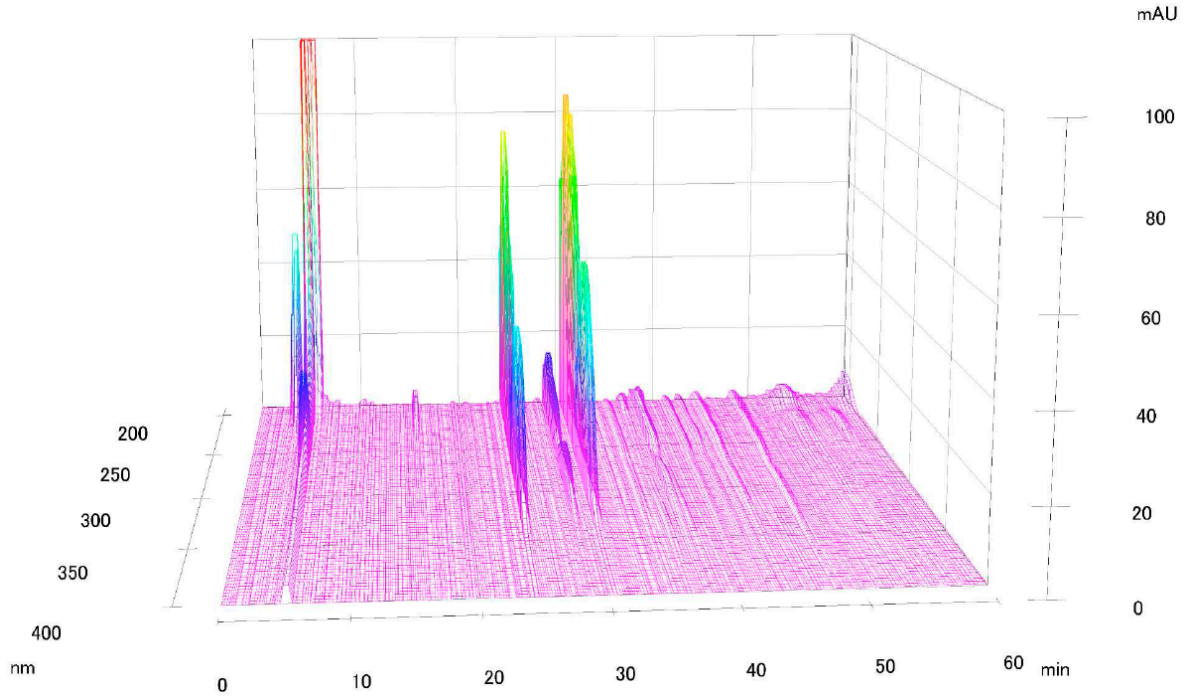

Figure S9. HPLC fingerprint of the methanol extract of Saposhnikovia Root and Rhizome

