Electronic Supplementary Information (ESI)

A comparative study of main constituents and antidepressant effects of raw and vinegar-baked Bupleuri Radix in chronic unpredictable mild stress rats

Tianli Lei[a], Yadan Wang[a], Mingxiao Li[a], Xin Zhang[a], Chongning Lv[a], Lingyun Jia[a], Jing Wang[*b] and Jincai Lu[*a]

[a]*Department of Medicinal Plant, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, P.R. China. E-mail: jincailu@126.com Tel. /fax: +86 024 23986500.*

[b]*Department of Pharmaceutical Botany, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, P.R. China. E-mail: wangjingyk@126.com Tel. /fax: +86 024 23986499.*

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S1 HPLC-DAD chromatographic conditions

The chromatographic separation was carried out on a THERMO ODS-2 HYPERSIL C18 column (250 mm × 4.6 mm, 5 μm) at column temperature of 30℃. The mobile phase was acetonitrile (A) and aqueous solution (B) with a gradient program as follows: 0–15 min: 30% A; 15–30 min: 30–40% A; 30–50 min: 40–45% A; 50–55 min: 45–55% A; 55–70 min: 55–70% A; 70–80 min: 70–30% A at a flow rate of 1 mL/min. The injection volume of sample was 10 μL, and the detection wavelength for quantitative analysis was set at 210 nm based on the scan from 190-400 nm of DAD.

S2 The chromatographic separation and MS analysis

The main chemical composition of BR and VBBR was analyzed by UHPLC-Q-TOF-MS. Chromatographic separation was achieved on an ACQUITY BEH C_{18} column (100×2.1 mm, i.d., 1.7 μm, Waters, Milford, MA, USA). The mobile phase consisting of water (A) and acetonitrile (B) was run at a flow rate of 0.4 mL/min. The UHPLC eluting conditions were optimized as follows: 5% B (0–2 min), 5% to 15% B (2–3 min), 15% to 40% B (3–10 min), 40% to 45% B (10–13 min), 45% to 60% B (13–17 min), 60% to 100% B (17–18 min), 100% B for 1 min, and then returned to initial condition in 1 min. The column temperature was maintained at 35℃. The injection volume was 1 μL. MS analysis was performed by a Waters Xevo G2 QTOF (Waters, Manchester, UK) with the mass conditions as follows: capillary voltage was 3 kV and cone voltage were 35 V in negative ion mode; Collision energy 30 eV; TOF MS were scanned with the mass range of *m/z* 100 – 1500; Desolvation gas was set to 600.0 L/h at temperature of 300℃; Cone gas was set to 50.0 L/h, and source temperature was set to 100℃.

Table S1 The chronic unpredictable mild stress (CUMS) procedure and BR/VBBR treatment schedule.

Day	Stressor	Drug treatment
1	Food deprivation (24 h)	No treatment
2	Water deprivation (23 h)/empty water bottles (1 h)	No treatment
3	Physical restraint (activity restriction in bottle, 4 h)	No treatment
4	White noises (24 h)	No treatment
5	Tail pinch (1 cm apart from the tail, 5 min)	No treatment
6	Cold water swimming (15℃, 5 min)	No treatment
7	Wet bedding (24 h)	No treatment
8	Day/night inversion (24 h)	No treatment
9	Cold water swimming (15℃, 5 min)	No treatment
10	Water deprivation (23 h)/Empty water bottles (1 h)	No treatment
11	White noise (24 h)	No treatment
12	Physical restraint (activity restriction in bottle, 4 h)	No treatment
13	Tail pinch (1 cm apart from the tail, 5 min)	No treatment
14	Food deprivation (24 h)	No treatment
15	Cold water swimming (15℃, 5 min)	No treatment
16	Wet bedding (24 h)	No treatment
17	Water deprivation (23 h)/Empty water bottles (1 h)	No treatment
18	Day/night inversion (24 h)	No treatment
19	Food deprivation (24 h)	No treatment
20	Cold water swimming (15℃, 5 min)	No treatment
21	Physical restraint (activity restriction in bottle, 4 h)	No treatment
22	White noises (24 h)	treatment
23	Tail pinch (1 cm apart from the tail, 5 min)	treatment
24	Wet bedding (24 h)	treatment
25	Water deprivation (23 h)/Empty water bottles (1 h)	treatment

