Supplementary materials

Chemical Characterization and Quality Control of the TCM Mixtures

Used in the Study

All the TCM mixtures were under careful quality control to assure their identity throughout all the experiments. Each TCM mixture was prepared in a single extraction and the dried extracts were well stored as the supply of test materials for animal experiments and chemical analysis.

Water extract powder of *salvia miltiorrhiza* (20 mg), *panax ginseng* (20 mg) and *shuanglong* formula (20 mg) were dissolved in 5 mL of water, respectively. The solutions were filtered through a 0.45 μ m filter membrane and the filtrates were used as test solution. 4 μ L of test solution was injected into the column in each run.

Chromatographic separation was performed on an Acquity UPLC BEH C_{18} column (2.1×100 mm, 1.7 μ m, Waters Corp., Milford, USA) using an ACQUITYTM UPLC system (Waters), equipped with a binary solvent delivery system, an autosampler, and a PDA detector. The column was maintained at 50 °C and eluted at a flow rate of 0.4 mL/min, using a mobile phase of (A) 0.1% (by volume) formic acid in water and (B) acetonitrile. The gradient program was optimized as follows: 0-4 min, 5% B to 5%B; 4-9 min, 5% B to 50% B; 9-19 min, 50% B to 90% B; 19-20 min, 90% B to 90% B; 20-22 min, 90% B to 5% B; 22-28 min, equilibration with 5% B. The column eluent was directly inducted into the mass spectrometer without split.

Mass spectrometry was performed on a Waters LCT Premier orthogonal accelerated time of flight mass spectrometer (Waters, Millford, MA) with an electrospray ionization source (ESI) operating in negative ion mode (W mode of operation). The capillary voltage and the cone voltage were set at 2200 V and 35 V, respectively. Nitrogen was used as the drying gas. The desolvation gas rate was set to 700 L/h at a temperature of 350 , and the cone gas rate was set at 40 L/h and the source temperature was set at 120 . The scan time and inter-scan delay were set to 0.2 s and 0.02 s, respectively. Data was collected in centroid mode from m/z 100 to m/z 1500 with a LockSpray frequency of 10 s, and data averaging over 10 scans. The total ion

chromatograms of test solutions prepared from selected TCM materials were shown in **Figure S1-S3**.

As a result, seventy seven compounds of *shuanglong* formula (SLF) were identified on the basis of retention time, m/z and available conference standards (shown in **Table S1**), among which twenty six compounds were from *salvia miltiorrhiza* (shown in **Table S2**) and fifty one compounds from *panax ginseng* (shown in **Table S3**). For the quality control, eighteen major components among the TCM mixture SLF were assayed with external standard calibrations, and the other components were estimated with their peak areas.