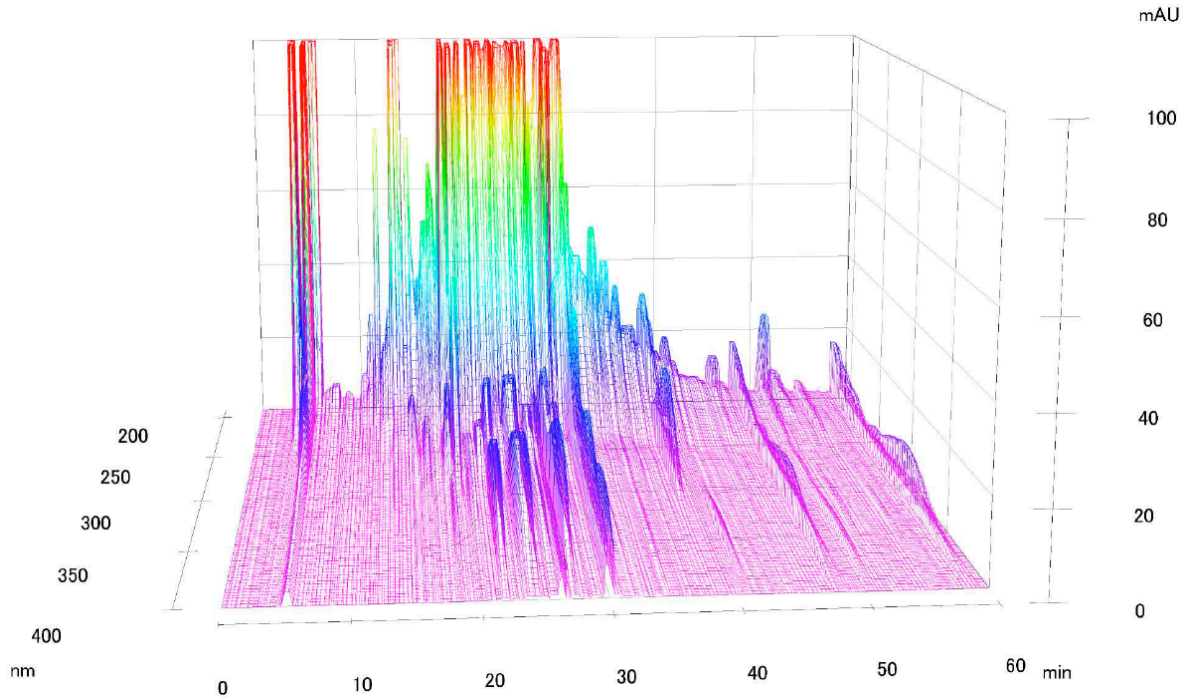

Figure S10. HPLC fingerprint of the methanol extract of Ephedra Herb

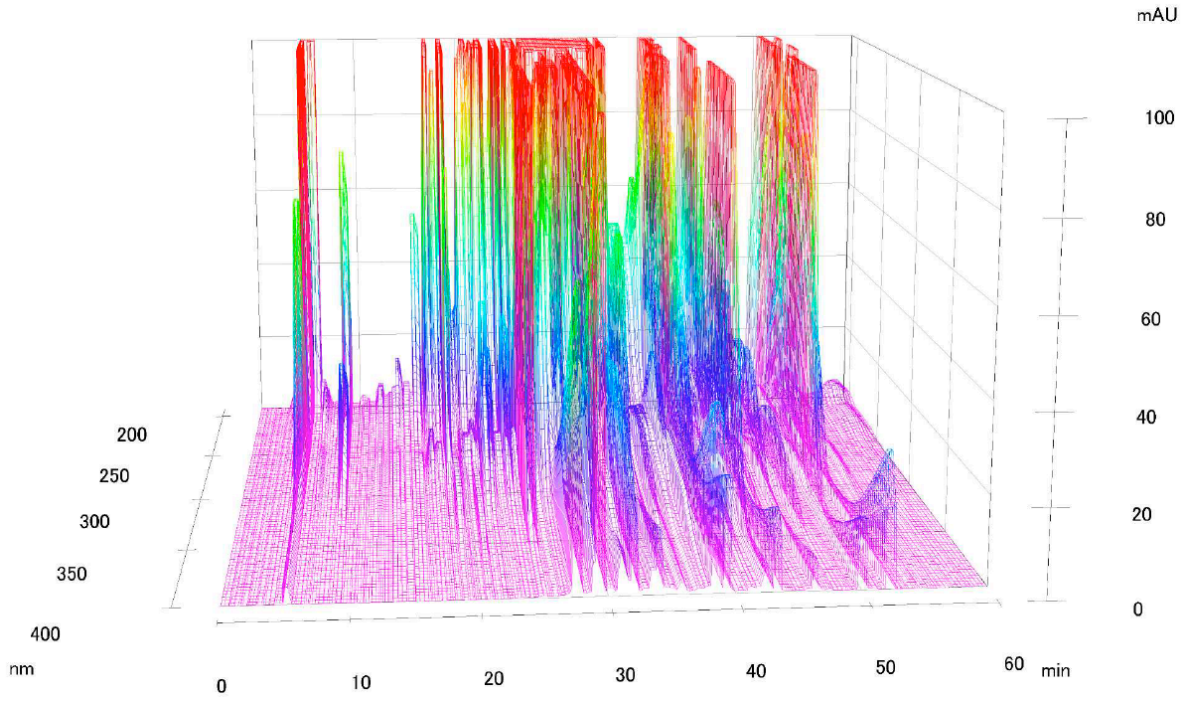

Figure S11. HPLC fingerprint of the methanol extract of Rhubarb

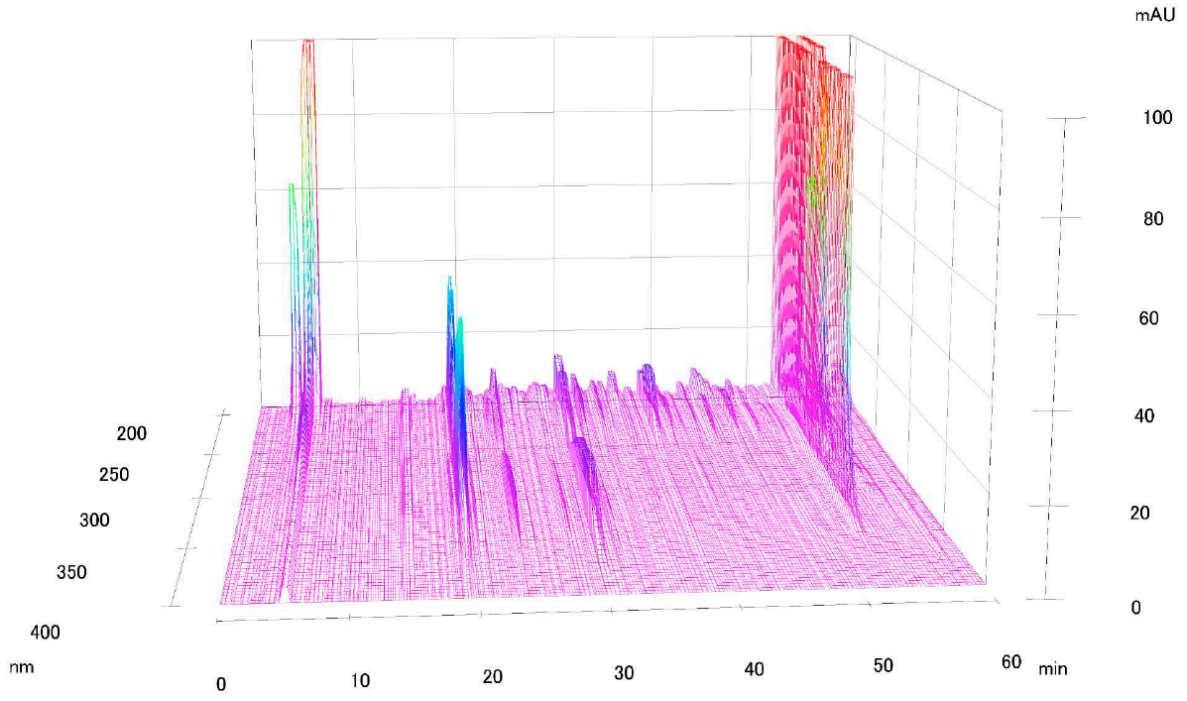

Figure S12. HPLC fingerprint of the methanol extract of Atractylodes Rhizome