26	Day/night inversion (24 h)	treatment
27	Tail pinch (1 cm apart from the tail, 5 min)	treatment
28	Food deprivation (24 h)	treatment
29	Physical restraint (activity restriction in bottle, 4 h)	treatment
30	Cold water swimming (15℃, 5 min)	treatment
31	Wet bedding (24 h)	treatment
32	Water deprivation (23 h)/Empty water bottles (1 h)	treatment
33	White noises (24 h)	treatment
34	Day/night inversion (24 h)	treatment
35	Food deprivation (24 h)	treatment
36	Tail pinch (1 cm apart from the tail, 5 min)	treatment
37	Food deprivation (24 h)	treatment
38	Physical restraint (activity restriction in bottle, 4 h)	treatment
39	Cold water swimming (15℃, 5 min)	treatment
40	Wet bedding (24 h)	treatment
41	Water deprivation (23 h)/Empty water bottles (1 h)	treatment
42	White noises (24 h)	treatment

Table S2. UHPLC–QTOF-MS data and assignments of the compounds in BR and VBBR samples

No.	RT(min)	Identification	Molecular formula	MW	Measured value	$[M-H]^-$ theoretical exact mass	Tolerance (ppm)	MS/MS fragment ions	Source
1	4.358	Hyperoside	$C_{21}H_{20}O_{12}$	464.0955	464.0989	463.0877	7.56	463.0912 $[M-H]^-$	BR, VBBR
2	7.002	Clinoposaponin XIV	$C_{42}H_{68}O_{14}$	796.4609	796.4523	795.4529	–1.13	795.4520 $[M-H]^-$	BR
3	7.124	(-)-epinortrachelogenin	$C_{20}H_{22}O_7$	374.1366	374.1271	373.1287	9.65	373.1323 $[M-H]^-$ 355.1195 $[M-H-H_2O]^-$	BR
4	8.199	HOSSa	$C_{42}H_{70}O_{14}$	798.4766	798.4658	797.4687	4.01	797.4719 $[M-H]^-$ 635.4030[M–H–Glc]$^-$	BR, VBBR
5	8.775	3',4'-dimethoxy quercetin	$C_{18}H_{34}O_5$	330.0740	330.0718	329.0661	–1.52	329.0656 $[M-H]^-$	BR, VBBR
6	9.220	Chinoposaponin XVIII	$C_{48}H_{78}O_{18}$	942.5188	942.5212	941.5110	–3.93	941.5073 $[M-H]^-$	BR, VBBR
7	9.560	21β-hydroxy-SSb_2	$C_{48}H_{68}O_{14}$	796.4609	796.4750	795.4472	–3.65	795.4443 $[M-H]^-$ 633.4103[M−H−Glc]-	BR, VBBR
8	9.900	SSc	$C_{48}H_{78}O_{17}$	926.5239	926.5229	925.5161	5.08	925.5208 $[M-H]^-$ 779.4444[M–H–Rha]$^-$ 763.4682[M–H–Glc]$^-$ 617.3965[M−H−Fuc−Glc]$^-$	BR, VBBR
9	9.986	SSf	$C_{48}H_{80}O_{17}$	928.5396	928.5372	927.5317	2.16	927.5337[M−H]$^-$ 781.4613[M–H–Rha]$^-$ 765.4606[M–H–Glc]$^-$ 619.4165[M−H−Fuc−Glc]$^-$	BR, VBBR
10	10.817	SSb_3	$C_{43}H_{72}O_{14}$	812.4922	812.4911	811.4844	5.18	811.4886[M−H]$^-$ 649.4146[M–H–Glc]$^-$	BR, VBBR
11	10.992	SSb_4	$C_{43}H_{72}O_{14}$	812.4922	812.4928	811.4844	3.70	811.4874[M−H]$^-$	BR, VBBR

