Anti-obesity effects of *Ecklonia cava* extract in high-fat diet-induced obese rats

(Supplementary Data)

Table S1. Base sequences of adipogenic and lipogenic primers with their PCR conditions.

Primers	Base Sequences	Polymerase Chain Reaction Conditions
PPAR-γ	F: CCA GAG TCT GCT GAT CTG CG R: GCC ACC TCT TTG CTC TGA TC	Denaturation at 95 C for 45 s, annealing at 58 °C for 45 s, and synthesizing at 72 °C for 45 s with 35 cycles
FAS	F: GGCTCAGCATGGTCGCTT R: CTCCCGCCAGCTGTCATT	Denaturation at 94 °C for 30 s, annealing at 60 °C for 45 s, and synthesizing at 72 °C for 30 s with 35 cycles
LPL	F: GATTTCTCTGTACGGCACAGTGG R: TTTGTGGAAACCTCGGGC	Denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s, and synthesizing at 72 °C for 30 s with 35 cycles
SREBP-1C	F: GTAGCGTCTGCACGCCCTA R:CTTGGTTGTTGATGAGCTGGAG	Denaturation at 94 °C for 45 s, annealing at 58 °C for 45 s, and synthesizing at 72 °C for 45 s with 35 cycles
β -actin	F: ATGCTCCTGCTTGAGT A GT, R: GAGGAAGAGGATGCGGCAGT	Denaturation at 95 °C for 45 s, annealing at 55 °C for 45 s, and synthesizing at 72 °C for 45 s with 30 cycles

*F, forward; R, reverse.

Table S2. The important bioactive compound in *E. cava* was identified by GCMS analysis.

	Compounds	RT	Area (%)	Functions	Chemical Formula	Ref.
1	Benzoyl bromide	27.080	10.84	Antibacterial, Antifungal, antioxidant, and anti-obesity activity.	C_7H_5BrO	[1] [2]
2	2-Propanol	27.764	3.23	Antibacterial properties and anti-prions activity with combinations.	C_3H_8O	PubChem
3	Benzene, 1,3-bis(1,1-dimethylethyl)-	36.529	2.95	It could be used as the base data for the effect of γ-irradiation on the medicinal herb.	$C_{14}H_{22}$	[3]
4	Docosane	37.478	2.34	The active ingredient in plant extract has antioxidant and anti-obesity and antibacterial activities.	$C_{22}H_{46}$	[4,5]
5	Dodecanal	53.494	3.14	Antibacterial activity and plant extracts which are recently reported for their significant anti-obesity, dodecanal was abundantly present.	$C_{12}H_{24}O$	[6-8]
6	Methyl salicylate	57.019	62.5	Analgesic, counter-irritant, and anti-inflammatory activities.	$C_8H_8O_3$	[8,9]
7	Tris(tert-butyldimethylsilyloxy)arsane	59.083	1.76	Antifungal, antibacterial, and antifungal. Abundantly present in the polyherbal extract which was reported for its anti-obesity effects.	$C_{18}H_{45}AsO_3Si_3$	[10,11]
	Arsenous acid	59.083	1.76	Anticancer activity	AsH_3O_3	[12]
8	Benzaldehyde, 4-propyl-	59.749	2.02	Antimicrobial activity	$C_{10}H_{12}O$	[13]
9	2,4-Di-tert-butylphenol	83.226	9.26	Antifungal, antioxidant, Anti-inflammatory activities.	$C_{14}H_{22}O$	[14,15]

RT—retention time.

Table S3. Effects of *Ecklonia cava* ethanol (70%) extract on major organs including liver, spleen, and kidney of rats fed with normal diet (NC), a high-fat diet (HFD), or HFD accompanied with treatment ECE at different doses of 125 mg (T1), 250 mg (T2), and 500 mg (T3) per kg B.W.

Groups	Liver wt. (gm/rat)	Spleen wt. (gm/rat)	Kidney wt. (gm/rat)
NC	12.67 ± 1.34	0.78 ± 0.14	1.60 ± 0.22*
HFD	17.82 ± 1.80^{+++}	0.97 ± 0.22	1.92 ± 0.22^{+}
HCA (250 mg/kg)	13.89 ± 2.5*	0.98 ± 0.26	1.82 ± 0.17
EC (125 mg/kg)	14.78 ± 2.97	1.0 ± 0.19	1.80 ± 0.33
EC (250 mg/kg)	13.84 ± 2.71*	0.82 ± 0.129	1.72 ± 0.15
EC (500 mg/kg)	12.76 ± 1.89***	0.79 ± 0.16	1.64 ± 0.04*

Data are presented as means ± SEM (n = 8 for each group). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. HFD group, + $p < 0.05$, ++ $p < 0.01$, and +++ $p < 0.001$ vs normal control group.

Table S4. Effects on visceral fat, including mesenteric fat, subcutaneous fat, peritoneal fat and epididymal fat weights of rats fed with normal diet (NC), a high-fat diet (HFD) or HFD accompanied with treatment ECE at different doses of 125 mg (T1), 250 mg (T2), and 500 mg (T3) per kg B.W.

