Supplementary information

Title: Antidepressant-like effects of dietary gardenia blue pigment derived from genipin and tyrosine

Methods

Materials

Genipin (>98%) was purchased from Linchuan Zhixin Biotechnology Ltd. (Jiangxi, China). Tyrosine and Lipopolysaccharides from Escherichia coli 055: B5 was purchased from Sigma-Aldrich Co. (USA).

Characterization of the GTD

FT-IR spectra were recorded on an infrared spectrometer (Nicolet iS10, Thermo Fisher Scientific, USA) with the wave number range 4000-400 cm^{-1} using KBr pellets. Appropriate amounts of GP, tyrosine and GTD were mixed with potassium bromide powder and ground into powders, and the disks were compressed for scanning.

Q-TOF-MS was determined by the Agilent 6500 Quadrupole - Time of Flight Mass Spectrometry (Agilent Technologies, US). GTD powder (10 mg) was weighed and dissolved in 0.5 mL of methanol. Then, it was filtered through a 0.22 μm pore size filter, and the filtrate was taken as a test solution. Electrospray ionization ion source (ESI) was used to acquire data in positive and positive ion modes. High-purity nitrogen is used as the atomizing gas and the auxiliary gas, and argon gas is used as the collision gas. In positive ion mode, the drying temperature is 350 °C, the drying gas flow rate is 10.0 $L \cdot min^{-1}$, the atomizing gas pressure is 30 psi, the ion transport voltage is 175 V, the capillary voltage is 4000 V, and the mass scan range is m/z 100 to 1700. In positive ion mode, the capillary voltage is 4000 V. Other parameters are consistent with the positive ion mode scan.

GTD powder (20 mg) was taken in a sterile sample tube, 0.5 mL of deuterated DMSO was added, shaken and fully dissolved. ^{1}H NMR and ^{13}C NMR were measured by the Bruker Avance DRX500 analyzer (Bruker, Switzerland). Therefore, we preliminarily determined the possible structure of the GTD by FTIR, Q-TOF-MS, ^{1}H

NMR and ^{13}C NMR data.

Weight changes and 1% sucrose water preference of CUMS rats

The body weights of rats were weighed on the 7th, 14th, 21st, 28th, 35th, and 42nd days in the experiment, respectively, to observe the changes of body weight. After that, the 1% sucrose water preference of each group was measured before the experiment and on the 7th, 14th, 21st, 28th, 35th and 42nd days. Concretely, two water bottles containing 200 ml of 1% sucrose water and 200 ml of pure water were simultaneously administered after 24 hours of water ban. Each rat was free to drink water. The percentage of 1% sucrose water per rat was calculated by measuring the consumption of water within 1 h.

Results

Chemical structure prediction of Genipin-Tyrosine Deriant (GTD).

On the FTIR spectrum (**Figure S1**), genipin has strong absorption peaks at 3398, 3237, 1682, 1622, 1443 and 1300 cm^{-1}. The characteristic absorption peaks of L-tyrosine are 3205, 2599-3127 (wide peak of amino acid carboxyl group), 1590 (benzene ring C=C), 1330, 1042 and 841 cm^{-1} (benzene ring pair disubstituted). In contrast to the genipin the major peaks of the GTD on the FTIR spectrum were altered. The absorption at 3398 cm^{-1} shifted to 3424 cm^{-1}, and 1443 cm^{-1} shifted to 1439 cm^{-1}. Similarly, the absorption of the amino acid benzene ring C = C moved from 1590 cm^{-1} to 1569 cm^{-1}, 1042 cm^{-1} to 1133 cm^{-1}. GTD was strongly absorbed at 700 cm^{-1}, which was the characteristic absorption of cis-C = C double bond. Since an absorption peak at 700 cm^{-1} was also observed, we then inferred that there is a C = C double bond on the ortho position of the nitrogen atom in the products' structure.

To obtain the mass and molecular formula, the product was then analyzed by Q-TOF-MS. As shown in **Figure S2**, the molecular weight of GTD was 384.1474, and the molecular formula was $C_{20}H_{19}NO_6$.