								649.4142[M–H–Glc]$^-$	
12	11.261	Acetyl-SSb$_3$ or -SSb$_4$	$C_{45}H_{74}O_{15}$	854.5028	854.4988	853.4949	0.82	853.4956[M−H]$^-$ 811.4852[M−H−C_2H_2O]$^-$	BR
13	11.602	SSa	$C_{42}H_{68}O_{13}$	780.4660	780.4642	779.4582	3.46	779.4555[M−H]$^-$ 617.4043[M–H–Glc]$^-$	BR, VBBR
14	11.695	SSb$_2$	$C_{42}H_{68}O_{13}$	780.4660	780.4557	779.4582	5.52	779.4625[M−H]$^-$ 617.3898[M–H–Glc]$^-$ 541.3439[M-H-Glc−76]$^-$ 471.2726[M−H−Fuc−Glc]$^-$	BR, VBBR
15	12.282	2"-O-acetyl-SSa	$C_{44}H_{70}O_{14}$	822.4766	822.4741	821.4687	7.18	821.4746[M−H]$^-$ 779.4615[M−H−C_2H_2O]$^-$ 761.4412[M−H−C_2H_2O−H_2O]$^-$ 617.3954[M−H−C_2H_2O−Glc]$^-$	BR, VBBR
16	12.626	SSb$_1$	$C_{42}H_{68}O_{13}$	780.4660	780.4689	779.4582	–8.85	779.4513[M−H]− 617.4095[M–H–Glc]$^-$	BR, VBBR
17	12.737	3"-O-acetyl-SSa	$C_{44}H_{70}O_{14}$	822.4766	822.4858	821.4687	7.55	821.4749[M−H]$^-$ 779.4491[M−H−C_2H_2O]$^-$ 761.3676[M−H−C_2H_2O−H_2O]$^-$ 617.3722[M−H−C_2H_2O−Glc]$^-$	BR, VBBR
18	12.963	2"-O-acetyl-SSb$_2$	$C_{44}H_{70}O_{14}$	822.4766	822.4757	821.4687	6.45	821.4740[M−H]$^-$ 779.4449[M−H−C_2H_2O]$^-$ 761.3449[M−H−C_2H_2O−H_2O]$^-$ 617.4070[M−H−C_2H_2O−Glc]$^-$	BR, VBBR
19	13.141	SSe	$C_{42}H_{68}O_{12}$	764.4711	764.4659	763.4633	0.79	763.4639[M−H]$^-$ 601.4020[M–H–Glc]$^-$	BR, VBBR
20	13.643	4"-O-acetyl-SSa	$C_{44}H_{70}O_{14}$	822.4766	822.4759	821.4687	6.57	821.4741[M−H]$^-$	BR, VBBR

								779.4526[M−H−C_2H_2O]$^-$ 761.4424[M−H−C_2H_2O−H_2O]$^-$ 617.3922[M−H−C_2H_2O−Glc]$^-$ 541.9836[M−H−Glc−76]$^-$	
21	14.324	Acetyl-SSm	$C_{44}H_{70}O_{13}$	806.4816	806.4758	805.4738	0	805.4738[M−H]$^-$ 763.4640[M−H−C_2H_2O]$^-$ 745.4531[M−H−C_2H_2O−H_2O]$^-$ 601.4105[M−H−C_2H_2O−Glc]$^-$	BR, VBBR
22	14.488	SSd	$C_{42}H_{68}O_{13}$	780.4660	780.4645	779.4582	–1.80	779.4568[M−H]$^-$ 617.3663[M–H–Glc]$^-$	BR, VBBR
23	14.893	2"-O-acetyl-SSd	$C_{44}H_{70}O_{14}$	822.4766	822.4752	821.4687	0.37	821.4690[M−H]$^-$ 779.4592[M−H−C_2H_2O]$^-$ 761.4507[M−H−C_2H_2O−H_2O]$^-$ 617.4232[M−H−C_2H_2O−Glc]$^-$	BR, VBBR
24	15.345	Diacetyl-SSb_2	$C_{46}H_{72}O_{15}$	864.4871	864.4859	863.4793	–7.53	863.4728[M−H]$^-$	BR, VBBR
25	15.384	3"-O-acetyl-SSd	$C_{44}H_{70}O_{14}$	822.4766	822.4752	821.4687	9.86	821.4768[M−H]$^-$ 779.4572[M−H−C_2H_2O]$^-$ 761.4506[M−H−C_2H_2O−H_2O]$^-$ 617.4125[M−H−C_2H_2O−Glc]$^-$	BR, VBBR
26	16.025	4"-O-acetyl-SSd	$C_{44}H_{70}O_{14}$	822.4766	822.4763	821.4687	4.87	821.4727[M−H]$^-$ 779.4723[M−H−C_2H_2O]$^-$ 761.4377[M−H−C_2H_2O−H_2O]$^-$ 617.4034[M−H−C_2H_2O−Glc]$^-$	BR, VBBR
27	16.297	6"-O-acetyl-SSd	$C_{44}H_{70}O_{14}$	822.4766	822.4768	821.4687	3.65	821.4717[M−H]$^-$ 779.4604[M−H−C_2H_2O]$^-$ 617.4078[M−H−C_2H_2O−Glc]$^-$	BR, VBBR

