

Supplementary Materials

Total flavonoid of *Epimedium koreanum* Nakai plays a therapeutic role in chronic renal failure by promoting AMPK activation

Yudan Zhao^a, Ruiqi Zhang^a, Lintong Mu^a, Wanyue Yang^a, Xin Zhang^a, Ling Han^c, Chongning Lv^{*a,b}, Jincai Lu^{*a,b}

^a*School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, PR China.*

^b*Liaoning Provincial Key Laboratory of TCM Resources Conservation and Development, Shenyang Pharmaceutical University, Shenyang 110006, PR China.*

^c*NERC for the Pharmaceutics of Traditional Chinese Medicines, Benxi 117004, PR China*

Corresponding authors

Tel./Fax: +86 24 43520728

Email addresses: jincailu@syphu.edu.cn (Jincai Lu); lcnmil@outlook.com (Chongning Lv)

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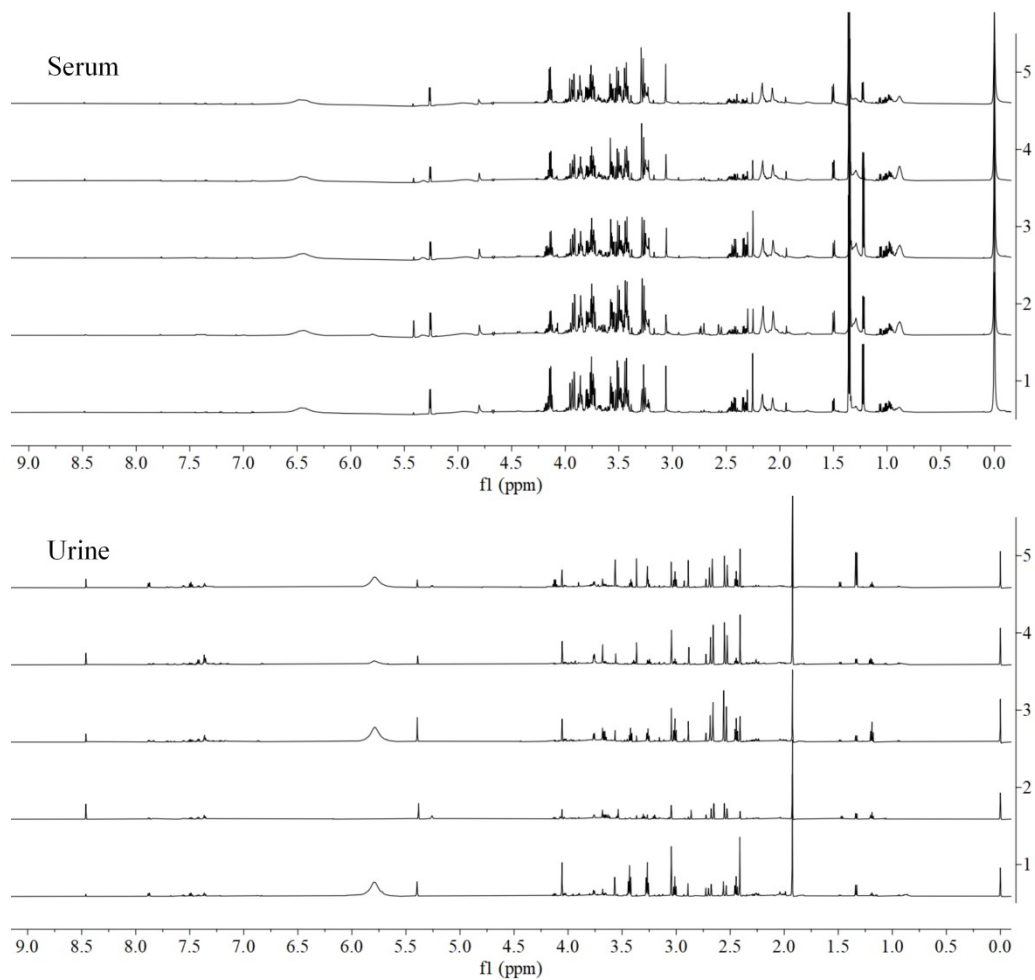


Fig. S1 The representative ¹H NMR spectra of serum and urine samples for the five groups. 1-Control group, 2-Adenine group, 3-Adenine+TFE (150 mg/kg) group, 4-Adenine+TFE (300 mg/kg) group, 5-Adenine+PFD group.

