

Jiannao pills for chronic restraint stress-induced anxiety

Supplementary Table 1. The mobile phase parameters in ultra-high performance liquid chromatography (UHPLC) experiments

Time (min)	Flow rate (mL/min)	Phase A: water (%)	Phase B: acetonitrile (%)
0	400	95	5
3.5	400	85	15
6	400	70	30
6.5	400	70	30
12	400	30	70
12.5	400	30	70
18	400	0	100
22	400	0	100
25	400	0	100
26	400	95	5
30	400	95	5

Supplementary Table 2. Analysis of the chemical compositions of Jiannao pills under the positive electrospray ionization mode

ID	NameEN	Formula	Mzmed	Rtmed
1	(1R,2E,7R,10E,12S,13S,15R)-12,15-dihydroxy-7-methyl-8-oxabicyclo[11.3.0]hexadeca-2,10-dien-9-one	C ₁₆ H ₂₄ O ₄	319.1304	755.149
2	(1R,2R,5S,8R,14R,15R,16S)-16-hydroxy-1,2,14,17,17-pentamethyl-8-(prop-1-en-2-yl)pentacyclo[11.7.0.0 ^{2,10} .0 ^{5,9} .0 ^{14,18}]icosane-5,15-dicarboxylic acid	C ₃₀ H ₄₆ O ₅	504.3676	903.9535
3	(1r,3R,4s,5S)-4-(((2E)-3-(3,4-Dihydroxyphenyl)-2-propenoyl)oxy)-1,3,5-trihydroxycyclohexanecarboxylic acid	C ₁₆ H ₁₈ O ₉	377.0848	186.528
4	(2S,3R,4R,5R,6S)-2-((2R,3R,4S,5R,6R)-2-[2-(3,4-dihydroxyphenyl)ethoxy]-3,5-dihydroxy-6-(hydroxymethyl)oxan-4-yl]oxy-6-methyloxane-3,4,5-triol	C ₂₀ H ₃₀ O ₁₂	485.162	144.59
5	(2Z)-6-hydroxy-2-((4-hydroxy-3-methoxyphenyl)methylidene)-1-benzofuran-3-one	C ₁₆ H ₁₂ O ₅	285.0753	1791.16
6	(S)-[10]-Gingerol	C ₂₁ H ₃₄ O ₄	351.2523	619.37
7	1-Hydroxyepiacorone	C ₁₅ H ₂₄ O ₃	253.1797	373.999
8	2-(2-oxo-8,9-dihydrofuro[2,3-h]chromen-8-yl)propan-2-yl acetate	C ₁₆ H ₁₆ O ₅	306.1327	397.468
9	2-(Methoxycarbonyl)-5-methyl-2,4-bis(3-methyl-2-butenyl)-6-(2-methyl-1-oxopropyl)-5-(4-methyl-3-pentenyl)cyclohexanone	C ₂₉ H ₄₆ O ₄	459.3472	630.22
10	2-Acetyl-5-methylpyridine	C ₈ H ₉ NO	136.0757	274.106
11	2-Aminoacetophenone	C ₈ H ₉ NO	136.0754	52.5511
12	2-Furoic acid	C ₅ H ₄ O ₃	113.0233	57.8203
13	2-Oxovaleric acid	C ₅ H ₈ O ₃	117.0544	44.4966
14	2,8-Dihydroxy-5,5,8-trimethyl-11-oxatetracyclo[7.3.1.0~1,9~.0~3,7~]tridecan-10-one	C ₁₅ H ₂₂ O ₄	289.1392	44.182
15	3-Feruloyl-1,5-quinolactone	C ₁₇ H ₁₈ O ₈	351.108	493.505
16	3-Hydroxyurs-12-en-23-oic acid	C ₃₀ H ₄₈ O ₃	479.3507	1019.905
17	4-[4-(3,4-dihydroxyphenyl)-2,3-dimethylbutyl]benzene-1,2-diol	C ₁₈ H ₂₂ O ₄	325.1415	601.041