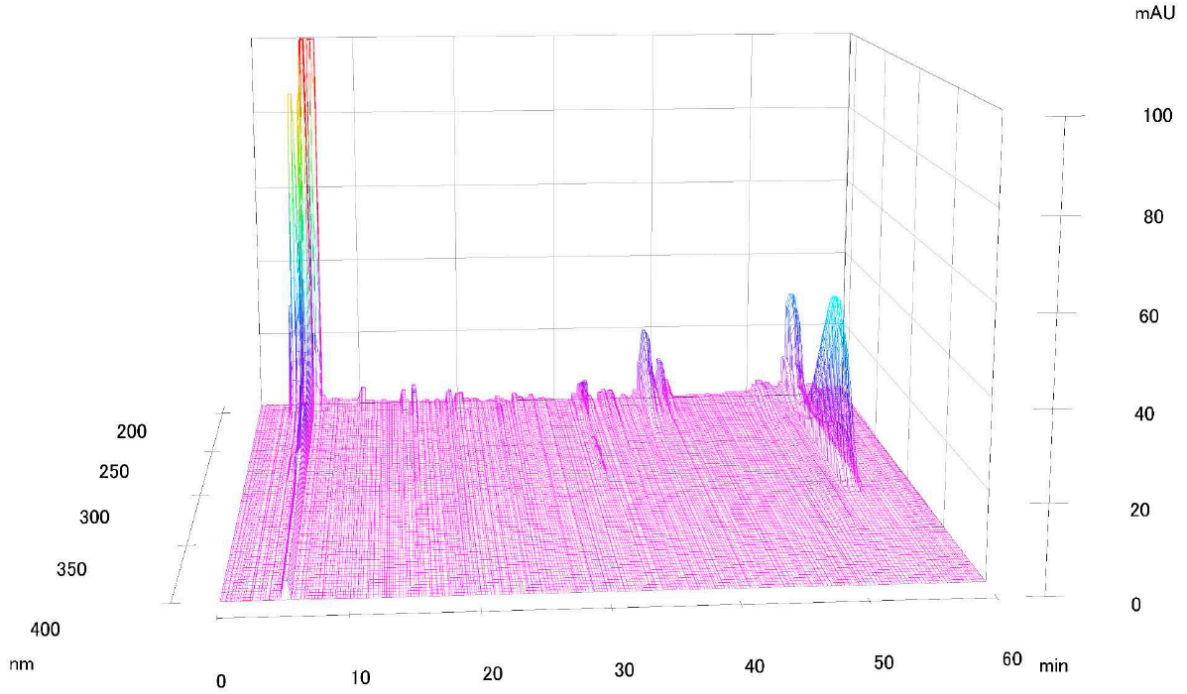

Figure S13. HPLC fingerprint of the methanol extract of Platycodon Root

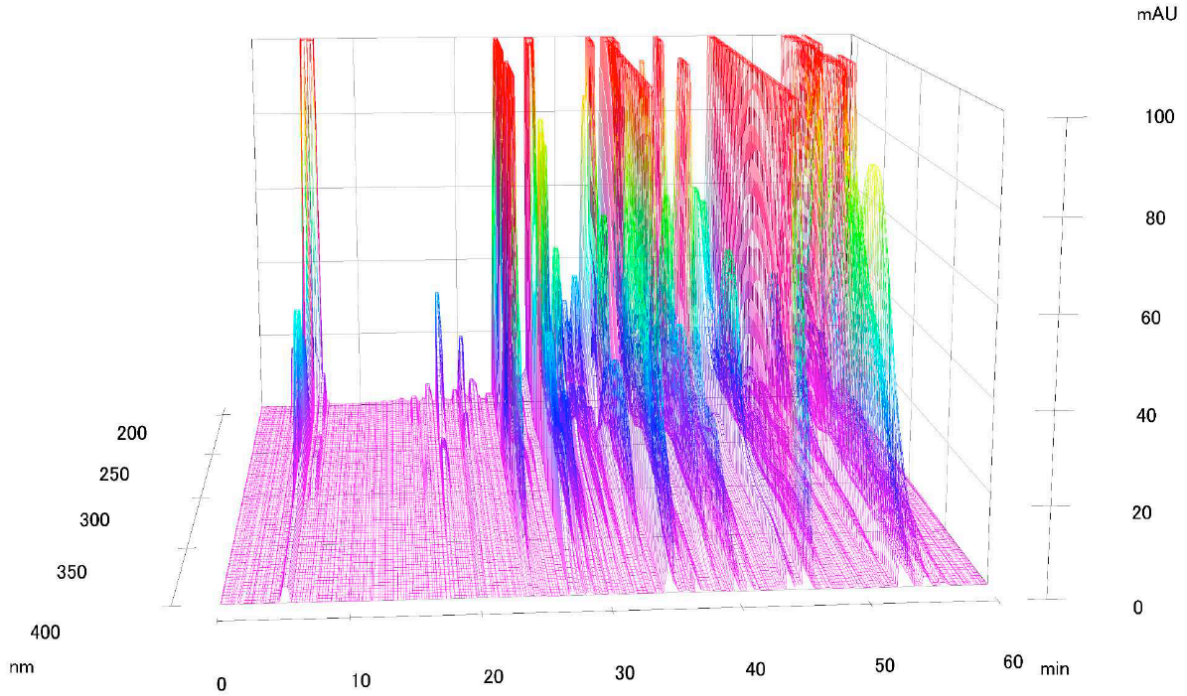

Figure S14. HPLC fingerprint of the methanol extract of Scutellaria Root

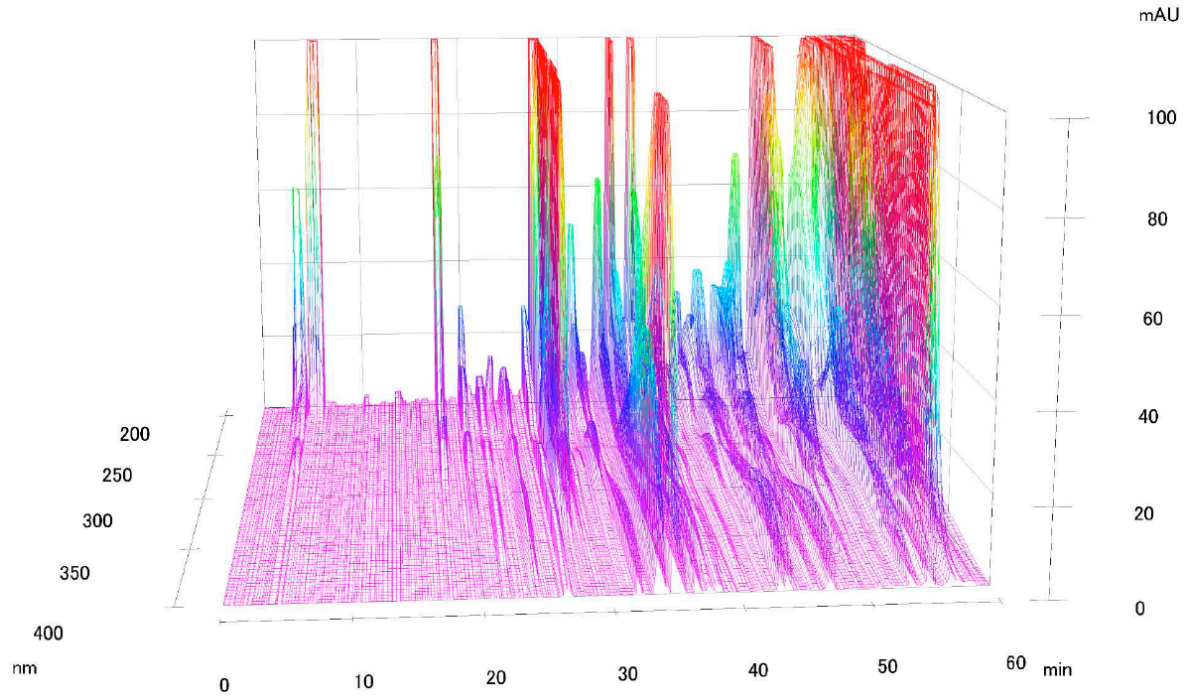

Figure S15. HPLC fingerprint of the methanol extract of Glycyrrhiza

Each sample solution (1 mg/mL, 10 μL) was injected to HPLC with the following conditions [column: TSK-Gel ODS-100V (4.6 × 250 mm, i.d., 5 μm); mobile phase: 10 mM aqueous phosphoric acid–CH_3CN 95:5 (0 min)–40:60 (60 min), linear gradient; flow rate: 0.8 mL/min; column temperature: 40 °C; detection: 200–400 nm by a photodiode array detector].

