

Supporting Information

Hypoglycemic Effects of *Gynura Divaricata* (L.) DC Polysaccharide and Action Mechanisms via Modulation of Gut Microbiota in Diabetic Mice

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Table S1**Table S1.** Sequence of qPCR primers.

Gene	Genetic sequence
GAPDH	F: GAGTCAACGGATTTGGTCGT
	R: GACAAGCTTCCCGTTCTCAG
PEPCK	F: GGATGATATTGCTTGGATGAG
	R: GAAGATGGTTGGAGGTTGT
G6Pase	F: TGGAGGAAGGAATGAATGTT
	R: CGGAAGTGTTGCTGTAGT

Table S2. Specific feed formula for the high sugar and high fat diet (Xietong Pharmaceutical Bioengineering Co., LTD, Nanjing, China)

Ingredient	gm
Casein	200
L-Cystine	3
Corn Starch	0
Maltodextrin	125
Sucrose	72.8
Cellulose	50
Soybean Oil	25
Lard	245
Mineral Mix S10026B	50
Vitamin Mix V10001C	1
Choline Bitartrate	2
FD&C Blue Dye	0.05

Table S3 Sequence of qPCR primers in T2DM mice.

Gene	Genetic sequence
GAPDH	GAGTCAACGGATTTGGTCGT GACAAGCTTCCCGTTCTCAG
AMPK	CGAGTGTTCCGAGGAGGAGGTC GAGGTGGTAGGCGACGGCTAG GGGAAGCGAGACGGCACTTTC
PI3K	TACCACTACGGAGCAGGCATAGC
AKT	CCATGTGGAAACGCCTGAGGAG TGGGTGAGCCTGATCGGAAGTC
PEPCK	GCAGTGAGGAAGTTCGTGGAAGG GTCAGTGAGAGCCAGCCAACAG
G6Pase	GCAGTGAGGAAGTTCGTGGAAGG GTCAGTGAGAGCCAGCCAACAG
GLUT4	AGCCAGCCTACGCCACCATAG CAGCAGAGCCACGGTCATCAAG
IRS2	CGGACGCCAAGCACAAGTACC TCCTGCTCCTGCTCGTTCTCC
InsR	ATCCGCCGCTCCTATGCTCTG GTTGCCTCAGGTTCTGGTTGTCC
GS	CGGGTGTGCGAAGACTTTGGG TGGTGCCTCTTGCTCAGTTTGTC
GSK-3 β	CTCGGTACTACAGGGCACCAGAG CCACCAACTGATCCACACCACTG
Foxo1	GTACGCCGACCTCATCACCAAG GCACGCTCTTCACCATCCACTC

Figure S1.

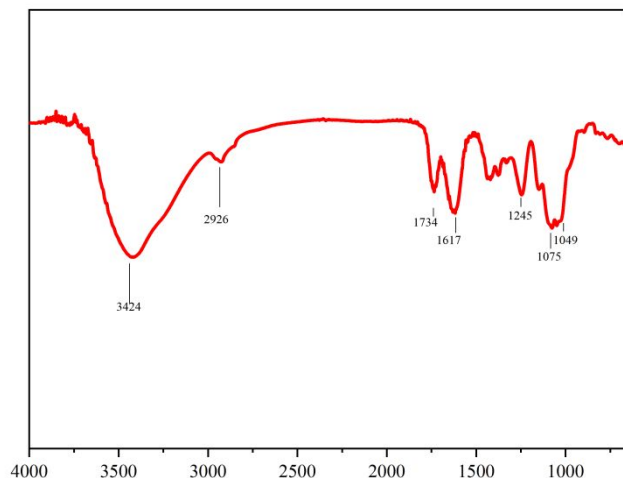


Figure S1. FT-IR spectrum of GDP.

In the IR spectrum of GDP (Figure S1), the broad band at 3424 cm⁻¹ and the absorption peak at 2926 cm⁻¹ are attributed to the stretching vibration of O–H and saturated C–H, respectively.¹ The peaks at 1734 cm⁻¹ 和 1617 cm⁻¹ are attributed to the stretching vibration of C=O in the methyl ester of the polysaccharide and the asymmetric stretching vibration of the free carboxyl anion (COO⁻), respectively,² indicating the presence of uronic acid in GDP; The weak absorption peak at 1245 cm⁻¹ is the asymmetric stretching vibration peak of S=O, indicating the presence of sulfate groups. The absorptions near 1075 cm⁻¹ and 1049 cm⁻¹ suggest the presence of pyranose rings in GDP.³