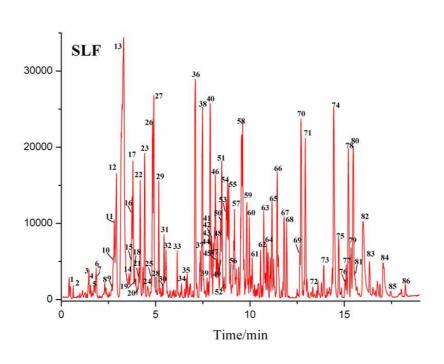


Figure S1 The total ion chromatogram of shuanglong formula (SLF) on UPLC/TOF MS

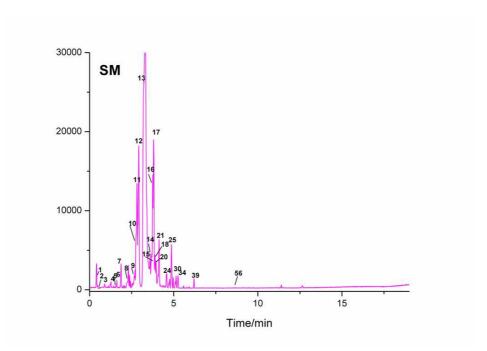


Figure S2 The total ion chromatogram of salvia miltiorrhiza (SM) on UPLC/TOF MS

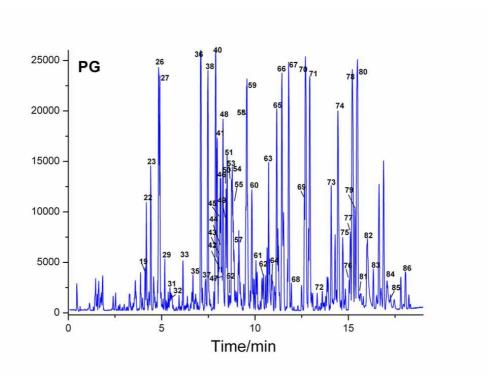


Figure S3 The total ion chromatogram of panax ginseng (PG) on UPLC/TOF MS

Table S1 Identification and quantitative assay of major components of shuanglong formula used in the study

snuangiong formula used in the study							
NO.	R.T.(min)	Selected ion	m/z	Elemental composition	Identified result	content (mg/g)	
1*	0.54	[M-H] ⁻	197.0272	$C_9H_{10}O_5$	3-(3,4-dihydroxyhenyl) lactic acid	0.22	
2	0.64	$[M-H]^-$	313.0923	$C_{17}H_{14}O_{6}$	salvianolic acid F		
3	1.26	[M-H] ⁻	137.0224	$C_7H_6O_3$	protocatechuic aldehyde	_	
4	1.55		383.0956		not determined	_	
5	1.62	[M+HCOO]	315.1078	$C_{16}H_{14}O_4$	isoimperatrin	_	
6	1.83	$[M-H]^-$	417.1034	$C_{20}H_{18}O_{10}$	salvianolic acid D	_	
7	1.87	$[M-H]^-$	371.0975	$C_{19}H_{16}O_{8}$	salvianolic acid M		
8	2.31	$[M-H]^-$	537.1421	$C_{27}H_{22}O_{12}$	salvianolic acid H		
9	2.7	$[M-H]^-$	555.1463	$C_{27}H_{24}O_{13}$	salvianolic acid K		
10	2.8	$[M-H]^-$	537.1498	$C_{27}H_{22}O_{12}$	salvianolic acid I	_	
11	2.8	$[M-H]^-$	717.2053	$C_{36}H_{30}O_{16}$	salvianolic acid L	_	
12*	2.91	$[M-H]^-$	359.1027	$C_{18}H_{16}O_{8}$	rosmarinic acid	0.38	
13*	3.3	$[M-H]^-$	717.2090	$C_{36}H_{30}O_{16}$	salvianolic acid B	6.34	
14	3.43	$[M-H]^-$	537.1498	$C_{27}H_{22}O_{12}$	salvianolic acid J		
15	3.69	$[M-H]^-$	445.0963	$C_{21}H_{18}O_{11} \\$	baicalin		
