Supplementary materials for:

Modulation of gut microbiota and intestinal barrier function during alleviation of antibiotic associated diarrhea with Rhizoma *Zingiber officinale* (Ginger) extract

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1. Multicomponent quantification of the ginger by HPLC

Analyses were performed using a Shimadzu HPLC system. Chromatography was carried out at 25ºC on an Inertsil ODS-SP C_{18} column (250mm × 4.6mm, with 5 μm particle size). The mobile phase consisted of (A) water spiked with 0.01% (v/v) phosphoric acid and (B) acetonitrile. The gradient elution was as follows: 45% B from 0 to 10 min, 45-48% B from 10 to 15 min, 48-60% B from 15 to 17 min, 60% B from 17 to 43 min, 60-67% B from 43 to 45 min, 67-69% B from 45 to 48 min, 69-71% B from 48 to 58 min, at a flow rate of 0.5 mL·min^{-1}. The signal was monitored at 282 nm.

Standards of 6-gingerol (Batch number 111833-201806, purity ≥ 99.9%) was purchased from the National Institutes for Food and Drug Control (Beijing, China). HPLC-grade methanol was purchased from Thermo Fisher Scientific. Water was purified by a Milli-Q Plus water purification system (Millipore, USA).

The contents of 6-gingerol, 10-gingerol and 8-gingerol were simultaneously determined by method of quantitative analysis of multi-components by single marker (QAMS). As a result, the contents of 6-gingerol, 10-gingerol and 8-gingerol were 0.11%, 0.08% and 0.09%, respectively, which were in accord with the quality standard of the Chinese Pharmacopoeia (CP) (2015).

2. The HPLC fingerprint analysis of ginger

The analysis was performed using Shimadzu HPLC system. Chromatography was carried out at 25ºC on an Inertsil ODS-SP C_{18} column (250mm × 4.6mm, with 5 μm particle size). The mobile phases used were solvent A (water), solvent B (acetonitrile), with gradient elution as follows: 5% – 20% B at 0 –5 min, 20% – 40% B at 5 – 20 min, 40% – 60% B at 20–35 min, 60% –75% B at 60–75 min, 75-90% B at 55–60 min, 95% B at 60–73 min. The flow rate was kept at 0.5 mL·min^{-1}. The signal was monitored 254 nm. The injection volume of samples was 15 μL.

Ten batches of ginger were collected from different areas of China, the producing area and batch number were shown in **Table S1**, all the samples were authenticated as *Zingiber officinale* Rosc. by Professor Kui-Jun Zhao at the Department of Pharmacy, Beijing Friendship Hospital affiliated to Capital Medical University.

Table S1 The sample information all the ginger sample

Sample name	Producing area	Batch number
S01	Sichuan province, China	18100803
S02	Sichuan province, China	18092604
S03	Sichuan province, China	18100602
S04	Henan province, China	18100402
S05	Henan province, China	18092603
S06	Henan province, China	181010.2
S07	Henan province, China	18092903
S08	Shandong province, China	18100205
S09	Shandong province, China	18091229
S10	Shandong province, China	18100604

Professional software "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine" (Version 2004A, SES software) was used for evaluating the similarities between different samples. The reference chromatogram was generated with average data.

Sample S1 was selected as a representative sample to validate the method for fingerprint analysis. Method precision and reproducibility were evaluated by the analysis of six injections of the sample solution and six sample solution prepared independently from sample S1, respectively. RSD values of the relative retention time (RRT) and the relative peak area (RPA) for some characteristic peaks, including 5 peaks (**Figure S1**, peak no.2, 7, 9, 17, 21) were calculated. And the stability study of the sample was performed within 24h (0, 4, 8, 12, 16, 24 h). RSDs of RRT and RPA in the

precision test (n = 6) were found in the range of 0.23-2.55% and 1.64-2.67%, respectively. Reproducibility (n = 6) for both RRT (0.74 – 2.05% RSD) and RPA (1.58 – 2.56 % RSD) were acceptable. The good precision, reproducibility and stability of analysis were demonstrated. **Table S2** summarizes the obtained data.