References:

- (1) Wan, C.; Qian, W. W.; Liu, W.; Pi, X.; Tang, M. T.; Wang, X. L.; Gu, Q.; Li, P.; Zhou, T. Exopolysaccharide from *Lactobacillus rhamnosus* ZFM231 alleviates DSS-induced colitis in mice by regulating gut microbiota. *J. Sci. Food Agri.* **2022**, *102* (15), 7087-7097.
- (2) Li, G.; Wang, F.; Wang, M. M.; Tang, M. T.; Zhou, T.; Gu, Q. Physicochemical, structural and rheological properties of pectin isolated from citrus canning processing water. *Int. J. Biol. Macromol.* **2022**, *195*, 12-21.
- (3) Zhao, H. K.; Wei, X. Y.; Xie, Y. M. Supercritical CO₂ extraction, structural analysis and bioactivity of polysaccharide from *Grifola frondosa*. *J. Food Compos. Anal.* **2021**, *102*, 104067.

Figure S2.

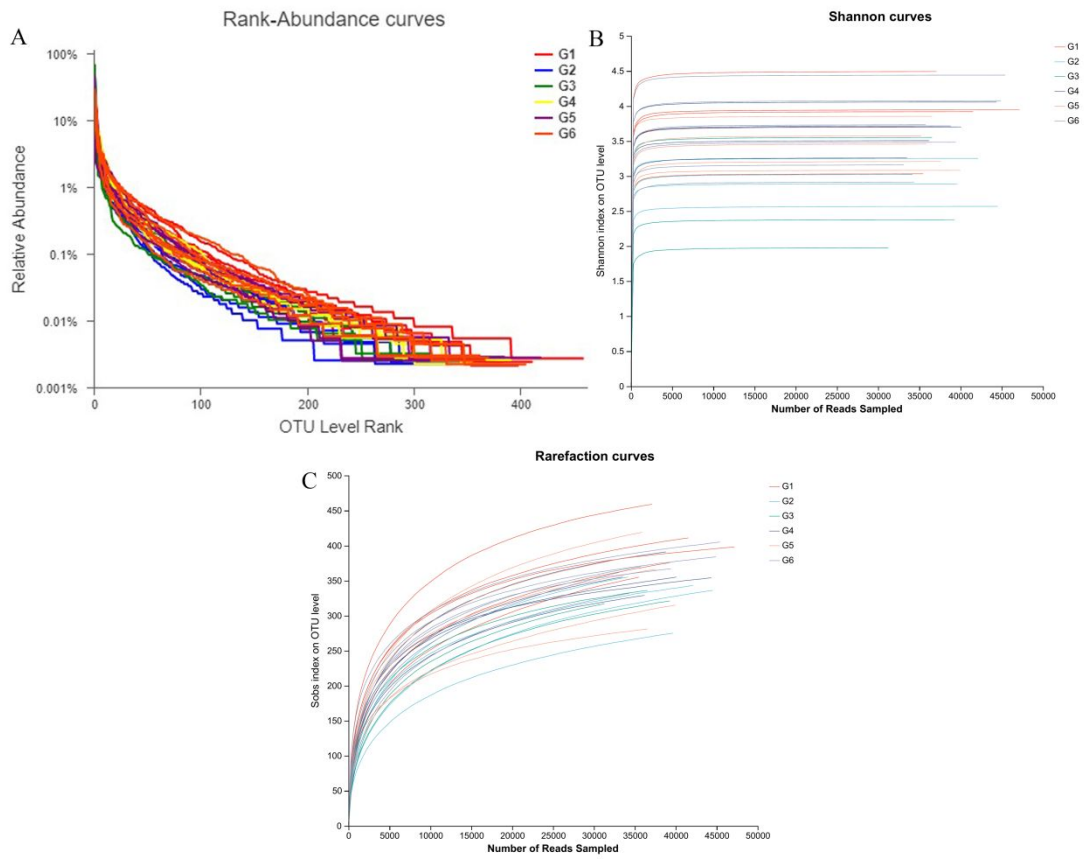


Figure S2. Rank-Abundance curve (A), Shannon Wiener rarefaction curve (B) and Sob rarefaction curve (C) of mice intestinal flora. (G1: NM group, G2: MD group, G3: GDPL group, G4: GDPM group, G5: GDPH group, G6: MET group)

Figure S3.