Table S1. Effects of 4 weeks of bofutsushosan (BTS) extract administration on the physical and biochemical parameters of mice fed a high-fat diet

Parameter	Unit	Day	Young mice (9 weeks old)			Aged mice (43–63 weeks old)		
			Normal (CE2 diet)	Control (HFD diet)	BTS (HFD diet+2% BTS)	Normal (CE2 diet)	Control (HFD diet)	BTS (HFD diet+2% BTS)
Body weight	g	0	23.8 ± 0.5	23.3 ± 0.4	23.7 ± 0.5	32.9 ± 1.1	32.1 ± 0.7	31.7 ± 0.7
		3	24.1 ± 0.4	24.8 ± 0.5	23.6 ± 0.4	33.4 ± 1.3	34.5 ± 0.7	32.1 ± 0.7*
		6	23.9 ± 0.4	25.0 ± 0.3	23.8 ± 0.4	32.5 ± 1.3	35.7 ± 0.7$^{\#}$	32.8 ± 0.7*
		9	24.4 ± 0.5	25.2 ± 0.3	24.5 ± 0.5	32.6 ± 1.3	36.4 ± 0.8$^{\#}$	32.9 ± 0.8*
		13	24.5 ± 0.4	25.8 ± 0.3$^{\#}$	24.9 ± 0.5	31.9 ± 1.4	37.4 ± 0.8$^{\#\#}$	33.7 ± 1.0*
		17	24.8 ± 0.4	26.1 ± 0.2$^{\#}$	24.9 ± 0.6	32.2 ± 1.5	38.6 ± 1.0$^{\#\#}$	34.3 ± 0.9**
		20	24.7 ± 0.3	27.0 ± 0.3$^{\#\#}$	25.4 ± 0.6*	32.2 ± 1.2	39.3 ± 0.9$^{\#\#}$	34.7 ± 0.9**
		24	25.2 ± 0.4	27.5 ± 0.4$^{\#\#}$	25.7 ± 0.5*	33.0 ± 1.4	40.2 ± 1.0$^{\#\#}$	35.0 ± 1.0**
		28	25.3 ± 0.4	28.2 ± 0.4$^{\#\#}$	25.8 ± 0.4**	32.8 ± 1.5	40.8 ± 1.1$^{\#\#}$	35.2 ± 1.0**
Body weight gain	g		1.6 ± 0.2	4.9 ± 0.3$^{\#\#}$	2.1 ± 0.1**	-0.1 ± 0.6	8.7 ± 0.5$^{\#\#}$	3.5 ± 0.7**
Food intake	g/day		4.8 ± 0.0	2.7 ± 0.0	2.6 ± 0.0*	4.2 ± 0.1	3.3 ± 0.1$^{\#\#}$	3.0 ± 0.1*
	g/kg bw/day		197.0 ± 3.8	103.7 ± 1.1$^{\#\#}$	103.4 ± 1.8	129.4 ± 4.0	89.3 ± 1.7$^{\#\#}$	88.1 ± 0.9
	kcal/kg bw/day		668.1 ± 12.7	526.5 ± 5.3$^{\#\#}$	514.8 ± 9.1	438.7 ± 13.6	453.3 ± 8.7	438.5 ± 4.3
Visceral fat	g	24	0.39 ± 0.05	1.22 ± 0.04$^{\#\#}$	0.70 ± 0.15**	1.20 ± 0.28	4.02 ± 0.25$^{\#\#}$	2.71 ± 0.21**
Subcutaneous fat	g	24	0.22 ± 0.02	0.73 ± 0.05$^{\#\#}$	0.47 ± 0.09*	0.75 ± 0.15	2.42 ± 0.13$^{\#\#}$	1.63 ± 0.14**
Total fat	g	24	0.61 ± 0.07	1.95 ± 0.08$^{\#\#}$	1.17 ± 0.23**	1.96 ± 0.42	6.44 ± 0.37$^{\#\#}$	4.34 ± 0.35**
Liver weight	g	28	1.38 ± 0.03	1.27 ± 0.03$^{\#}$	1.23 ± 0.03	1.62 ± 0.09	1.98 ± 0.10$^{\#}$	1.64 ± 0.08*
Epididymal WAT	g	28	0.43 ± 0.01	0.94 ± 0.03$^{\#\#}$	0.60 ± 0.07**	0.95 ± 0.17	2.53 ± 0.20$^{\#\#}$	1.75 ± 0.11**
Interscapular BAT	mg	28	148.6 ± 14.9	150.2 ± 17.2	124.6 ± 13.2	185.0 ± 15.9	238.3 ± 22.0	188.0 ± 14.0
GLU	mg/dL	28	170.4 ± 3.3	224.0 ± 7.9$^{\#\#}$	210.8 ± 13.6	176.4 ± 12.8	222.1 ± 16.3	206.7 ± 9.7
TG	mg/dL	28	64.0 ± 17.1	140.7 ± 34.6	74.8 ± 7.2	109.0 ± 23.4	64.4 ± 9.4	72.8 ± 9.2
NEFA	μEq/L	28	607.4 ± 71.6	964.7 ± 146.6	863.6 ± 62.8	711.8 ± 47.7	724.3 ± 48.9	744.5 ± 73.0
T-CHO	mg/dL	28	70.4 ± 3.9	187.2 ± 5.5$^{\#\#}$	180.6 ± 8.4	70.8 ± 2.4	197.7 ± 3.0$^{\#\#}$	161.8 ± 9.0**
AST	IU/L	28	32.4 ± 1.2	33.3 ± 1.5	33.0 ± 1.8	38.4 ± 2.7	70.1 ± 8.6$^{\#}$	52.0 ± 6.5
ALT	IU/L	28	21.0 ± 1.3	13.8 ± 1.3$^{\#\#}$	12.4 ± 0.8	27.0 ± 3.6	33.9 ± 6.4	21.3 ± 2.7
ALP	IU/L	28	292.6 ± 4.3	227.5 ± 8.3$^{\#\#}$	242.4 ± 10.7	197.8 ± 8.2	136.1 ± 5.4$^{\#\#}$	132.2 ± 6.6

