Polysaccharide from *Angelica sinensis* with medicinal and edible purposes ameliorated NAFLD by the bile acids mediated activation of FXR

Supplementary materials

1. Analytical procedure of bile acids

The bile acid concentrations were determined by LC-MS/MS. Chromatographic separation was achieved on a Waters HSS T3 column (1.7 μm, 100 mm × 2.1 mm, Waters, USA) and the mobile phase A and B is water (containing acetic acid, pH 4.3) and 90% methanol aqueous solution (containing acetic acid, pH 4.3), respectively. The flow rate is 0.3 mL/min. Gradient elution procedure: 0-1 min, 25% B; 1-1.5 min, 25-50% B; 1.5-4 min, 50-90% B; 4-5.5 min, 90-25% B. IS was chosen as internal standard (Fig S1A). The MS/MS analysis was performed with negative ionization mode and the optimized monitoring ions of cholic acid (CA), taurocholic acid (TCA) and glycylcholic acid (GCA) were 407/407, 514/80 and 464/74, respectively. The standard curve was drawn by comparing the peak area of each bile acid in the standard mixture with the peak area of the internal standard. Our results showed that LC-MS/MS was a suitable method for the quantification of bile acids with satisfactory linearity ($r > 0.99$) (**Figure S1 B-D**).

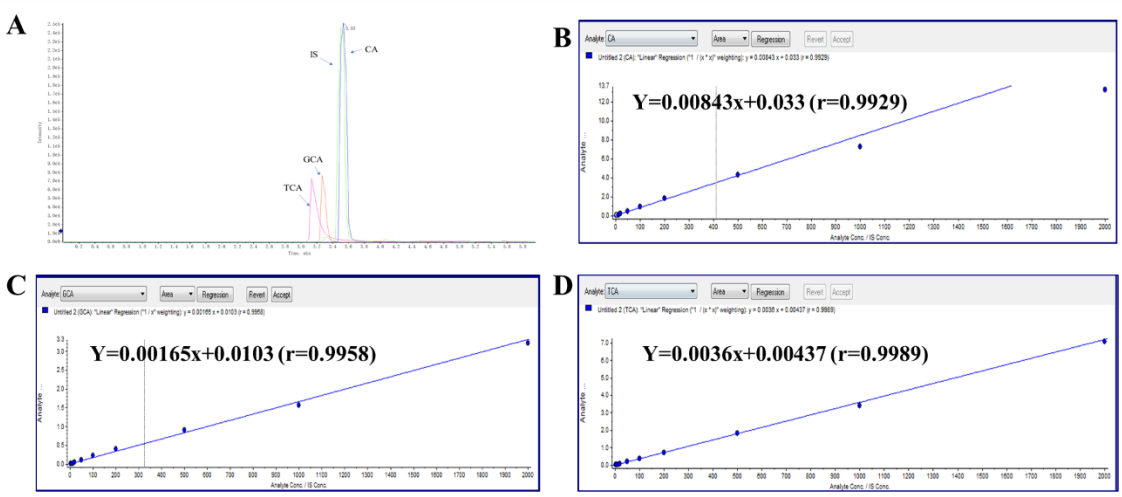

Fig S1 The bile acid concentrations were determined by LC-MS/MS. (A) Chromatogram of internal standard IS and three bile acid standard mixed solution including cholic acid (CA), taurocholic acid (TCA) and glycylcholic acid (GCA); (B) Standard curve of CA; (C) Standard curve of TCA; (D) Standard curve of GCA.

2. Binding of ASP and bile acid

ASP (10 mg) was dissolved in 2 mL 0.01 M HCl and incubated at 37 ℃ for 60 min to simulate the condition of stomach. Then, the solution was adjusted to pH=7.0 by 0.01 M NaOH, followed by mixing the solution with 4 mL of 1% trypsin and 4 mL of 500 μM bile acid mixed solution (CA, TCA and GCA were mixed by 0.05 M pH=7.0 phosphate buffer solution) and then incubated at 37 ℃ for 60 min to simulate intestinal conditions. The mixtures were dialyzed (Mw cutoff 14 kDa) against distilled water at 37 ℃. After dialysis for 3 h, 800 μL water was taken and incubated at 95 ℃ for 5 min to inactivate the enzyme. Finally, unbound bile acids were obtained by solid phase extraction and were determined by LC-MS/MS. Our results showed that the binding rate of ASP with three bile acids has reached 88.37% ± 4.48% (CA), 93.98% ± 2.90%

(TCA) and 95.81% ± 1.52% (GCA) (**Figure S2A**), and the amount of bile acid binding per 100 mg ASP was 17.67 ± 0.90 (CA), 18.80 ± 0.58 (TCA) and 19.16 ± 0.30 (GCA) μM (**Figure S2B**).