16*	3.71	$[M-H]^-$	491.1032	$C_{26}H_{22}O_{10}$	salvianolic acid C	0.63	
17	3.8	$[M-H]^-$	717.2072	$C_{36}H_{30}O_{16}$	salvianolic acid E	_	
18	3.87	$[M-H]^-$	455.3541	$C_{30}H_{48}O_3$	ursolic acid	_	
19	3.87	[M+HCOO]	831.4751	$C_{41}H_{70}O_{14} \\$	ginsenoside F ₄	_	
20	4.06	[M+HCOO]	419.0966	$C_{19}H_{18}O_{8}$	methyl rosmarinate	_	
21	4.12	$[M-H]^-$	731.2222	$C_{37}H_{32}O_{16}$	9'-methyl lithospermate B		
22	4.17	[M+HCOO]	1007.6335	$C_{48}H_{82}O_{19}$	20-gluco-ginsenoside Rf	_	
23	4.41	[M+HCOO]	977.5273	$C_{47}H_{80}O_{18}$	notoginsenoside R_1	_	
24*	4.59	[M+HCOO]	537.1498	$C_{27}H_{22}O_{12}$	lithospermic acid	0.55	
25*	4.77	$[M-H]^-$	493.1253	$C_{26}H_{22}O_{10}$	salvianolic acid A	0.55	
26*	4.83	[M+HCOO]	991.5528	$C_{48}H_{82}O_{18}$	ginsenoside Re	0.44	
27*	4.89	[M+HCOO]	845.5647	$C_{42}H_{72}O_{14}$	ginsenoside Rf	0.17	
28	4.9	[M+HCOO]	459.3927	$C_{29}H_{50}O$	β -sitosterol		
29	5.16	$[M-H]^-$	885.5041	$C_{45}H_{74}O_{17}$	ginsenoside malonyl-Rg ₁		
30	5.25	[M-H] ⁻	491.0979	$C_{19}H_{16}O_8$	isosalvianolic acid C		
31*	5.44	$[M-H]^-$	1031.5356	$C_{51}H_{84}O_{21}$	ginsenoside malonyl-Rd	0.072	
32	5.55	$[M-H]^-$	885.5643	$C_{45}H_{74}O_{17}$	ginsenoside malonyl-Rg1 isomer	_	
33	6.15	$[M+HCOO]^-$	815.4805	$C_{41}H_{70}O_{13} \\$	ginsenoside F ₃		
34	6.38	$[M-H]^-$	555.1244	$C_{27}H_{24}O_{13}$	dimethyl salvianolate B		
35	6.67		1033.6500		not determined		
36*	7.09	[M+HCOO]	845.5662	$C_{42}H_{72}O_{14} \\$	ginsenoside Rg ₁	3.39	
37	7.33	$[M+HCOO]^-$	1285.6573	$C_{59}H_{100}O_{27}$	notoginsenoside R ₄	_	
38	7.47	[M+HCOO]	815.5520	$C_{41}H_{70}O_{13} \\$	notoginsenoside R_2	_	