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18	4-Methylumbelliferone	$C_{10}H_8O_3$	194.0808	58.8171
19	4,4,8,10,14-pentamethyl-17-(4,5,6-trihydroxy-6-methylheptan-2-yl)-2,5,6,7,9,15-hexahydro-1H-cyclopenta[a]phenanthrene-3,16-dione	$C_{30}H_{46}O_5$	469.3321	894.073
20	5-O-Methylvisammioside	$C_{22}H_{28}O_{10}$	453.1754	618.911
21	7-methoxy-4-methylcoumarin	$C_{11}H_{10}O_3$	191.0704	280.182
22	Acetophenone	C_8H_8O	121.0648	275.703
23	Adenine	$C_5H_5N_5$	136.0616	43.8439
24	Alpha-Methoxy-1H-indole-3-propanoic acid	$C_{12}H_{13}NO_3$	220.0965	219.866
25	Apigenin	$C_{15}H_{10}O_5$	271.0601	863.069
26	Arecaidine	$C_7H_{11}NO_2$	142.0861	86.5438
27	Armillarilin	$C_{24}H_{30}O_7$	431.2068	694.335
28	Benzaldehyde	C_7H_6O	107.049	58.8171
29	CHOLIC ACID	$C_{24}H_{40}O_5$	447.2518	914.806
30	Colubrinic acid	$C_{30}H_{46}O_4$	453.3361	583.902
31	Curcumenol	$C_{15}H_{22}O_2$	257.1515	584.6185
32	dehydrocostus lactone	$C_{15}H_{18}O_2$	248.1646	402.903
33	Deoxyvasicinone	$C_{11}H_{10}N_2O$	187.0864	289.183
34	Dulciol C	$C_{28}H_{34}O_7$	483.238	710.8175
35	Emodin	$C_{15}H_{10}O_5$	271.0601	497.016
36	Ent-16a-Hydroxy-17-acetoxy-19-kauranal	$C_{22}H_{34}O_4$	363.2511	783.445
37	Eudesmin	$C_{22}H_{26}O_6$	409.1627	783.2875
38	Furocoumarinic acid glucoside	$C_{17}H_{18}O_9$	367.1018	420.923
39	Gamma-L-Glutamyl-L-pipecolic acid	$C_{11}H_{18}N_2O_5$	259.1302	512.518
40	Ganoderic acid Y	$C_{30}H_{46}O_3$	455.3524	949.123
41	Ganoderol A	$C_{30}H_{46}O_2$	439.3568	890.32
42	Ginkgolic acid (C13:0)	$C_{20}H_{32}O_3$	321.24	865.137
43	INDOLE-3-ETHANOL	$C_{10}H_{11}NO$	162.0913	172.112
44	Indoleacetic acid	$C_{10}H_9NO_2$	176.0703	302.03
45	Kaempferol	$C_{15}H_{10}O_6$	287.0554	447.863
46	Kaempferol-3-O-beta-glucopyranosyl-7-O-alpha-rhamnopyranoside	$C_{27}H_{30}O_{15}$	595.1657	363.62
47	KOBUSONE	$C_{14}H_{22}O_2$	245.1509	648.209
48	L-Gulose	$C_6H_{12}O_6$	203.0523	39.79025
49	L-Tyrosine	$C_9H_{11}NO_3$	182.0808	51.9955
50	Laurelliptine	$C_{18}H_{19}NO_4$	314.138	222.094
51	Lycorine	$C_{16}H_{17}NO_4$	288.1224	148.599
52	Mannitol	$C_6H_{14}O_6$	205.0684	34.5347
53	monolinolein	$C_{21}H_{38}O_4$	393.2397	889.803
54	N-(p-Hydroxyphenyl)ethyl p-hydroxycinnamide	$C_{17}H_{17}NO_3$	284.1286	207.267
55	N-Feruloylglycyl-L-phenylalanine	$C_{21}H_{22}N_2O_6$	399.1527	409.486
56	Neobavaisoflavone	$C_{20}H_{18}O_4$	323.1257	618.605
57	Nicotinic acid	$C_6H_5NO_2$	124.0393	101.329
58	Norharman	$C_{11}H_8N_2$	169.0758	256.746
59	Norisoboldine	$C_{18}H_{19}NO_4$	314.1363	500.748
60	Phytolaccinic acid	$C_{31}H_{48}O_6$	517.3511	1026.33