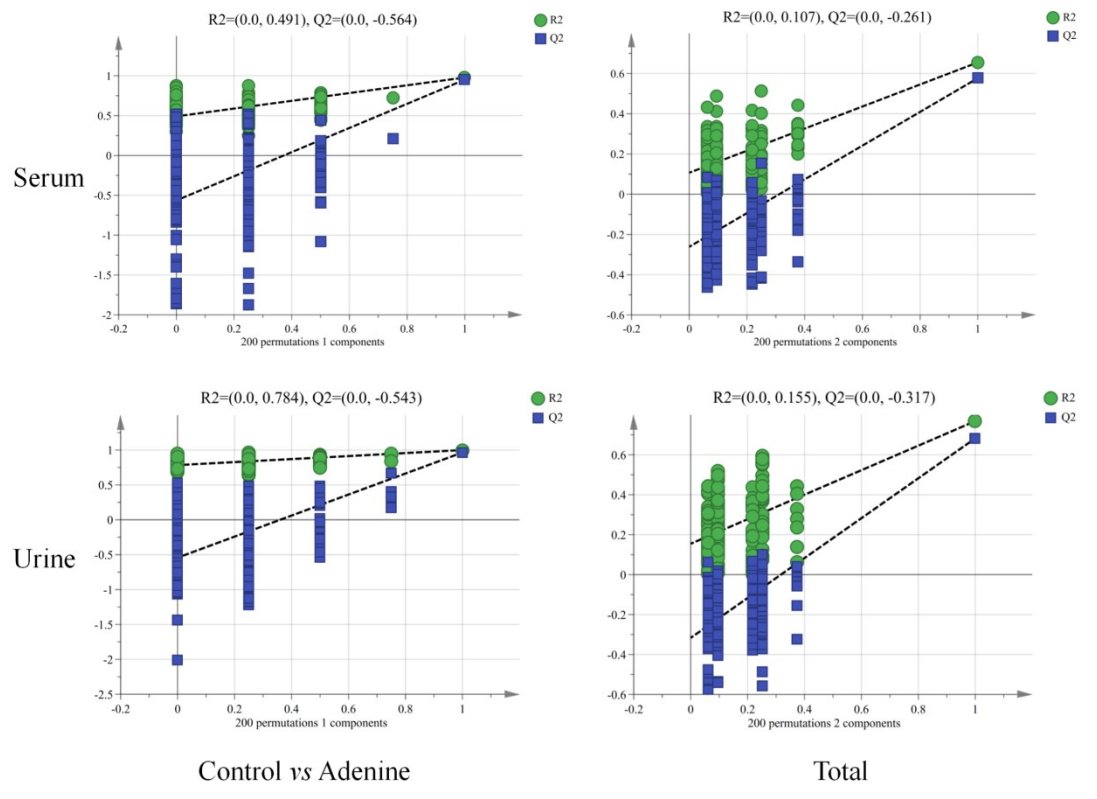


Fig. S2 The permutation tests (200 times) for the OPLS-DA models. Control vs Adenine means the permutation tests for the OPLS-DA models which established for Control group and adenine group; Total means the permutation tests for the OPLS-DA models which established for the five groups. All Q² values are less than zero, indicating that the models fit well.