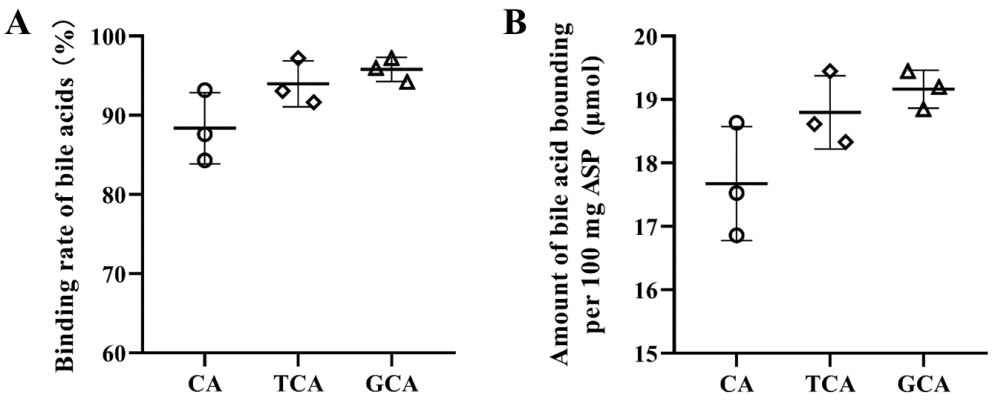

Fig S2 Binding of ASP and bile acids (n=3). (A) Binding rate of ASP and bile acids; (B) Amount of bile acid binding per 100 mg ASP.

3. ASP remodeled the composition and abundance of the gut microbiota in HFD-fed mice

Extraction of genome DNA, PCR amplification and Illumina MiSeq sequencing were carried out according to the standardized protocol of Genewiz (Suzhou, China). The Quantitative Insights into Microbial Ecology (QIIME, v1.8.0) data analysis package was used for 16S rDNA data analysis. Our data showed that Bacteroidetes, Firmicutes and Proteobacteria were the most abundant phyla at the phylum level in all groups (**Figure S3B**). Heatmaps of gut microbiota composition at the genus and species levels showed specific differences among the control, HFD, and ASP groups (**Figure S3C**). These results suggested that the microbiota profile in HFD-induced NAFLD mice was remodeled by ASP after oral administration.

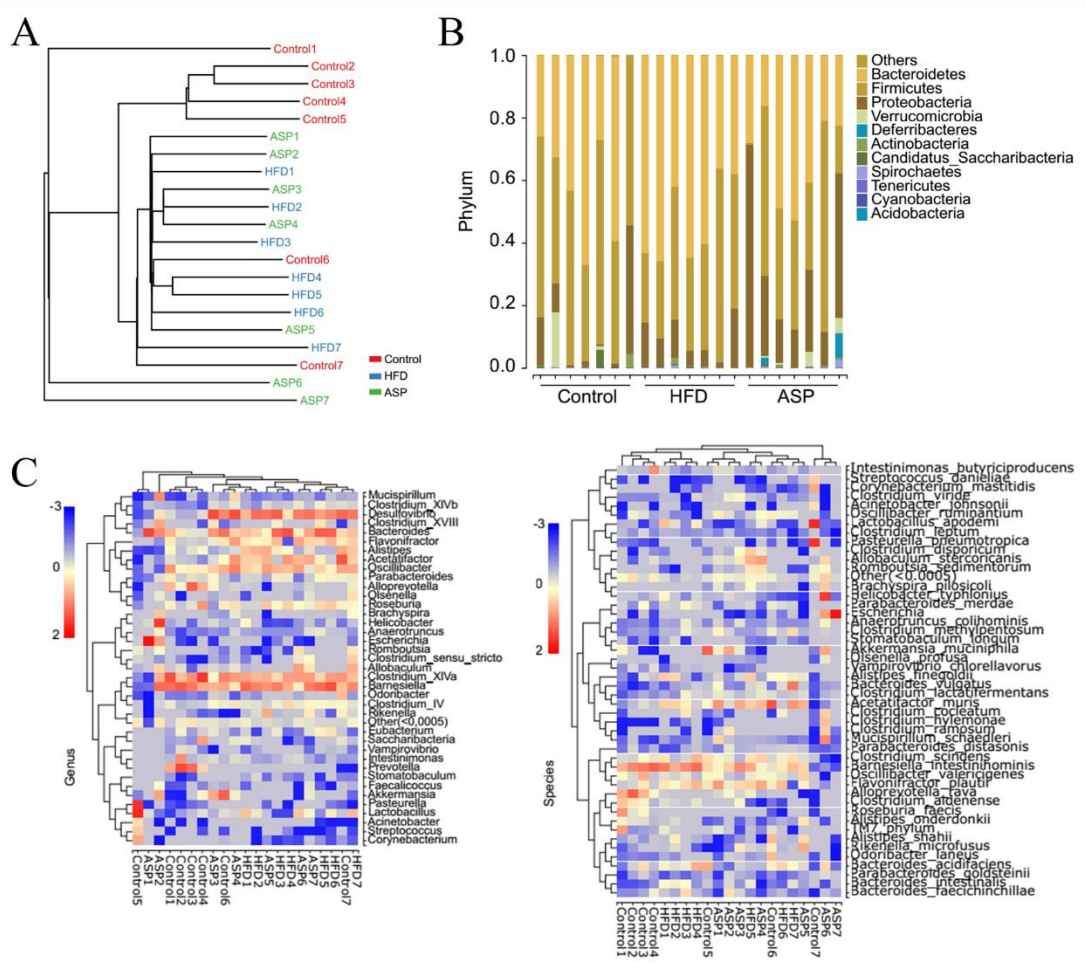

Figure S3 ASP treatment changed gut microbiota profile in HFD-induced NAFLD mice. (A) An unweighted unifrac cluster tree based on UPGMA showed similarity of intestinal microbiota in three groups. (B) Changes in the composition of the gut microbiota in different groups at the phylum level; stacked bar charts represent the relative abundance of major taxa. (C) Heatmap displayed different relative abundance among all genus and species. Different colors indicate different metabolite expressions (n = 7 per group).