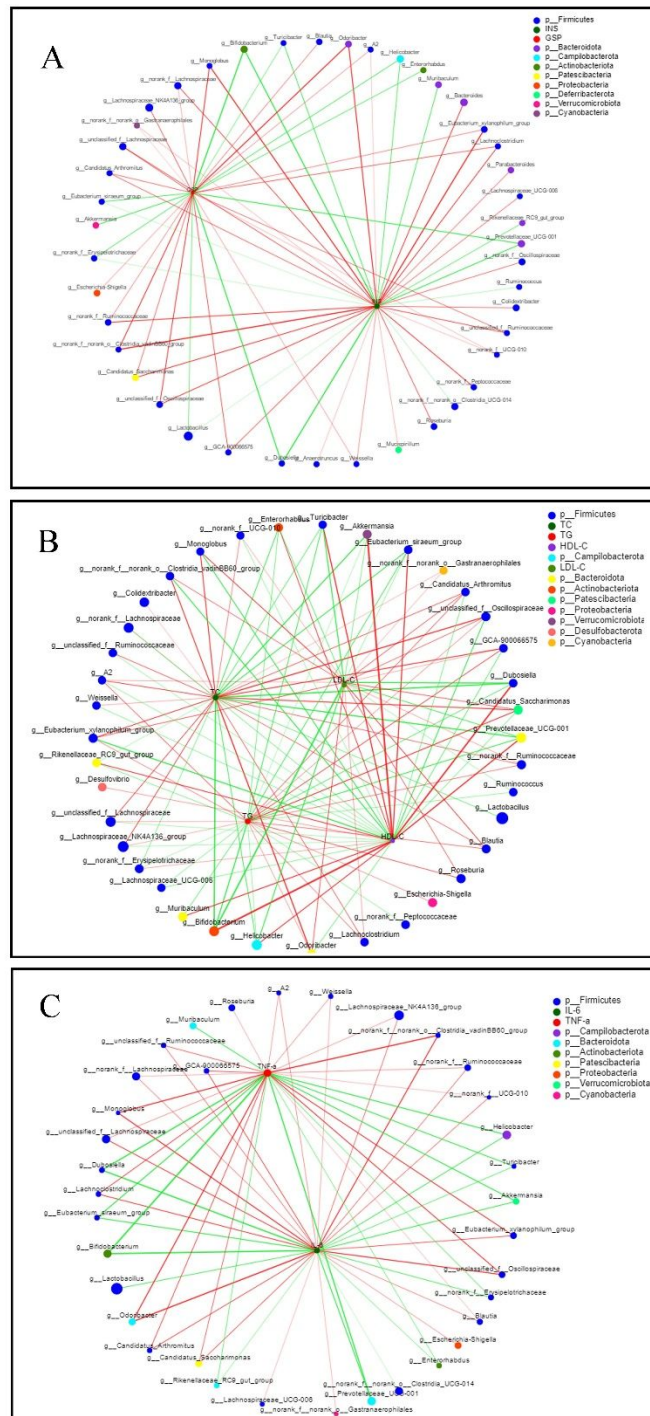


Figure S3. Visual correlation network diagram between gut microbiota of mice at the genus level and environmental factors. (A) INS and GSP ; (B) TG, TC, HDL-C and LDL-C ; (C) IL-6 and TNF- α . The red line represents positive correlation, the green lines represent negative correlation, and thickness of the line represents intensity of the correlation.

Analysis on the correlation network diagram between species and environmental factors shows that there are close correlations between gut microbiota of the T2DM mice at the genus level and blood glucose related indicators, including GSP and INS, serum TG, TC, HDL-C and LDL-C, as well as inflammation related factors. As presented in Figure S3, *Monoglobus* and g__unclassified_f__*Oscillospiraceae* are positively correlated with GSP and INS levels, while *Lactobacillus*, *Bacteroides* and *Prevotellaceae*_UCG-001 are negatively related to GSP and INS levels. *Bacteroides* and *Helicobacter* are positively correlated with HDL-C, but negatively correlated with TG, TC, and LDL-C. *Monoglobus* and g__unclassified_f__*Oscillospiraceae* are positively correlated with TG, TC, and LDL-C, and negatively correlated with HDL-C. *Weissella*, *Monoglobus* and *Odoribacter* show a positive correlation with the levels of inflammatory factors IL-6 and TNF- α , while *Dubosiella* and *Bacteroides* show a negative correlation with the IL-6 and TNF- α levels.