Table S1 Accurate m/z, fragment ions of analytes using UHPLC-Q-TOF/MS from TFE

No.	Compound	t _R (min)	Molecular formula	Quasi molecular ion	m/z Calculated	m/z Experimental	Error (ppm)	Fragment Ions
1	Magnoflorine	4.189	C ₂₀ H ₂₄ NO ₄	M ⁺	342.1700	342.1705	-1.51	297.1121 [M+H-(CH ₃) ₂ NH] ⁺
2	Hyperoside	6.087	C ₂₁ H ₂₀ O ₁₂	[M+Na] ⁺	487.0847	487.0848	-0.22	303.0489 [M+H-Gal] ⁺
3	Epimedoside E	8.260	C ₃₇ H ₄₆ O ₁₉	[M+H] ⁺	795.2706	795.2712	-0.75	633.1777 [M+H-Glu] ⁺
4	Epimedoside A	8.569	C ₃₂ H ₃₈ O ₁₅	[M+H] ⁺	663.2283	663.2285	-0.23	517.1701 [M+H-Rha] ⁺
5	Ikarisoside A/Baohuoside II	10.422	C ₂₆ H ₂₈ O ₁₀	[M+H] ⁺	501.1755	501.1737	3.65	—
6	Epimedin A	12.309	C ₃₉ H ₅₀ O ₂₀	[M+H] ⁺	839.2968	839.2977	-1.05	677.2441 [M+H-Glu] ⁺ 531.1863 [M+H-Glu-Rha] ⁺ 369.13336 [M+H-2Glu-Rha] ⁺ 313.0705 [M+H-2Glu-Rha-isobutenyl] ⁺ 677.2451 [M+H-Xyl] ⁺
7	Epimedin B	12.827	C ₃₈ H ₄₈ O ₁₉	[M+H] ⁺	809.2863	809.2883	-2.53	531.1869 [M+H-Xyl-Rha] ⁺ 369.1339 [M+H-Xyl-Rha-Glu] ⁺ 313.0711 [M+H-Xyl-Rha-Glu-isobutenyl] ⁺ 677.2437 [M+H-Rha] ⁺
8	Epimedin C	13.390	C ₃₉ H ₅₀ O ₁₉	[M+H] ⁺	823.3019	823.3026	-0.84	531.1859 [M+H-2Rha] ⁺ 369.1332 [M+H-2Rha-Glu] ⁺ 313.0705 [M+H-2Rha-Glu-isobutenyl] ⁺