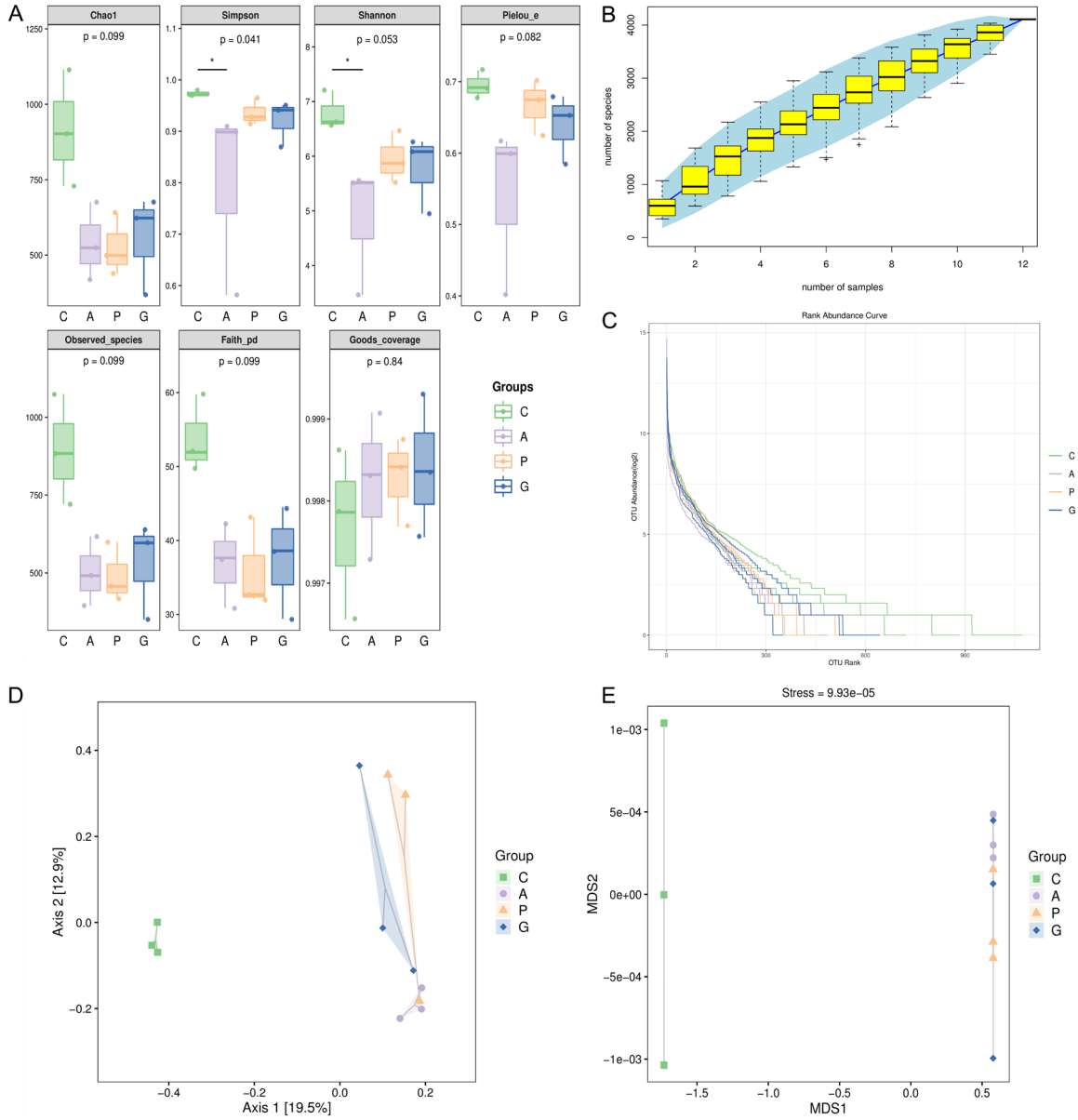
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61	Pyrrole-2-carboxylic acid; LC-tDDA; CE10	$C_5H_5NO_2$	112.0393	189.265
62	Rhaponticin	$C_{21}H_{24}O_9$	421.1467	514.613
63	Seneciphylline	$C_{18}H_{23}NO_5$	334.1652	147.91
64	Tazettine	$C_{18}H_{21}NO_5$	332.1489	135.102
65	Tulipinolide	$C_{17}H_{22}O_4$	291.157	380.942
66	Venoterpine	$C_9H_{11}N_0$	150.0912	60.4652
67	Wogonin	$C_{16}H_{12}O_5$	285.0753	1661.37
68	Zoapatanol	$C_{20}H_{34}O_4$	339.2514	737.5955

Supplementary Table 3. Analysis of the chemical compositions of Jiannao pills under the negative electrospray ionization mode