Table S2 Injection precision, reproducibility and stability fingerprint analysis

Peak	RSDs of RRT (%) (n=5)			RSDs of RPA (%) (n=5)		
no.	Precision	Reproducibility	Stability	Precision	Reproducibility	Stability
1	1.35	1.10	1.57	1.64	2.43	2.26
2	2.55	2.94	1.42	2.27	2.97	1.71
3	1.07	2.27	2.05	2.26	1.56	1.58
4	0.24	1.07	0.74	2.67	2.80	1.80
5	0.23	0.43	0.71	2.19	2.57	2.56

The fingerprint of ginger was then established (**Figure S1**). **Table S3** shows the values of similarity of each sample. The closer the similarity values to 1, the more similar the chromatogram to the reference chromatogram. As is shown in **Table S3**, the similarity values of all the 10 samples was higher than 0.90.

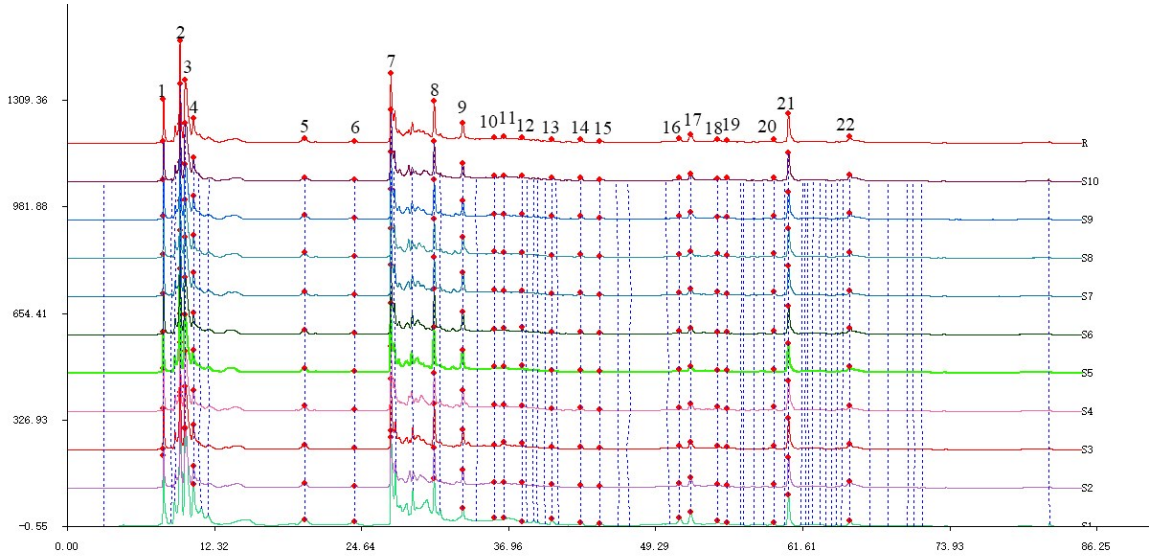

Figure S1. Chemical fingerprint of rhubarb. Sample nos. 1-10 (S1-S10) were used to

construct fingerprint. R stands for the reference chromatogram.

Table S3 The similarities of chromatograms of each sample.

No.	Similarity	No.	Similarity
1	0.932	6	0.982
2	0.949	7	0.982
3	0.980	8	0.967
4	0.952	9	0.975
5	0.977	10	0.977