9	Icariin	13.809	C ₃₃ H ₄₀ O ₁₅	[M+H] ⁺	677.2440	677.2471	-4.59	531.1870 [M+H-Rha] ⁺ 369.1341 [M+H-Rha-Glu] ⁺ 313.0713 [M+H-Rha-Glu-isobutenyl] ⁺ 719.2545 [M+H-Glu] ⁺
10	Korepimodoside C	15.916	C ₄₁ H ₅₂ O ₂₁	[M+H] ⁺	881.3074	881.3089	-1.72	531.1860 [M+H-Glu-Rha-OAc] ⁺ 369.1336 [M+H-2Glu-Rha-OAc] ⁺ 313.0706 [M+H-2Glu-Rha-OAc-isobutenyl] ⁺ 531.1863 [M+H-Glu-Rha-2OAc] ⁺
11	Epimedokoreanoside I	17.858	C ₄₃ H ₅₄ O ₂₂	[M+H] ⁺	923.3179	923.3205	-2.76	369.1337 [M+H-2Glu-Rha-2OAc] ⁺ 313.0709 [M+H-2Glu-Rha-2OAc-isobutenyl] ⁺ 531.1852 [M+H-Glu-Rha-3OAc] ⁺
12	Korepimodoside B	21.752	C ₄₅ H ₅₆ O ₂₃	[M+H] ⁺	965.3285	965.3296	-1.13	369.1331 [M+H-2Glu-Rha-3OAc] ⁺ 313.0705 [M+H-2Glu-Rha-3OAc-isobutenyl] ⁺ 531.1854 [M+H-Glu-Rha-3OAc] ⁺
13	Caohuoside A/B	21.851	C ₄₅ H ₅₆ O ₂₃	[M+H] ⁺	965.3285	965.3297	-1.23	369.1331 [M+H-2Glu-Rha-3OAc] ⁺ 313.0703 [M+H-2Glu-Rha-3OAc-isobutenyl] ⁺ 531.1855 [M+H-Glu-Rha-3OAc] ⁺
14	Caohuoside A/B	21.995	C ₄₅ H ₅₆ O ₂₃	[M+H] ⁺	965.3285	965.3299	-1.44	369.1332 [M+H-2Glu-Rha-3OAc] ⁺ 313.0704 [M+H-2Glu-Rha-3OAc-isobutenyl] ⁺ 369.1337 [M+H-Glu-Rha] ⁺
15	Sagittatoside A	22.900	C ₃₃ H ₄₀ O ₁₅	[M+H] ⁺	677.2440	677.2446	-0.89	313.0706 [M+H-Glu-Rha-isobutenyl] ⁺ 515.1907 [M+H-Xyl] ⁺
16	Sagittatoside B	23.308	C ₃₂ H ₃₈ O ₁₄	[M+H] ⁺	647.2334	647.2339	-0.72	369.1335 [M+H-Xyl-Glu] ⁺ 313.0705 [M+H-Xyl-Glu-isobutenyl] ⁺
17	2''-O-rhamnosyl icariside II	23.407	C ₃₃ H ₄₀ O ₁₄	[M+H] ⁺	661.2491	661.2491	-0.03	369.1331 [M+H-2Rha] ⁺
18	Baohuoside I	23.981	C ₂₇ H ₃₀ O ₁₀	[M+H] ⁺	515.1912	515.1913	-0.25	369.1336 [M+H-Rha] ⁺

Table S2 Hemodynamic parameters of the rat kidneys

Group	PSV (cm/s)	EDV (cm/s)	RI
Control	24.01 ± 1.55	11.57 ± 1.27	0.52 ± 0.06
Adenine	13.73 ± 1.36 ^{###}	3.92 ± 0.76 ^{###}	0.72 ± 0.03 [#]
Adenine+TFE (150 mg/kg)	17.31 ± 1.51 [*]	6.51 ± 0.66 [*]	0.62 ± 0.04
Adenine+TFE (300 mg/kg)	17.33 ± 0.94 [*]	7.27 ± 1.18 [*]	0.64 ± 0.07
Adenine+PFD	18.06 ± 2.51 ^{**}	7.3 ± 1.2 ^{**}	0.60 ± 0.05 [*]

Data were expressed as mean ± SD. ^{###}*p* < 0.001, [#]*p* < 0.05, vs control group; ^{**}*p* < 0.01, ^{*}*p* < 0.05, vs adenine group.