28	16.515	Diacetyl-SSd	$C_{46}H_{72}O_{15}$	864.4871	864.4841	863.4793	–6.14	863.4740$[M-H]^-$ 821.4637$[M-H-C_2H_2O]^-$ 803.4763$[M-H-C_2H_2O-H_2O]^-$ 761.3729$[M-H-2C_2H_2O-H_2O]^-$	BR, VBBR
29	16.849	Diacetyl-SSd	$C_{46}H_{72}O_{15}$	864.4871	864.4843	863.4793	–5.91	863.4742$[M-H]^-$ 821.4572$[M-H-C_2H_2O]^-$ 803.3924$[M-H-C_2H_2O-H_2O]^-$ 761.4001$[M-H-2C_2H_2O-H_2O]^-$	BR
30	17.310	Diacetyl-SSd	$C_{46}H_{72}O_{15}$	864.4871	864.4850	863.4793	5.79	863.4843$[M-H]^-$ 821.4383$[M-H-C_2H_2O]^-$	BR

Table S3(a). ^{1}H NMR data and assignments of the metabolites in hippocampus samples.

No.	Metabolites	Moieties	δ^{1}H/ppm and multiplicity
1	Lipids	CH_3, $(CH_2)_n$	0.90(m)
2	Leucine	δCH_3, αCH, αCH	0.98(d), 3.73(m), 1.70(m)
3	Isoleucine	δCH_3, γCH_3	1.01(d), 0.95(m)
4	Valine	$\gamma'CH_3$, γCH_3	0.99(d) ,1.05(d)
5	Lactate	βCH_3, αCH	1.33(d), 4.12(q)
6	Alanine	βCH_3,-CH-	1.48(d), 3.73(m)
7	Arginine	γCH_2	1.73(m)
8	Acetate	CH_3	1.92(s)
9	γ-Aminobutyric acid	CH_3, CH_2, -CH-	1.91(m),2.30(t), 3.02(m)
10	Glutamate	αCH, βCH_2, γCH_2	2.05(m), 2.36(m), 3.77(m)
11	Glutamine	CH_2	2.14(m)
12	Acetoacetate	$COCH_3$	2.29(s)
13	Succinate	αCH_2	2.36(s)
14	Citrate	Half CH_2, Half CH_2	2.50(d), 2.66(d)
15	Sarcosine	CH_3, CH_2	2.70(s), 3.60(s)
16	Dimethylglycine	CH_3	3.01(s)
17	2-Oxoglutarate	CH_2	3.03(m), 2.42(m)
18	Creatine	N-CH_3, N-CH_2-	3.02(s), 3.94(s)
19	Creatinine	N-CH_3, N-CH_2-	3.04(s), 4.07(s)
20	Choline	$N(CH_3)_3$, OCH_2	3.21(s), 4.05(m)
21	Phosphatidylcholine	$N(CH_3)_3$	3.22(s)
22	Glycerophosphocholine	$N(CH_3)_3$	3.23(s)
23	Taurine	S-CH_2, N-CH_2	3.27(t), 3.43(t)
24	TMAO	CH_3	3.27(s)
25	Betaine	CH_2, CH_3	3.27(s), 3.89(s)
26	Threonine	γCH_2	4.27(m), 3.58(d)
27	Myo-inositol	CH_2, CH	3.29(t), 3.54(dd), 3.62(m)
28	Aspartate	βCH_2, αCH	2.68(dd), 2.82(dd)
29	Glycine	CH_2	3.55(s)
30	Glycerol	CH_2, CH	3.57(m), 3.79(m)
31	β-glucose	1-CH	4.65(d)
32	Fumarate	CH=	6.52(s)
33	Tyrosine	1-CH	6.90(d), 7.20(d)
34	Histidine	5-CH, 2-CH	7.11(s), 7.90(s)
35	Phenylalanine	2,6-CH, 3,5-CH, 4-CH	7.33(d), 7.38(t), 7.43(t)
36	Uracil	α-CH, β-CH	7.54(d), 5.81(d)
37	Xanthurenate	-CH=N	7.90(s)