3. Ginger improves gut barrier integrity in antibiotic-associated diarrhea (AAD) rats

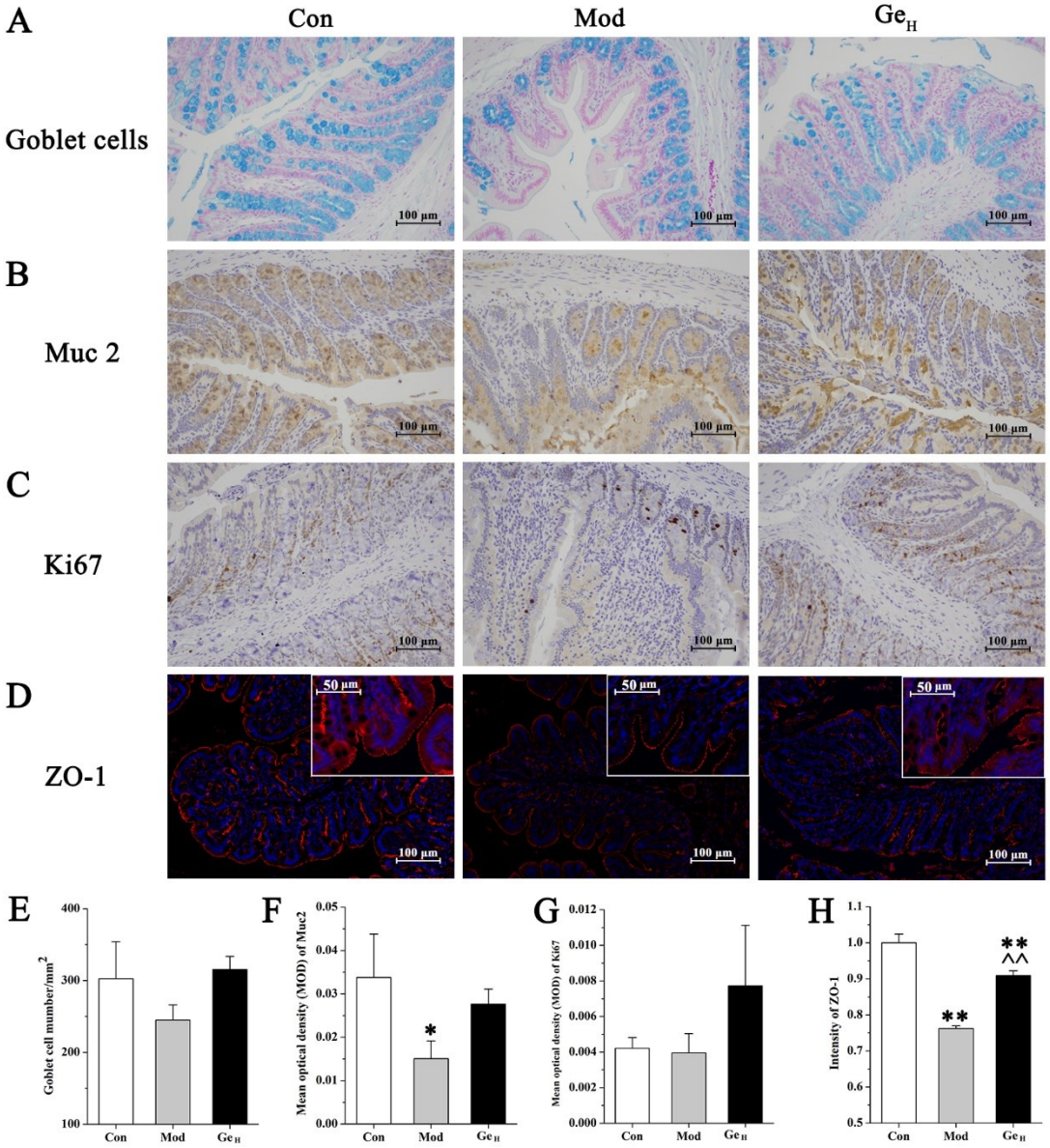

Figure S2 Ginger extract improves gut barrier integrity in antibiotic-associated diarrhea (AAD) rats on 21th day. (A) Alcian blue staining for detecting goblet cells numbers were shown in normal rats. (B) Immunohistochemistry of Muc2 protein located in colonic tissues was detected. (C) Cell proliferation marker Ki67 was detected by immunohistochemistry in colon sections. (D) Immunoblot analysis for the protein level of ZO-1 in colon tissue. The values represent the mean ± S.D. (n=5/group), and MOD denotes the mean optical density of the areas of interest. Original magnification: 200×. * and ** represent $P < 0.05$ and $P < 0.01$ versus Con group, respectively. ^^ represents $P < 0.01$ versus Mod group.