39	7.56	$[M-H]^-$	329.2471	$C_{20}H_{26}O_4$	salviol	_
40	7.88	[M+HCOO]	829.5691	$C_{42}H_{72}O_{13}$	ginsenoside Rg ₂	
41	7.9	[M+HCOO]	815.4859	$C_{41}H_{70}O_{13}$	ginsenoside F ₅	
42	7.94	[M+HCOO]	1255.6456	$C_{58}H_{98}O_{26}$	ginsenoside Ra ₁	_
43	7.97	[M+HCOO]	683.4987	$C_{36}H_{62}O_9$	ginsenoside Rh ₁	
44	8.05	[M+HCOO]	1285.7575	$C_{59}H_{100}O_{27}$	ginsenoside Ra ₃	
45*	8.11	[M+HCOO]	1153.7021	$C_{54}H_{92}O_{23}$	ginsenoside Rb ₁	1.12
46*	8.15	$[M-H]^-$	1193.7024	$C_{57}H_{94}O_{26}$	ginsenoside malonyl-Rb ₁	0.27
47	8.18	[M+HCOO]	1165.6365	$C_{55}H_{92}O_{23}$	ginsenoside Rs ₂	
48*	8.27	[M+HCOO]	1001.5022	$C_{49}H_{80}O_{18} \\$	ginsenoside R ₀	0.59
49	8.28	[M+HCOO]	683.4992	$C_{36}H_{62}O_{9}$	ginsenoside F ₁	
50	8.42	[M+HCOO]	1255.6363	$C_{58}H_{98}O_{26}$	ginsenoside Ra ₂	
51*	8.47	[M+HCOO]	1123.5933	$C_{53}H_{90}O_{22}$	ginsenoside Rb ₂	3.99
52*	8.5	$[M-H]^-$	1163.5982	$C_{56}H_{92}O_{25}$	ginsenoside malonyl-Rb ₂	1.41
53	8.77	[M+HCOO]	1123.5909	$C_{53}H_{90}O_{22}$	ginsenoside Rb ₃	
54*	8.8	[M+HCOO]	1123.5868	$C_{53}H_{90}O_{22}$	ginsenoside Rc	0.54
55*	8.88	$[M-H]^-$	1163.5994	$C_{56}H_{92}O_{25}$	ginsenoside malonyl-Rc	0.099
56	8.96	$[M-H]^-$	339.0806	$C_{18}H_{12}O_7$	salvianolic acid G	
57	9.16	[M+HCOO]	1195.6102	$C_{56}H_{94}O_{24}$	panas quinquefolium R_1	
58	9.5	[M+HCOO]	1165.7053	$C_{55}H_{92}O_{23}$	ginsenoside Rs ₁	
59*	9.56	[M+HCOO]	991.5594	$C_{48}H_{82}O_{18} \\$	ginsenoside Rd	0.26
60	9.82		503.2664		not determined	
61	10.1	[M+HCOO]	991.5609	$C_{48}H_{82}O_{18} \\$	Rd isomer	
62	10.56	$[M-H]^-$	961.5531	$C_{48}H_{82}O_{19}$	20-gluco-ginsenoside Rf	
02	10.50	[141-11]	901.3331	C481182O19	isomer	
63	10.72		1033.5769		not determined	
64	10.91		961.6228		not determined	
65	11.16	[M+HCOO]	811.5568	$C_{42}H_{70}O_{12}$	ginsenoside Rg ₄	
66	11.43	[M+HCOO]	811.5567	$C_{42}H_{70}O_{12}$	ginsenoside Rg ₅	
67	11.81		665.4831		not determined	
68	11.96	[M+HCOO]	829.488	$C_{42}H_{72}O_{13}$	Rg ₂ isomer	
69	12.62	[M+HCOO]	505.3546	$C_{30}H_{52}O_3$	protopanaxadiol	
70	12.7	[M+HCOO]	829.5677	$C_{42}H_{72}O_{13}$	ginsenoside F ₂	
71	12.92	[M+HCOO]	829.5697	$C_{42}H_{72}O_{13}$	ginsenoside Rg ₃	
72	13.32	[M+HCOO]	799.4894	$C_{41}H_{70}O_{12}$	ginsenoside Mc	
73	13.91		595.2895		not determined	
74	14.43		1191.5946		not determined	
75	14.69		723.4442		not determined	
76	15.03	[M+HCOO]	667.4415	$C_{36}H_{62}O_{8}$	ginsenoside Rh ₂	
77	15.11	[M+HCOO]	851.4934	$C_{51}H_{98}O_6$	tripalmitin	_
78	15.21	[M+HCOO]	811.4869	$C_{42}H_{70}O_{12} \\$	ginsenoside Rg ₆	
79	15.43	[M+HCOO]	649.3914	$C_{36}H_{60}O_{7}$	ginsenoside Rh ₃	_
80	15.48	[M+HCOO]	811.4849	$C_{42}H_{70}O_{12} \\$	ginsenoside Rk ₁	_
81	15.58	[M+HCOO]	667.4438	$C_{36}H_{62}O_{8}$	Compound K	