The chemical shifts (δ) for ^1H NMR spectrum (500 MHz, DMSO) were shown in in **Figure S3**:7.72 (1H, m, H-1), 7.67 (1H, m, H-3), 6.90 (1H, d, 6.8Hz, H-7), 6.85 (1H, d, 6.8Hz, H-6), 7.01 (2H, d, 8.3Hz, H-2', 6'), 6.55 (2H, d, 8.3Hz, H-3', 5'), 4.23 (2H,

t,6.8Hz, H-10), 3.92 (3H, s, H-12) , 3.92 (1H, m, H-8'), 3.42 (1H, m, H-7'), 2.90 (1H, m, H-7'). The ^{13}C NMR spectrum contained signals for two groups of 21 carbons. The chemical shifts (δ) for ^{13}C NMR spectrum (125 MHz, DMSO) were shown in **Figure S4**: 131.5 (C-1), 131.4 (C-3), 115.1 (C-4), 129.9 (C-5), 104.8 (C-6), 129.4 (C-7), 131.4 (C-8), 120.9 (C-9), 61.8 (C-10), 166.8 (C-11), 52.6 (C-12), 129.8 (C-1'), 129.5 (C-2', 6'), 115.3 (C-3',5'), 155.3 (C-4'), 37.9 (C-7'), 78.2 (C-8'), 166.8 (C-9').

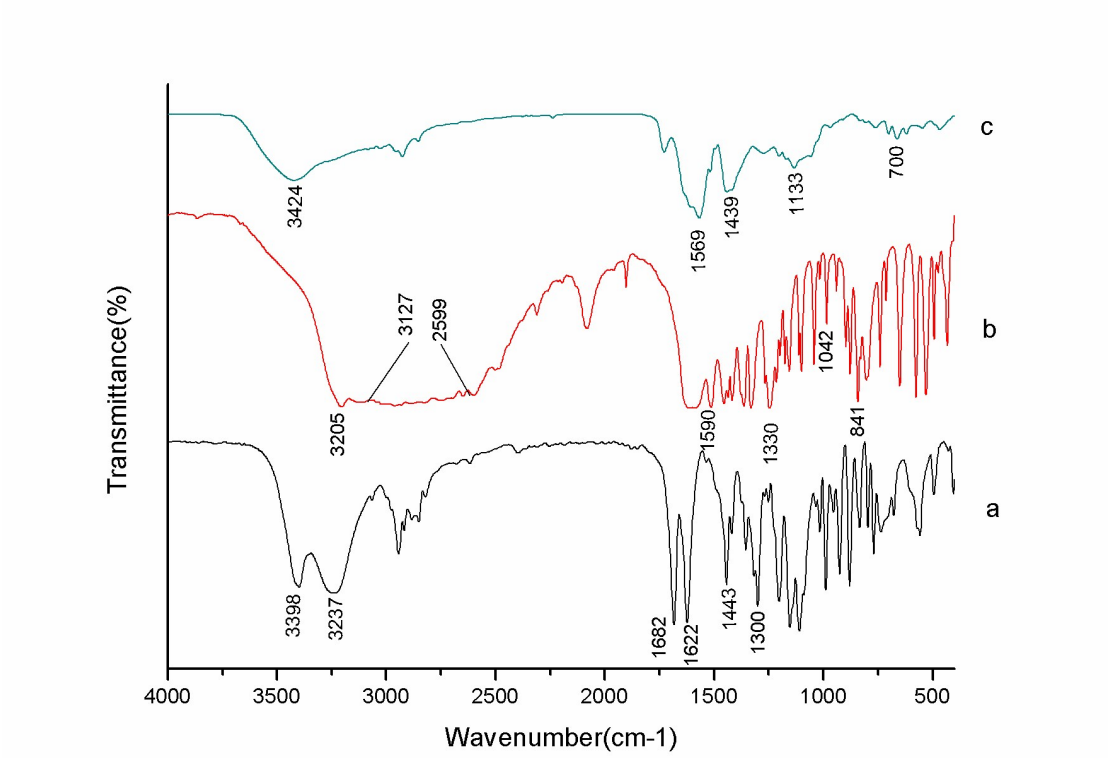

Figure S1 FTIR spectrum of genipin, L-Tyrosine and genipin-tyrosine derviwant. (a) genipin; (b) L-Tyrosine; (c) genipin-tyrosine dervirant.