4. Ginger Treatment Modulates Gut microbiota in AAD Rats

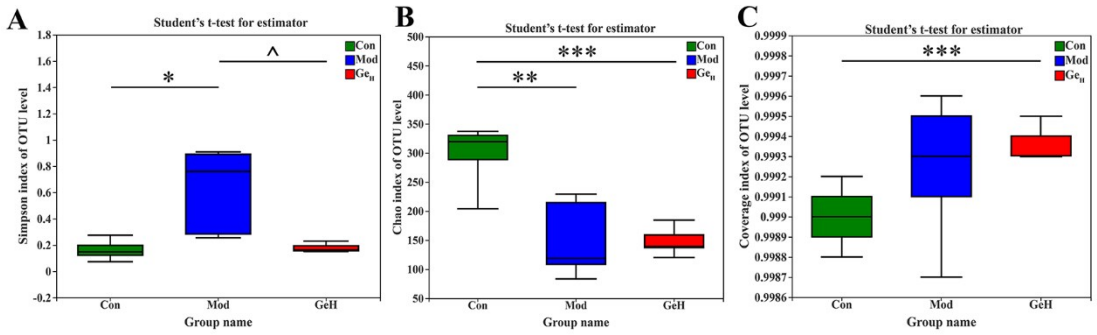

Figure S3 α-Diversity analysis for the different bacterial communities microbial richness based on the Simpson index (A), Chao (B) and Coverage index (C) of OUT level, respectively. The values are expressed as means ± S.D, n=5/group. *, ** and ** represent $P < 0.05$, 0.01 and 0.01 versus AAD Con group, respectively. ^ represents $P < 0.05$ versus Mod group.