Mean ± S.E. (n = 5–8).

$^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* Normal, $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* Control (*t*-test).

Table S2. Effects of two months of bofutsushosan (BTS) extract administration on the physical and biochemical parameters of mice fed a high-fat diet

Parameter	Unit	Day	Young mice (9 weeks old)		Aged mice (64–65 weeks old)	
			Control (HFD diet)	BTS (HFD diet+2% BTS)	Control (HFD diet)	BTS (HFD diet+2% BTS)
Body weight	g	0	22.3 ± 0.2	22.3 ± 0.2	34.9 ± 0.9	34.2 ± 0.8
		3	24.2 ± 0.4	22.2 ± 0.1**	38.4 ± 1.0	34.8 ± 0.7*
		7	24.5 ± 0.5	23.1 ± 0.3*	39.7 ± 1.2	35.6 ± 0.8*
		13	25.5 ± 0.4	24.0 ± 0.2*	42.1 ± 1.1	36.6 ± 0.9**
		20	26.7 ± 0.6	25.1 ± 0.4*	43.8 ± 1.3	37.4 ± 1.1**
		28	28.0 ± 0.7	25.9 ± 0.4*	45.7 ± 1.4	38.1 ± 1.1**
		34	29.4 ± 0.8	26.5 ± 0.3**	46.5 ± 1.4	39.0 ± 1.2**
		42	30.5 ± 0.7	27.5 ± 0.5**	47.0 ± 1.6	39.8 ± 1.3**
		49	31.7 ± 0.8	28.6 ± 0.5**	47.7 ± 1.6	40.5 ± 1.4**
		57	32.6 ± 1.0	29.5 ± 0.6*	48.2 ± 1.6	40.6 ± 1.3**
		62	33.4 ± 0.9	30.2 ± 0.6*	49.0 ± 1.5	41.3 ± 1.4**
Body weight gain	g		11.1 ± 0.9	7.9 ± 0.7*	14.1 ± 0.9	7.1 ± 1.0**
Food intake	g/day		2.7 ± 0.1	2.7 ± 0.1	3.5 ± 0.1	3.2 ± 0.1
	g/kg bw/day		96.8 ± 2.0	102.5 ± 2.4	79.5 ± 1.2	84.4 ± 0.8*
	kcal/kg bw/day		491.5 ± 10.0	510.0 ± 12.0	403.5 ± 6.3	419.9 ± 4.2
Liver weight	g	62	1.50 ± 0.07	1.43 ± 0.03	2.99 ± 0.28	2.21 ± 0.13*
Liver TG	mg/g protein	62	163.4 ± 11.1	115.1 ± 10.9**	264.2 ± 24.6	187.1 ± 40.9
AST	IU/L	62	38.7 ± 1.7	43.0 ± 4.5	152.4 ± 22.0	70.0 ± 4.9**
ALT	IU/L	62	15.1 ± 1.2	16.0 ± 0.7	97.8 ± 23.1	35.3 ± 4.3*
ALP	IU/L	62	207.4 ± 3.8	209.6 ± 6.0	217.3 ± 19.8	150.7 ± 6.7*

Mean ± S.E. (n = 5–8).
$^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* Control (*t*-test).