Figure S2 Q-TOF-MS for genipin-tyrosine dervirant

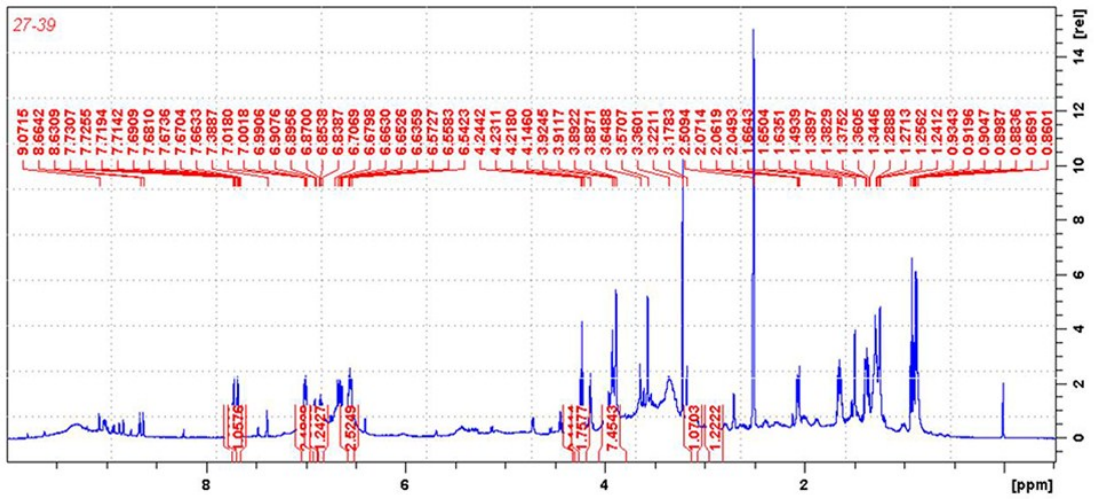

Figure S3 ^{1}H NMR spectrum for GTD

GTD was taken in a sterile sample tube, 0.5 ml of deuterated DMSO was added, and ^{1}H NMR were measured.

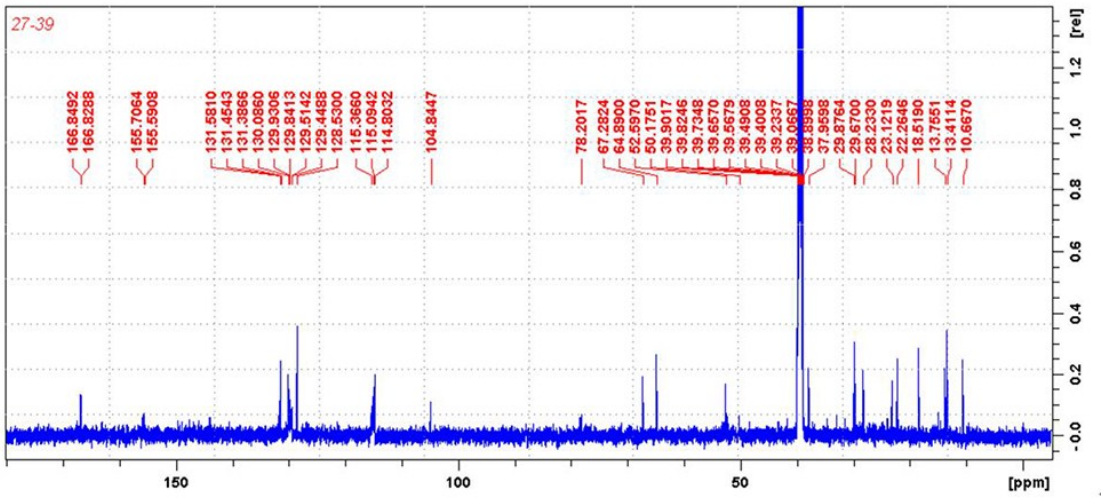

Figure S4 ^{13}C NMR spectrum for GTD.

GTD was taken in a sterile sample tube, 0.5 ml of deuterated DMSO was added, and ^{13}C NMR were measured.

GTD improved 1% sucrose water preference in CUMS rats

The 1% sucrose water preference of rats was shown in **Table S1**. There was no significant difference in 1% sucrose preference between the groups before the experiment. After the chronic stress stimulation, the 1% sucrose water preference of

stressed rats was significantly lower (p<0.05 or p<0.01) compared control group between 14th to 28th days. After 14 days of continuous administration, the rats 1% sucrose preference in each administration groups was significantly increased compared with the model group(p<0.05 or p<0.01).