[a] Multiplicity for ^{1}H resonances: s, single; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets.

Table S3 (b). ^{1}H NMR data and assignments of the metabolites in liver samples.

No.	Metabolites	Moieties	δ^{1}H/ppm and multiplicity
1	Lipids	CH_3, $(CH_2)_n$	0.90(m)
2	Leucine	δCH_3, αCH, αCH	0.98(d), 3.73(m), 1.70(m)
3	Isoleucine	δCH_3, γCH_3	1.02(d), 0.95(m)
4	Valine	$\gamma'CH_3$, γCH_3	0.99(d), 1.05(d)
5	β-hydroxybutyrate	CH_3, CH_2, -CH-	1.21(d), 2.41(d), 2.31(d)
6	Lactate	βCH_3, αCH	1.33(d), 4.12(q)
7	Alanine	βCH_3, -CH-	1.49(d), 3.73(m)
8	Arginine	γCH_2	1.73(m)
9	Acetate	CH_3	1.93(s)
10	Glutamate	αCH, βCH_2, γCH_2	2.07(m), 2.36(m), 3.73(m)
11	Glutathione	CH_2, CH_2	2.12(m), 2.56(m)
12	Methionine	S-CH_3	2.14(s)
13	Succinate	αCH_2	2.37(s)
14	Pyruvate	CH_3	2.41(s)
15	Dimethylamine	CH_3	2.73(s)
16	Dimethylglycine	CH_3	3.01(s)
17	Creatine	N-CH_3, N-CH_2-	3.02(s), 3.94(s)
18	2-Oxoglutarate	CH_2	3.03(m), 2.43(m)
19	O-Acetylcarnitine	NCH_3	3.19(s)
20	Choline	$N(CH_3)_3$, OCH_2	3.21(s), 4.05(m)
21	Glycerophosphocholine	$N(CH_3)_3$	3.23(s)
22	phosphoethanolamine	NCH_3, CH_2	3.23(t), 3.89(m)
23	Taurine	S-CH_2, N-CH_2	3.27(t), 3.41(t)
24	TMAO	CH_3	3.27(s)
25	Betaine	CH_2, CH_3	3.27(s), 3.89(s)
26	Scyllo-inositol	CH_3	3.37(s)
27	Aspartate	βCH_2, αCH	2.70(dd), 2.82(dd)
28	Glycine	CH_2	3.55(s)
29	Glycerol	CH_2, CH	3.58(m), 3.78(m)
30	Oxidized glutathione	CH_2, CH_3	3.79(t), 2.57(m)
31	β-glucose	1-CH	4.65(d)
32	α-glucose	1-CH	5.24(d)
33	Tyrosine	CH	6.90(d), 7.20(d)
34	Histidine	βCH_2, CH_2	7.11(s), 3.15(dd)
35	Phenylalanine	2,6-CH, 3,5-CH,4-CH	7.34(d), 7.38(m), 7.44(m)
36	Phenylacetylglycine	-CH=, CH=, -CH_2, -CH	7.42(s) , 7.39(m), 3.68(s), 3.77(d)
37	Uracil	α-CH, β-CH	7.54(d), 5.81(d)
38	Xanthurenate	-CH=N	7.91(s)

[a] Multiplicity for ^{1}H resonances: s, single; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets.