Table S3. Effects of crude drug extracts of each component of bofutsushosan (BTS) extract on oleic acid-albumin-induced triglyceride accumulation in HepG2 cells

Treatment	TG/protein (% of control)			
Conc. (μg/mL)	0	10	30	100
BTS ext.	100.0 ± 1.2	97.2 ± 1.6	95.3 ± 2.3	89.1 ± 1.6**
H_2O extract				
1. Japanese Angelica Root	100.0 ± 1.2	101.5 ± 2.2	101.3 ± 1.3	98.2 ± 1.5
2. Peony Root	100.0 ± 2.9	100.4 ± 1.0	98.3 ± 1.0	93.2 ± 1.1
3. Cnidium Rhizome	100.0 ± 2.3	100.5 ± 3.5	97.1 ± 2.3	98.8 ± 1.7
4. Gardenia Fruit	100.0 ± 1.5	104.0 ± 3.4	97.9 ± 3.1	100.1 ± 2.5
5. Forsythia Fruit	100.0 ± 2.1	105.0 ± 0.9	105.7 ± 4.2	88.9 ± 4.4**
6. Mentha Herb	100.0 ± 1.2	98.5 ± 2.5	91.2 ± 2.1*	88.6 ± 1.4**
7. Ginger	100.0 ± 1.8	95.6 ± 1.4	99.0 ± 2.9	103.3 ± 2.1
8. Schizonepeta Spike	100.0 ± 2.2	93.3 ± 1.3	91.4 ± 1.7*	86.9 ± 3.0**
9. Saposhnikovia Root and Rhizome	100.0 ± 2.4	96.3 ± 1.4	103.8 ± 4.4	107.2 ± 1.3
10. Ephedra Herb	100.0 ± 2.1	102.5 ± 2.6	102.2 ± 2.5	98.2 ± 1.1
11. Rhubarb	100.0 ± 3.0	97.3 ± 2.2	89.4 ± 0.7**	89.5 ± 1.6**
12. Sodium Sulfate	100.0 ± 2.2	99.3 ± 1.7	100.8 ± 1.9	112.8 ± 2.4
13. Atractylodes Rhizome	100.0 ± 2.6	108.2 ± 1.6	114.2 ± 4.1*	115.5 ± 3.6
14. Platycodon Root	100.0 ± 1.8	95.0 ± 2.2	96.5 ± 1.7	119.3 ± 2.5
15. Scutellaria Root	100.0 ± 2.5	97.8 ± 4.2	97.1 ± 4.0	85.3 ± 3.8**
16. Glycyrrhiza	100.0 ± 2.1	110.1 ± 3.4	101.7 ± 0.5	103.2 ± 1.6
MeOH extract				
1. Japanese Angelica Root	100.0 ± 0.9	100.7 ± 0.9	99.0 ± 0.6	90.2 ± 2.5**
2. Peony Root	100.0 ± 3.7	99.6 ± 2.4	100.8 ± 0.5	101.7 ± 3.7
3. Cnidium Rhizome	100.0 ± 2.2	99.5 ± 1.5	96.6 ± 1.0	96.9 ± 1.3
4. Gardenia Fruit	100.0 ± 2.0	97.3 ± 1.3	96.4 ± 1.3	94.8 ± 1.4
5. Forsythia Fruit	100.0 ± 0.5	81.7 ± 1.6*	59.0 ± 3.0**	131.4 ± 4.2**
6. Mentha Herb	100.0 ± 1.8	92.3 ± 1.3	94.7 ± 1.0	83.5 ± 3.0**
7. Ginger	100.0 ± 2.8	98.4 ± 1.9	113.1 ± 1.4	162.8 ± 3.7**
8. Schizonepeta Spike	100.0 ± 4.0	91.6 ± 0.5*	80.4 ± 1.2**	63.4 ± 1.0**
9. Saposhnikovia Root and Rhizome	100.0 ± 2.3	103.1 ± 1.4	100.6 ± 1.4	99.0 ± 3.6
10. Ephedra Herb	100.0 ± 1.1	99.5 ± 2.3	106.1 ± 2.1	107.0 ± 1.9
11. Rhubarb	100.0 ± 1.3	94.1 ± 1.1	88.2 ± 4.3**	116.6 ± 3.6*
13. Atractylodes Rhizome	100.0 ± 2.0	113.9 ± 2.7	135.1 ± 6.0**	186.3 ± 4.5**
14. Platycodon Root	100.0 ± 2.6	113.0 ± 1.9*	130.6 ± 3.8**	160.2 ± 3.2**
15. Scutellaria Root	100.0 ± 4.1	105.4 ± 2.3	96.6 ± 1.7	86.5 ± 1.6**
16. Glycyrrhiza	100.0 ± 6.1	114.3 ± 1.8**	132.6 ± 2.5**	122.6 ± 6.4**
Conc. (μM)	0	1	10	100
Bezafibrate[45–47]	100.0 ± 1.1	93.4 ± 1.4	86.7 ± 1.2**	72.7 ± 0.6**

Each value represents the mean ± S.E. (n = 4).
Significantly different from the control, $^{*}p < 0.05$, $^{**}p < 0.01$.