82	15.97		433.2725		not determined	
83	16.33	[M+HCOO]	625.2377	$C_{28}H_{36}O_{13}$	eleatheroside B	
84	17.08	$[M-H]^-$	455.2816	$C_{30}H_{48}O_3$	oleanolic acid	_
85	17.46	[M+HCOO]	461.26677	$C_{27}H_{44}O_3$	tigogenin	_
86	18.22	[M+HCOO]	649.4343	$C_{36}H_{60}O_{7}$	ginsenoside Rk ₂	_

^{*} The indicative major components were assayed with external standard calibrations and the other components were estimated and controlled with their peak area.

Table S2 Identification and quantitative assay of major components of salvia miltiorrhiza extract used in the study

NO	D.M.(;)			Elemental	•	content
NO.	R.T.(min)	Selected ion	m/z	composition	Identification	(mg/g)
1*	0.54	[M-H] ⁻	197.0272	$C_9H_{10}O_5$	3-(3,4-dihydroxyhenyl) lactic acid	0.81
2	0.64	$[M-H]^-$	313.0923	$C_{17}H_{14}O_6$	salvianolic acid F	
3	1.26	$[M-H]^-$	137.0224	$C_7H_6O_3$	protocatechuic aldehyde	
4	1.55	$[M-H]^-$	383.0956	$C_{21}H_{20}O_7$	not determined	
5	1.62	[M+HCOO]	315.1078	$C_{16}H_{14}O_4$	isoimperatrin	
6	1.83	$[M-H]^-$	417.1034	$C_{20}H_{18}O_{10} \\$	salvianolic acid D	
7	1.87	$[M-H]^-$	371.0975	$C_{19}H_{16}O_{8}$	salvianolic acid M	
8	2.31	$[M-H]^-$	537.1421	$C_{27}H_{22}O_{12}$	salvianolic acid H	
9	2.7	$[M-H]^-$	555.1463	$C_{27}H_{24}O_{13}$	salvianolic acid K	
10	2.8	$[M-H]^-$	537.1498	$C_{27}H_{22}O_{12}$	salvianolic acid I	
11	2.8	$[M-H]^-$	717.2053	$C_{36}H_{30}O_{16}$	salvianolic acid L	
12*	2.91	$[M-H]^-$	359.1027	$C_{18}H_{16}O_{8}$	rosmarinic acid	1.93
13*	3.3	$[M-H]^-$	717.2090	$C_{36}H_{30}O_{16}$	salvianolic acid B	23.69
14	3.43	$[M-H]^-$	537.1498	$C_{27}H_{22}O_{12}$	salvianolic acid J	
15	3.69	$[M-H]^-$	445.0963	$C_{21}H_{18}O_{11} \\$	baicalin	
16*	3.71	$[M-H]^-$	491.1032	$C_{26}H_{22}O_{10} \\$	salvianolic acid C	2.19
17	3.8	$[M-H]^-$	717.2072	$C_{36}H_{30}O_{16}$	salvianolic acid E	
18	3.87	$[M-H]^-$	455.3541	$C_{30}H_{48}O_3$	ursolic acid	
20	4.06	[M+HCOO]	419.0966	$C_{19}H_{18}O_{8}$	methyl rosmarinate	
21	4.12	$[M-H]^-$	731.2222	$C_{37}H_{32}O_{16}$	9'-methyl lithospermate B	
24*	4.59	[M+HCOO]	537.1498	$C_{27}H_{22}O_{12}$	lithospermic acid	2.21
25*	4.77	$[M-H]^-$	493.1253	$C_{26}H_{22}O_{10} \\$	salvianolic acid A	2.05
30	5.25	$[M-H]^-$	491.0979	$C_{19}H_{16}O_{8}$	isosalvianolic acid C	_
34	6.38	$[M-H]^-$	555.1244	$C_{27}H_{24}O_{13}$	dimethyl salvianolate B	_
39	7.56	$[M-H]^-$	329.2471	$C_{20}H_{26}O_4$	salviol	_
56	8.96	$[M-H]^-$	339.0806	$C_{18}H_{12}O_7$	salvianolic acid G	

^{*} The indicative major components were assayed with external standard calibrations and the other components were estimated and controlled with their peak area.