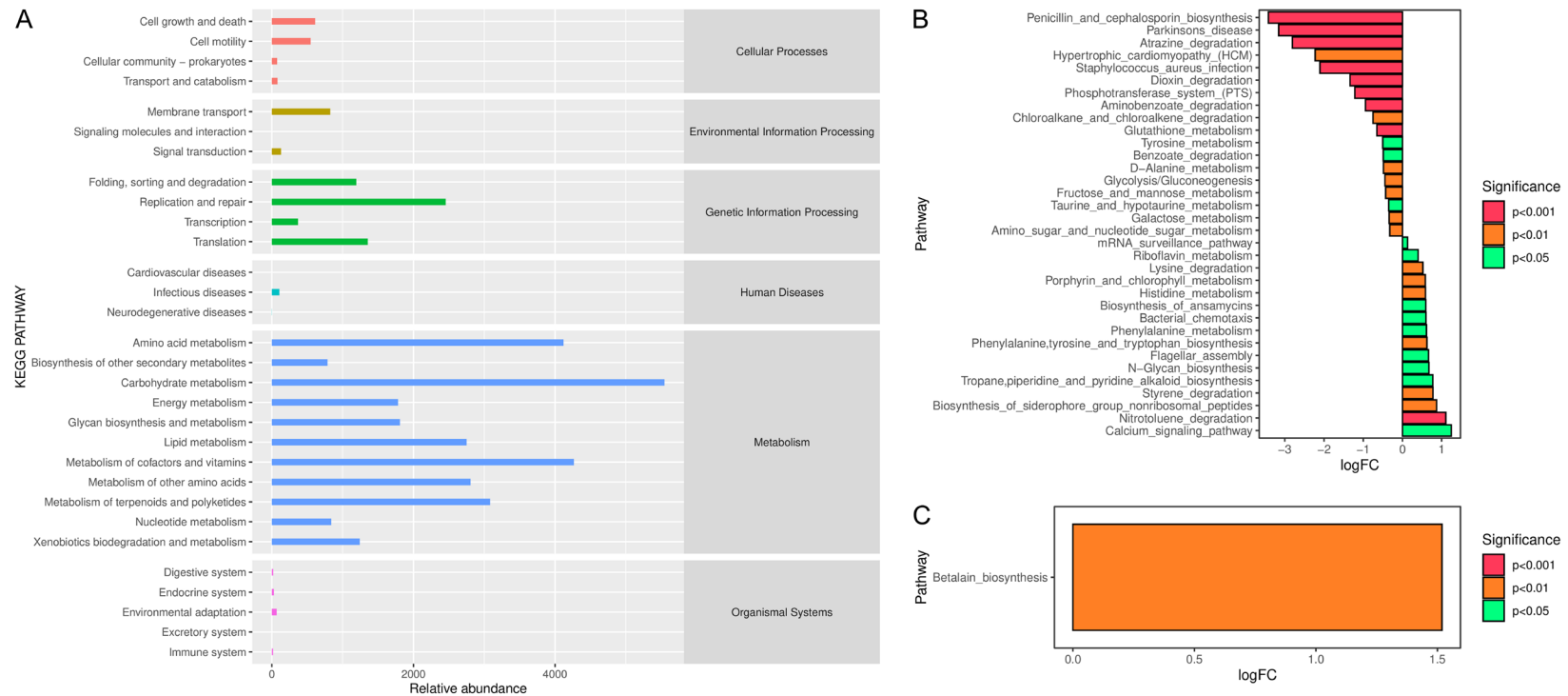
ID	NameEN	Formula	Mzmed	Rtmed
1	2-Acetylpyrrole	C_6H_7NO	108.0455	108.136
2	3,4-di-O-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1196	370.547
3	3,4,5-trimethoxycinnamic acid	$C_{12}H_{14}O_5$	237.0763	432.601
4	Asiatic acid	$C_{30}H_{48}O_5$	487.3415	1024.52
5	Baicalein	$C_{15}H_{10}O_5$	269.0448	496.536
6	Dehydrotumulosic acid	$C_{31}H_{48}O_4$	483.3465	990.458
7	Genistein	$C_{15}H_{10}O_5$	269.0448	539.794
8	Isokobusone	$C_{14}H_{22}O_2$	221.1546	768.113
9	Oleanoic Acid	$C_{30}H_{48}O_3$	455.3536	1356.22
10	Oleic acid	$C_{18}H_{34}O_2$	281.2482	1025.49
11	p-Aminobenzoic acid	$C_7H_7NO_2$	136.0402	166.205
12	p-coumaric acid methyl ester geometric isomer (tentative, MSe)	$C_9H_8O_3$	163.0402	279.681
13	p-Hydroxybenzaldehyde	$C_7H_6O_2$	121.0295	221.131
14	Parishin E	$C_{19}H_{24}O_{13}$	459.1145	170.166
15	Pelargonidin-3-O-glucoside	$C_{21}H_{21}O_{10}$	431.0984	384.642
16	Podocarpusflavone A	$C_{31}H_{20}O_{10}$	551.0969	649.425
17	Pyrocatechol	$C_6H_6O_2$	109.0295	94.5063
18	Sibiricose A5	$C_{22}H_{30}O_{14}$	517.1567	206.918

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Supplementary Figure 1. Analysis of the diversity of intestinal microflora species of mice in each group. A. Group box diagram of alpha diversity index; B. Specaccum species accumulation curve; C. The curve of the abundance grade; D. The two-dimensional diagram of the principal coordinate analysis (PCoA); E. The two-dimensional diagram of the nonmetric multidimensional scaling (NMDS) analysis. C, Control group; A, Anxiety model group; G, Jiannao pills treatment group; P, Positive control group. * $P < 0.05$ when compared to group C.

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Supplementary Figure 2. Intestinal microflora function analysis of mice in each group. A. The diagram of the abundance of the secondary functional pathways by Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis; B. The difference of KEGG metabolic pathway between group C and group A; C. The difference in KEGG metabolic pathway between group A and group G. C, Control group; A, Anxiety model group; G, Jiannao pills treatment group; P, Positive control group.