Table S4. Effects of crude drug extracts of each component of bofutsushosan (BTS) on triglyceride contents in high-glucose-pretreated HepG2 cells

Treatment	TG/protein (% of control)			
Conc. (μg/mL)	0	30	100	300
BTS ext.	100.0 ± 1.8	96.3 ± 1.4	96.3 ± 2.2	90.2 ± 2.6**
H_2O extract				
1. Japanese Angelica Root	100.0 ± 5.5	84.5 ± 2.0	92.4 ± 0.9	103.5 ± 3.2
2. Peony Root	100.0 ± 1.1	95.3 ± 1.5**	90.6 ± 1.7**	85.1 ± 1.9**
3. Cnidium Rhizome	100.0 ± 2.3	92.4 ± 0.9	92.2 ± 1.9	99.9 ± 2.0
4. Gardenia Fruit	100.0 ± 1.6	90.9 ± 2.3*	89.2 ± 2.1**	93.0 ± 2.6
5. Forsythia Fruit	100.0 ± 2.5	93.7 ± 2.6	82.7 ± 3.2**	63.8 ± 0.8**
6. Mentha Herb	100.0 ± 2.2	94.9 ± 2.2	90.0 ± 0.6*	88.1 ± 4.2**
7. Ginger	100.0 ± 1.8	90.1 ± 0.4**	89.0 ± 1.6**	93.0 ± 1.9*
8. Schizonepeta Spike	100.0 ± 0.8	99.0 ± 0.6	89.3 ± 1.6**	89.3 ± 1.6**
9. Saposhnikovia Root and Rhizome	100.0 ± 2.0	88.3 ± 2.3**	85.6 ± 1.1**	90.4 ± 1.6**
10. Ephedra Herb	100.0 ± 2.2	87.8 ± 3.1**	82.2 ± 1.7**	71.3 ± 1.8**
11. Rhubarb	100.0 ± 1.2	89.9 ± 3.0**	85.3 ± 1.0**	64.6 ± 1.4**
12. Sodium Sulfate	100.0 ± 3.3	86.1 ± 1.7	91.1 ± 1.0	106.5 ± 3.4
13. Atractylodes Rhizome	100.0 ± 2.2	97.6 ± 1.3	96.9 ± 2.7	108.7 ± 2.7
14. Platycodon Root	100.0 ± 1.9	92.6 ± 0.9*	92.8 ± 1.3*	97.7 ± 2.1
15. Scutellaria Root	100.0 ± 1.2	91.8 ± 1.4**	87.4 ± 0.6**	87.2 ± 2.8**
16. Glycyrrhiza	100.0 ± 1.1	92.1 ± 2.8	91.6 ± 3.3*	98.4 ± 3.3
Conc. (μg/mL)	0	10	30	100
MeOH extract				
1. Japanese Angelica Root	100.0 ± 5.0	86.7 ± 2.8	87.7 ± 4.0	88.5 ± 1.6
2. Peony Root	100.0 ± 1.6	94.1 ± 3.6	91.1 ± 0.6*	83.7 ± 2.6**
3. Cnidium Rhizome	100.0 ± 6.9	69.0 ± 5.1**	66.4 ± 5.1**	83.3 ± 4.3**
4. Gardenia Fruit	100.0 ± 2.3	91.0 ± 1.8*	87.8 ± 3.0**	82.5 ± 2.5**
5. Forsythia Fruit	100.0 ± 2.2	85.6 ± 3.1**	66.2 ± 2.0**	47.5 ± 1.6**
6. Mentha Herb	100.0 ± 2.1	87.0 ± 2.8**	88.1 ± 1.7**	81.5 ± 0.8**
7. Ginger	100.0 ± 1.6	95.2 ± 3.7	95.3 ± 2.8	86.5 ± 1.9**
8. Schizonepeta Spike	100.0 ± 2.9	95.8 ± 2.3	91.5 ± 2.8	77.6 ± 2.0**
9. Saposhnikovia Root and Rhizome	100.0 ± 2.3	93.0 ± 3.6	90.8 ± 1.9	85.9 ± 1.2**
10. Ephedra Herb	100.0 ± 3.3	108.8 ± 3.5	102.7 ± 6.9	92.2 ± 3.2
11. Rhubarb	100.0 ± 1.2	93.1 ± 1.9	87.2 ± 4.3**	79.1 ± 2.9**
13. Atractylodes Rhizome	100.0 ± 1.7	101.6 ± 1.9	107.4 ± 0.7	119.5 ± 3.6
14. Platycodon Root	100.0 ± 1.7	95.5 ± 2.0	97.6 ± 2.6	96.3 ± 1.6
15. Scutellaria Root	100.0 ± 2.6	92.9 ± 0.5	93.8 ± 3.7	83.0 ± 2.8**
16. Glycyrrhiza	100.0 ± 2.2	92.9 ± 0.6	88.3 ± 3.2	52.5 ± 1.0**
Conc. (μM)	0	125	250	500
Metformin[48–50]	100.0 ± 1.2	92.1 ± 1.4*	88.6 ± 1.5**	87.8 ± 2.2**

Each value represents the mean ± S.E. (n = 4).
Significantly different from the control, $^{*}p < 0.05$, $^{**}p < 0.01$.