Table S3 Identification and quantitative assay of major components of panax ginseng extract used in the study

				Elemental		content
NO.	R.T.(min)	Selected ion	m/z	composition	Identified result	(mg/g)
19	3.87	[M+HCOO]	831.4751	$C_{41}H_{70}O_{14} \\$	ginsenoside F ₄	_
22	4.17	[M+HCOO]	1007.6335	$C_{48}H_{82}O_{19} \\$	20-gluco-ginsenoside Rf	_
23	4.41	[M+HCOO]	977.5273	$C_{47}H_{80}O_{18} \\$	notoginsenoside R ₁	_
26*	4.83	[M+HCOO]	991.5528	$C_{48}H_{82}O_{18} \\$	ginsenoside Re	0.61
27*	4.89	[M+HCOO]	845.5647	$C_{42}H_{72}O_{14} \\$	ginsenoside Rf	0.23
28	4.9	[M+HCOO]	459.3927	$C_{29}H_{50}O$	β -sitosterol	
29	5.16	$[M-H]^-$	885.5041	$C_{45}H_{74}O_{17}$	ginsenoside malonyl-Rg ₁	
31*	5.44	$[M-H]^-$	1031.5356	$C_{51}H_{84}O_{21}$	ginsenoside malonyl-Rd	0.11
32	5.55	$[M-H]^-$	885.5643	$C_{45}H_{74}O_{17}$	ginsenoside alonyl-Rg1 isomer	_
33	6.15	[M+HCOO]	815.4805	$C_{41}H_{70}O_{13}$	ginsenoside F ₃	_
35	6.67		1033.6500		not determined	_
36*	7.09	[M+HCOO]	845.5662	$C_{42}H_{72}O_{14}$	ginsenoside Rg ₁	4.64
37	7.33	[M+HCOO]	1285.6573	$C_{59}H_{100}O_{27}$	notoginsenoside R ₄	
38	7.47	[M+HCOO]	815.5520	$C_{41}H_{70}O_{13}$	notoginsenoside R ₂	
40	7.88	[M+HCOO]	829.5691	$C_{42}H_{72}O_{13}$	ginsenoside Rg ₂	_
41	7.9	[M+HCOO]	815.4859	$C_{41}H_{70}O_{13}$	ginsenoside F ₅	_
42	7.94	[M+HCOO]	1255.6456	$C_{58}H_{98}O_{26}$	ginsenoside Ra ₁	_
43	7.97	[M+HCOO]	683.4987	$C_{36}H_{62}O_{9}$	ginsenoside Rh ₁	_
44	8.05	[M+HCOO]	1285.7575	$C_{59}H_{100}O_{27}$	ginsenoside Ra ₃	_
45*	8.11	[M+HCOO]	1153.7021	$C_{54}H_{92}O_{23}$	ginsenoside Rb ₁	1.57
46*	8.15	$[M-H]^-$	1193.7024	$C_{57}H_{94}O_{26}$	ginsenoside malonyl-Rb ₁	0.35
47	8.18	[M+HCOO]	1165.6365	$C_{55}H_{92}O_{23}$	ginsenoside Rs ₂	_
48*	8.27	[M+HCOO]	1001.5022	$C_{49}H_{80}O_{18}$	ginsenoside R ₀	0.73
49	8.28	[M+HCOO]	683.4992	$C_{36}H_{62}O_{9}$	ginsenoside F ₁	_
50	8.42	[M+HCOO]	1255.6363	$C_{58}H_{98}O_{26}$	ginsenoside Ra ₂	_
51*	8.47	[M+HCOO]	1123.5933	$C_{53}H_{90}O_{22}$	ginsenoside Rb ₂	5.04
52*	8.5	$[M-H]^-$	1163.5982	$C_{56}H_{92}O_{25}$	ginsenoside malonyl-Rb ₂	1.77
53	8.77	[M+HCOO]	1123.5909	$C_{53}H_{90}O_{22}$	ginsenoside Rb ₃	
54*	8.8	[M+HCOO]	1123.5868	$C_{53}H_{90}O_{22}$	ginsenoside Rc	0.81
55*	8.88	$[M-H]^-$	1163.5994	$C_{56}H_{92}O_{25}$	ginsenoside malonyl-Rc	0.14
57	9.16	[M+HCOO]	1195.6102	$C_{56}H_{94}O_{24}$	panas quinquefolium R ₁	
58	9.5	[M+HCOO]	1165.7053	$C_{55}H_{92}O_{23}$	ginsenoside Rs ₁	_
59*	9.56	[M+HCOO]	991.5594	$C_{48}H_{82}O_{18} \\$	ginsenoside Rd	0.38
60	9.82		503.2664		not determined	
61	10.1	[M+HCOO]	991.5609	$C_{48}H_{82}O_{18}$	ginsenoside Rd isomer	
62	10.56	$[M-H]^-$	961.5531	$C_{48}H_{82}O_{19}$	20-gluco-ginsenoside Rf isomer	_
63	10.72		1033.5769		not determined	
64	10.91		961.6228		not determined	_