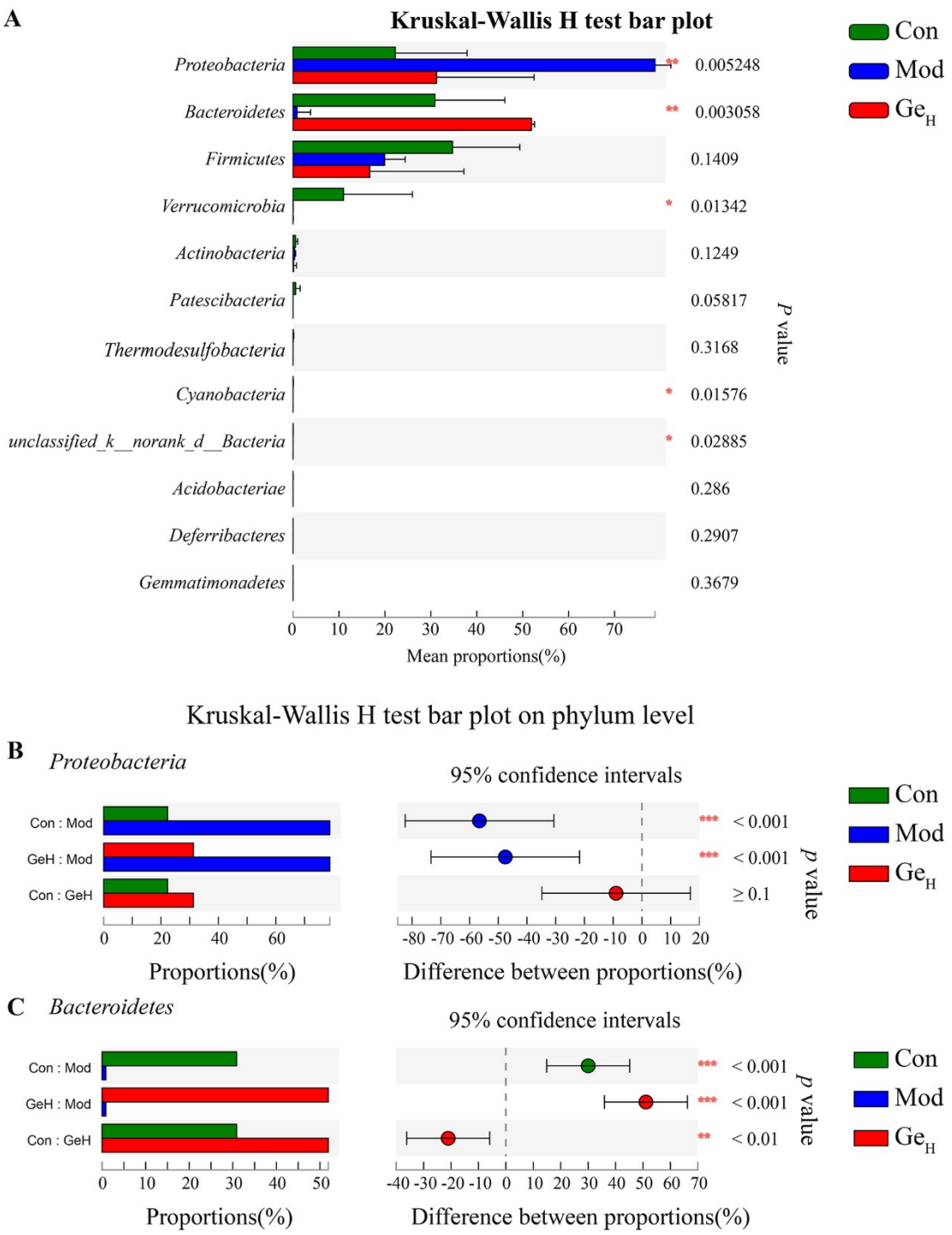

Figure S4 (A) Relative abundances of phyla that showed significant differences among samples from the Con, Mod and Ge_H groups. Kruskal-Wallis H test bar plot on phtlum level of *Proteobacteria* and *Bacteroidetes* (C). Kruskal-Wallis H test was used to evaluate the significance of differences between the indicated groups. $^{**}P < 0.01$;$^{***}P < 0.001$.

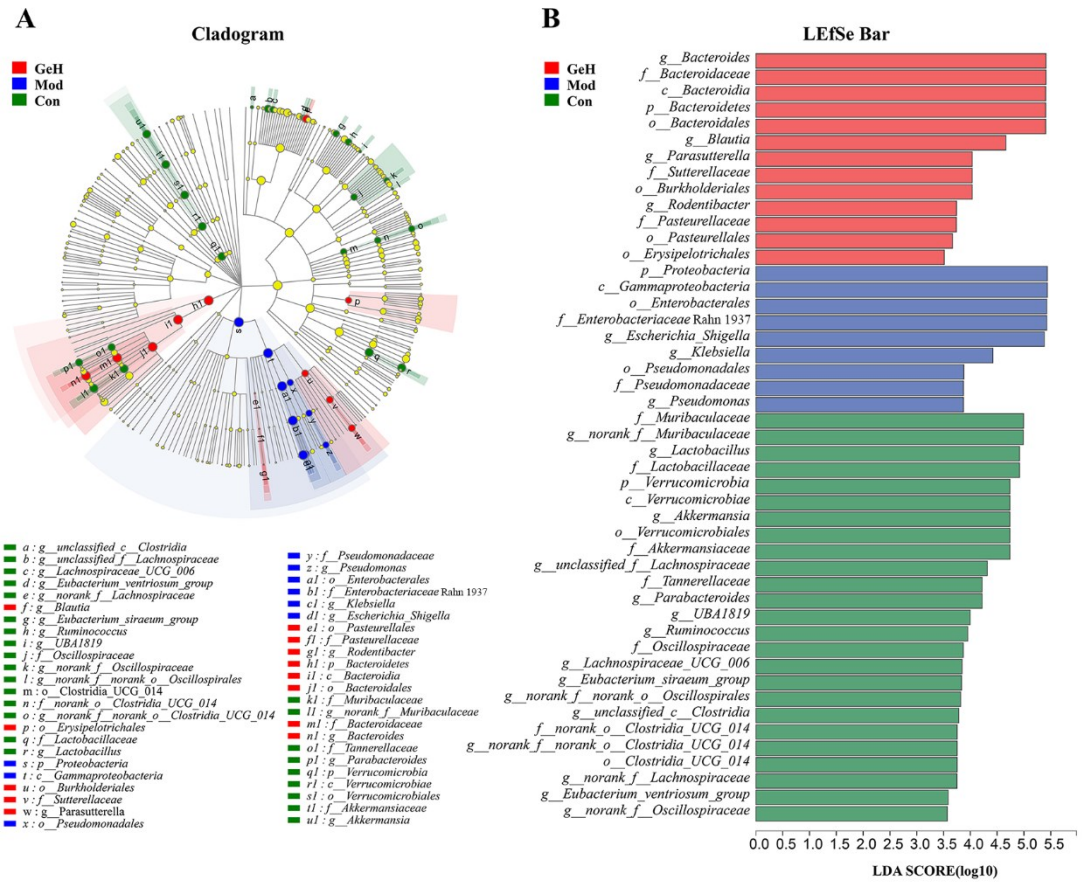

Figure S5 Cladogram showing the phylogenetic distribution of the bacterial lineages from Con, Mod and Ge_H groups (A). Taxonomic representation of statistically and biologically consistent differences between Con, Mod and Ge_H groups. Differences are represented by the color of the most abundant class. The diameter of each circle is proportional to taxon abundance. Circles indicate phylogenetic levels from phylum to genus. (B) Indicator bacteria with LDA scores of 3.5 or greater in bacterial communities.