Table S1 1% sucrose preference of CUMS rats ($\bar{X} \pm SE$, n = 10)

group	0 day	7 days	14 days	21days	28 days	35 days	42 days
Control	73.3±3.1	76.4±1.3	80.8±0.9	82.8±2.3	84.6±0.9	85.4±3.8	86.5±1.1
CUMS	72.0±3.7	69.1±4.3	65.1±3.6**	62.9±1.0**	60.8±4.9**	57.8±9.8*	56.2±3.0**
Fluoxetine 7.5mg/kg	74.2±2.2	71.6±2.3	69.9±2.3**	67.2±2.2**	64.9±3.9**	67.6±2.1$^{\#}$	72.5±6.3
Genipin 50mg/kg	72.7±2.8	69.4±2.5*	67.6±1.9**	64.3±1.5**	62.2±2.9**	63.4±0.5$^{\#}$	71.1±0.8**
GTD 25mg/kg	70.6±6.2	68.6±1.7**	66.2±3.7**	61.6±0.6**	57.4±2.1**	64.9±1.0$^{\#}$	69.7±0.4$^{\#\#}$
GTD 50mg/kg	73.2±1.9	70.7±2.8	69.5±0.7**	64.9 ± 2.4**	63.1±3.4**	66.7±4.2$^{\#}$	69.7±2.8$^{\#\#}$
GTD 100mg/kg	74.5±1.2	71.9±2.0	70.2±1.4**	67.8±2.0**	65.7±2.7**	68.2±2.9$^{\#}$	71.4±3.4$^{\#}$

Each group randomly received stress stimulations except for control group. Rats were subjected to 1% sucrose preference test every week. After stress stimulation of 28 days, 2-weeks treatment were administered to rats with fluoxetine (7.5 mg/kg), genipin (50 mg/kg) or GTD (25, 50, 100 mg/kg). * $p < 0.05$, ** $p < 0.01$ compared vs. control; $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$compared vs. CUMS.

GTD improved stress rat weight gain

The weight of stress groups after 14 days stress was decreased significantly compared with control group (**Table S2)** ($p < 0.05$ or $p< 0.01$). On the 35th and 42nd days, the GTD groups (25 mg/kg, 50 mg/kg and 100 mg/kg) and the fluoxetine group had significantly increased stress rats body weight ($p < 0.05$ or $p < 0.01$).

Table S2 Changes in body weight of CUMS rats ($\bar{X}\pm$SE, n = 10)

group	0 day	7 days	14 days	21days	28 days	35 days	42 days
Control	162.7±23.9	185.3±14.4	201.1±12.1	215.7±9,2	227.6±15.0	238.0±16.7	244.6±14.0
CUMS	165.9±2.1	172.1±2.7	177.4±4.1	183.7±6.5*	187±9.8**	191.8±6.6**	195.5±6.3**
Fluoxetine 7.5mg/kg	166.8±3.1	174.4±3.7	179.3±4.7	183.9±10.7	186.4±9.2	205.4±10.9	218.5±10.2$^{\#\#}$
Genipin 50mg/kg	165.2±3.6	174.6±6.6	178.0±5.2	181.9±5.0**	186.9±9.1**	189.4±9.5**	190.7±9.2**
GTD 25mg/kg	168.6±2.3	175±5.5	181.0±3.4	184.8±7.6*	188.5±8.8**	205.7±9.3*	224±12.9$^{\#}$
GTD 50mg/kg	166.3±3.5	174.9±6.2	180.2±7.8	184.9±8.3*	188.5±7.5**	205.6±8.6*$^{\#}$	220.8±11$^{\#\#}$
GTD 100mg/kg	168.6±3.4	173±5.2	178±6.6	182.3±4.8**	188.3±5.2**	203.5±5.6*	213.6±7.6$^{\#}$

Each group randomly received stress stimulations except for control group. Rats were weighed every week. After stress stimulation of 28 days, 2-weeks treatment were administered to rats with fluoxetine (7.5 mg/kg), genipin (50 mg/kg) or GTD (25, 50, 100 mg/kg). * $p < 0.05$, ** $p < 0.01$ compared vs. control; $^{\#}$ $p < 0.05$, $^{\#\#}$ $p <$ 0.01compared vs. CUMS.

Reference

1. Fujikawa S, Fukui Y, Koga K, et al. Structure of genipocyanin G1, a spontaneous reaction product between genipin and glycine[J]. *Tetrahedron Letters*, 1987, 28(40): 4699-4700.
2. Qi, X., Yao, N., Ren, Y., and Yuan-Lu, C. Study on Preparation Technology of Genipin-Tyrosine Derivative of Bio-Transformation Product in Zhi-zi-chi Soup, *Chinese patent medicine,* 2015, 37, 413-416.