65	11.16	[M+HCOO]	811.5568	$C_{42}H_{70}O_{12}$	ginsenoside Rg ₄	
66	11.43	[M+HCOO]	811.5567	$C_{42}H_{70}O_{12} \\$	ginsenoside Rg ₅	_
67	11.81		665.4831		not determined	_
68	11.96	[M+HCOO]	829.488	$C_{42}H_{72}O_{13} \\$	ginsenoside Rg2 isomer	_
69	12.62	[M+HCOO]	505.3546	$C_{30}H_{52}O_3$	protopanaxadiol	_
70	12.7	[M+HCOO]	829.5677	$C_{42}H_{72}O_{13} \\$	ginsenoside F ₂	_
71	12.92	[M+HCOO]	829.5697	$C_{42}H_{72}O_{13}$	ginsenoside Rg ₃	_
72	13.32	[M+HCOO]	799.4894	$C_{41}H_{70}O_{12}$	ginsenoside Mc	_
73	13.91		595.2895		not determined	_
74	14.43		1191.5946		not determined	_
75	14.69		723.4442		not determined	_
76	15.03	[M+HCOO]	667.4415	$C_{36}H_{62}O_{8}$	ginsenoside Rh ₂	_
77	15.11	[M+HCOO]	851.4934	$C_{51}H_{98}O_6$	tripalmitin	_
78	15.21	[M+HCOO]	811.4869	$C_{42}H_{70}O_{12} \\$	ginsenoside Rg ₆	_
79	15.43	[M+HCOO]	649.3914	$C_{36}H_{60}O_7$	ginsenoside Rh ₃	_
80	15.48	[M+HCOO]	811.4849	$C_{42}H_{70}O_{12} \\$	ginsenoside Rk ₁	_
81	15.58	[M+HCOO]	667.4438	$C_{36}H_{62}O_{8}$	Compound K	_
82	15.97		433.2725		not determined	_
83	16.33	[M+HCOO]	625.2377	$C_{28}H_{36}O_{13}$	eleatheroside B	_
84	17.08	$[M-H]^-$	455.2816	$C_{30}H_{48}O_3$	oleanolic acid	_
85	17.46	[M+HCOO]	461.26677	$C_{27}H_{44}O_3$	tigogenin	_
86	18.22	[M+HCOO]	649.4343	$C_{36}H_{60}O_{7}$	ginsenoside Rk ₂	

^{*} The indicative major components were assayed with external standard calibrations and the other components were estimated and controlled with their peak area.

Supplementary Figure S4

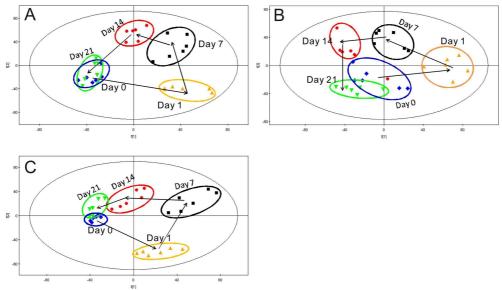


Figure S4 PLS-DA scores plot of non-normalized rat urine data of SLF group (A), SM group (B), PG group (C).

SLFG: SLF group; PGG: PG group; SMG: SM group