Table S3 The endogenous metabolites identified from serum and urine samples

No.	Chemical shift	Metabolites	Source
1	0.86(m),1.28(m)	VLDL/LDL	serum
2	0.87(m)	Lipids	urine
3	0.89(t),1.64(m),1.72(m),3.99(dd)	2-hydroxybutyrate	urine
4	0.90(t),1.1(d)	3-methyl-2-oxovalerate	urine
5	0.93(t),1.01(d),1.28(m),1.96(m),3.66(m)	L-Isoleucine	serum
6	0.94(t),1.72(m),3.72(t)	L-Leucine	serum,urine
7	0.99(d),1.04(d),2.29(m)	L-Valine	serum
8	1.19(t)	Ethanol	urine
9	1.20(d),2.32(d),2.41(d),4.16(m)	3-hydroxybutyrate	serum,urine
10	1.33(d),4.11(q)	Lactate	serum,urine
11	1.36(s)	α -hydroxyisobutyrate	urine
12	1.46(m),1.73(m),1.90(m),3.76(m)	L-Lysine	serum
13	1.48(d),3.78(q)	Alanine	serum,urine
14	1.92(d)	Acetate	serum,urine
15	2.04(m)	<i>N</i> -acetylglycoproteins	serum
16	2.05(s),2.53(s),2.69(dd),4.41(m)	<i>N</i> -acetyl-L-aspartate	serum
17	2.14(s)	<i>O</i> -acetylglycoproteins	serum
18	2.10(m),3.74(m)	Glutamate	serum
19	2.15(m),2.45(m),3.77(m)	Glutamine	serum
20	2.23(s)	Acetone	serum
21	2.28(s),3.45(s)	Acetoacetate	serum,urine
22	2.35(s)	Oxalacetate	urine
23	2.37(s)	Pyruvate	serum
24	2.41(s)	Succinate	serum,urine
25	2.45(t),3.01(t)	2-oxoglutarate	urine
26	2.54(d),2.68(d)	Citrate	serum
27	2.72(m),5.3(m)	Unsaturated fatty acid	serum
28	2.83(s)	Methylguanidine	urine

29	2.89(s)	Trimethylamine	urine
30	3.04(s),3.93(s)	Creatine	serum,urine
31	3.05(s),4.05(s)	Creatinine	serum,urine
32	3.12(d),3.80(d)	Ethanolamine	urine
33	3.13(s)	Malonate	urine
34	3.20(s)	Choline	urine
35	3.25(s)	Betaine	urine
36	3.26(s)	TMO	serum,urine
37	3.27(t),3.42(t)	Taurine	urine
38	3.37(s)	Scyllo-inositol	urine
39	3.56(s)	Glycine	serum,urine
40	3.68(s),3.76(m),7.37(m)	Phenylacetyl glycine	urine
41	3.72(s)	trans-aconitate	urine
42	3.90(d),6.84(d),7.70(d)	4-aminohippurate	urine
43	3.95(d),7.55(m),7.83(dd)	Hippurate	urine
44	4.42(d), 5.25(d)	Glucose	serum
45	4.44(s),8.08(m),8.84(m),9.12(s)	Trigonelline	urine
46	5.26(t)	Allantoate	serum,urine
47	5.40(s)	Allantoin	serum,urine
48	5.79(brs)	Urea	serum,urine
49	6.42(brs)	Maltol	serum
50	7.21(m),7.28(m),7.35(s),7.51(m),7.71(d)	Indoxylsulfate	urine
51	7.49(t),7.55(t),7.87(d)	Benzoate	urine
52	7.68(s)	Guanine	urine
53	7.89(s)	Xanthine	urine
54	8.19(m)	Hypoxanthine	urine
55	8.46(s)	Formate	serum,urine

Table S4 The The cytotoxicity of TFE and PFD

Group	IC ₂₀ (μg/mL)
TFE	25.8 ± 1.9
PFD	508 ± 6.7

Table S5 qRT-PCR primer sequence

Gene	Primer (5'-3')	Size (bp)
α -SMA	Forward: ACCATCGGGAATGAACGCTT	191
	Reverse: CTGTCAGCAATGCCTGGGTA	
E-cadherin	Forward: ATGAGGTCGGTGCCCGTATT	138
	Reverse: CGTTGGTCTTGGGGTCTGTGA	
Fibronectin	Forward: AAACCTCTACGGGTCGCTG	160
	Reverse: GCGCTGGTGGTGAAGTCAAA	
SIRT1	Forward: AGATTTCAAGGCTGTTGGTTCC	326
	Reverse: CAGCATCATCTTCCAAGCCATT	
NF- κ B p65	Forward: CAGATACCACTAAGACGCACCC	227
	Reverse: CTCCAGGTCTCGCTTCTTCACA	
β -actin	Forward: TGCTATGTTGCCCTAGACTTCG	240
	Reverse: GTTGGCATAGAGGTCTTTACGG	