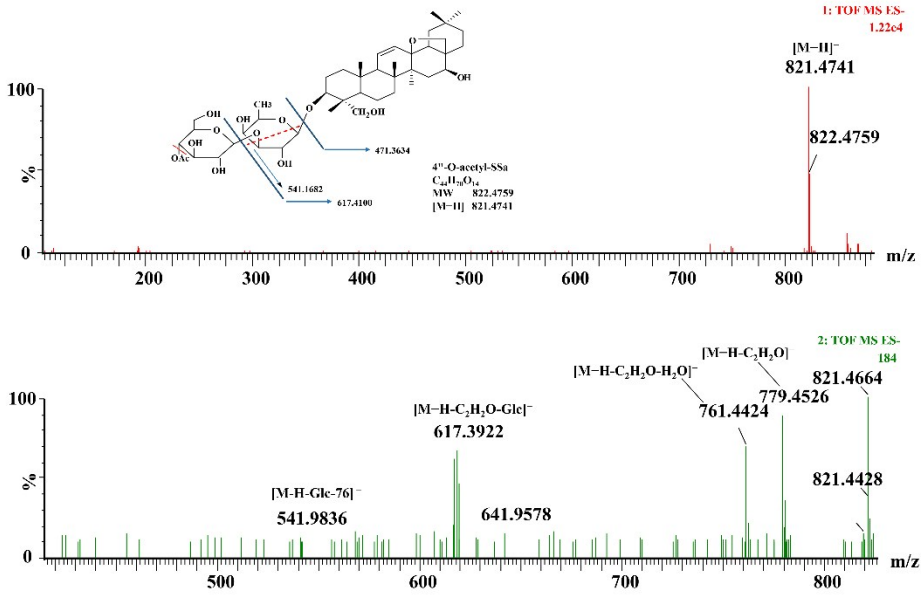

Fig. S1 ESI–QTOF-MS/MS spectra and possible fragment pathway of 4"-O-acetyl-SSa in negative mode, (A) MS of ion at *m/z* 821, (B) MS2 of ion at *m/z* 617.

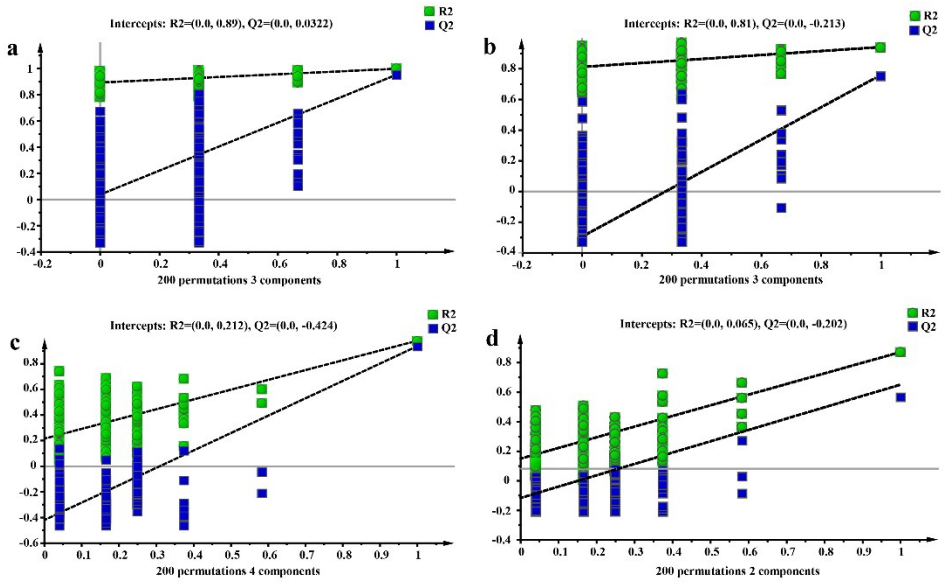

Fig. S2 PLS-DA model validation based on ^{1}H NMR data of hippocampus and liver. a, b represents data of hippocampus and liver for the CUMS group (MS) and the control group (NS); c, d represents data of hippocampus and liver for the CUMS group (MS), the control group (NS), Bupleuri Radix (BR), vinegar-baked Bupleuri Radix (VBBR) and FLU group (FLU).