	Subcutaneous wt. (gm/rat)	Mesenteric wt. (gm/rat)	Peritoneal wt. (gm/rat)	Epididymal wt. (gm/rat)
NC	4.25 ± 2.96**	3.75 ± 1.78***	4.13 ± 1.64***	3.42 ± 1.34***
HFD	14.9 ± 8.23^{++}	8.4 ± 2.92	9.14 ± 2.33^{+++}	7.46 ± 2.00^{+++}
HCA (250 mg/kg)	11.06 ± 6.54	5.59 ± 1.14*	7.25 ± 1.81^{++}	6.12 ± 1.76^{++}
EC (125 mg/kg)	8.53 ± 3.71	5.84 ± 2.44	7.50 ± 1.54^{++}	5.63 ± 1.16^{+}
EC (250 mg/kg)	7.17 ± 2.42*	5.11 ± 2.22*	7.32 ± 1.22^{++}	4.98 ± 1.19*
EC (500 mg/kg)	6.49 ± 1.84*	4.62 ± 0.90**	5.84 ± 1.57**	4.75 ± 0.97**

Data are presented as means ± SEM (n = 8 for each group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. HFD group, + $p < 0.05$, ++ $p < 0.01$, and +++ $p < 0.001$ vs. normal control group.

Table S5. Effects on lipid profile of rats fed with normal diet; and high-fat diet with or without treatment of different doses of ECE (125, 250, and 500 mg/kg b.w) for 8 weeks.

Parameter	TC (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	AI	Free-fatty acid (μM)	TG (mg/mL)
NC	121.53 ± 4.38***	102.87 ± 3.4***	18.67 ± 4.27***	120.53 ± 3.44***	0.26 ± 0.002***	282.86 ± 44.38***
HFD	193.54 ± 2.94^{+++}	77.05 ± 5.78^{+++}	116.49 ± 6.0^{+++}	192.54 ± 3.81^{+++}	0.79 ± 0.07^{+++}	682.79 ± 14.59^{+++}
HCA (250 mg/kg)	121.74 ± 4.37***	100.48 ± 7.1**	21.27 ± 5.14***	120.74 ± 1.34***	0.22 ± 0.01***	304.60 ± 86.25***
EC (125 mg/kg)	153.9 ± 3.7*** $^{+++}$	87.66 ± 5.59^{+++}	66.21 ± 3.5*** $^{+++}$	152.9 ± 4.4***$^{+++}$	0.52 ± 0.03	500.60 ± 42.48
EC (250 mg/kg)	125.51 ± 7.76***	91.76 ± 6.95*	33.74 ± 4.50***	124.51 ± 5.15***	0.42 ± 0.14*	331.28 ± 9.93***
EC (500 mg/kg)	119.82 ± 10.7***	97.75 ± 6.60**	22.07 ± 5.82***	118.82 ± 4.11***	0.37 ± 0.03**	226.37 ± 51.76***

Data are presented as means ± SEM (n = 8 for each group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. HFD group, + $p < 0.05$, ++ $p < 0.01$, and +++ $p < 0.001$ vs normal control group.

Table S6. Effects of *Ecklonia cava* ethanol (70%) extract (ECE) on plasma biomarkers as liver function enzymes in rats fed with normal diet; and high-fat diet with or without treatment of different doses of ECE (125, 250, and 500 mg/kg b.w) for 8 weeks.

Parameter	AST (U/L)	ALP (U/L)	ALT (U/L)	GGT (U/L)
NC	8.78 ± 0.96***	2.25 ± 0.35***	1.31 ± 0.18***	8.04 ± 1.25
HFD	17.62 ± 0.83^{+++}	7.92 ± 0.93^{+++}	7.79 ± 0.98^{+++}	15.92 ± 6.43
HCA (250 mg/kg)	11.84 ± 1.48**	2.97 ± 0.18***	1.84 ± 0.53***	7.23 ± 0.57
EC (125 mg/kg)	13.78 ± 1.37^{+}	4.49 ± 0.41^{+***}	3.37 ± 0.46***	8.10 ± 7.78
EC (250 mg/kg)	13.09 ± 0.29*	4.26 ± 0.09^{+***}	2.65 ± 0.27***	8.31 ± 5.8
EC (500 mg/kg)	10.87 ± 0.02**	2.61 ± 0.37***	1.68 ± 0.29***	8.50 ± 5.17

Data are presented as means ± SEM (n = 8 for each group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. HFD group, + $p < 0.05$, ++ $p < 0.01$ and +++ < 0.001 vs. normal control group.

Table S7. Comparison of different published studies related to anti-obesity effects of *Ecklonia cava* and current study.

Animal	Body + orgens + fat weight	Plasma biomarkers	Liver biomarkers	Glucose, Insuline	Histology Liver, Adipoe tissue	Gene expression	3T3-L1	Antioxident DPPH, ABTS assay	Ref
Sprague–Dawley rats	Yes	TC,TG,FFA, HDL, LDL, leptin, ghrelin, GIP	ALT, AST, GGT, AST	Yes	Yes	Yes	Yes	Both DPPH and ABTS	Our Manuscript
C57BL/6N mice	Yes	TC,HDL, leptin, GOT, GPT,	liver- TG	Yes	-	Yes	-	-	[16]
C57BL/6NTacSam mice	Yes	HDL, LDL, liptin	Liver- TG	Glucose	-	Yes	-	-	[17]
C57BL/6N mice	Yes	IL-6, TNF-a, IL-10	-	-	Adipos tissue	-	-	-	[18]
Male C57BL/6 mice	Yes	TG, TC, HDL, GOT,GPT	-	Glucose	Liver	Yes	-	-	[19]
C57BL/6 mice	Yes	TG, TC,HDL,LDL	ALT,AST	Glucose	-	Yes	-	-	[20]

Data presented in table is only for comparison prospective.

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