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Astilbin from *Smilax glabra* Roxb. alleviates high-fat diet–induced metabolic dysfunction

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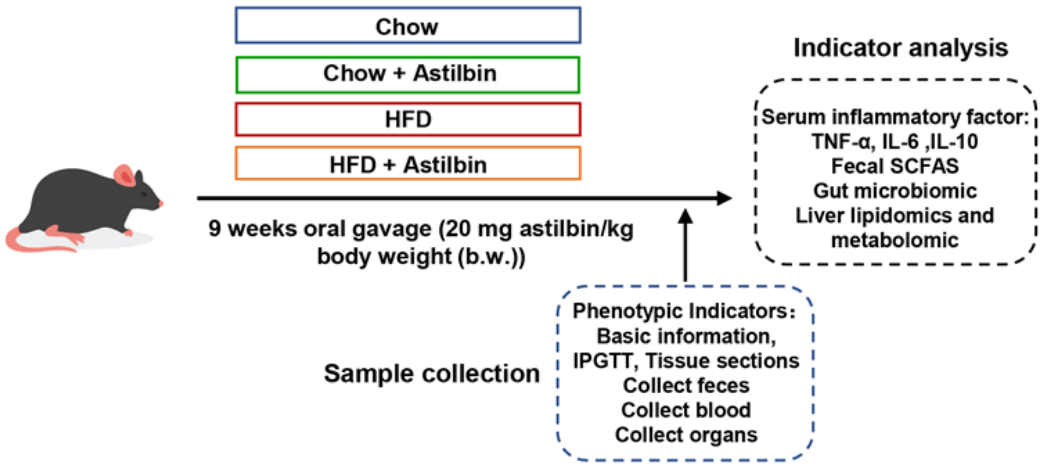
MRM assay for astilbin

We used an ACQUITY UPLC TQD ultra performance liquid chromatography tandem triple quadrupole mass spectrometer (Waters Corporation, USA) set up an MRM assay for astilbin and tested the purchased standards. A liquid-phase method was used as follows: column: Waters BEH Shield RP18 column, 2.1 × 100 mm^2 id, 5-μm film thickness (Waters, USA); mobile phases A and B: acetonitrile and 0.1% formic acid solution; flow rate: 0.3 mL/min; and column temperature: 45°C. The elution gradient was set as follows: 0-3 min, 5%-20% A; 3-5 min, 20%-40% A; 5-8 min, 40%-100% A, and the injection volume was 5 μL.

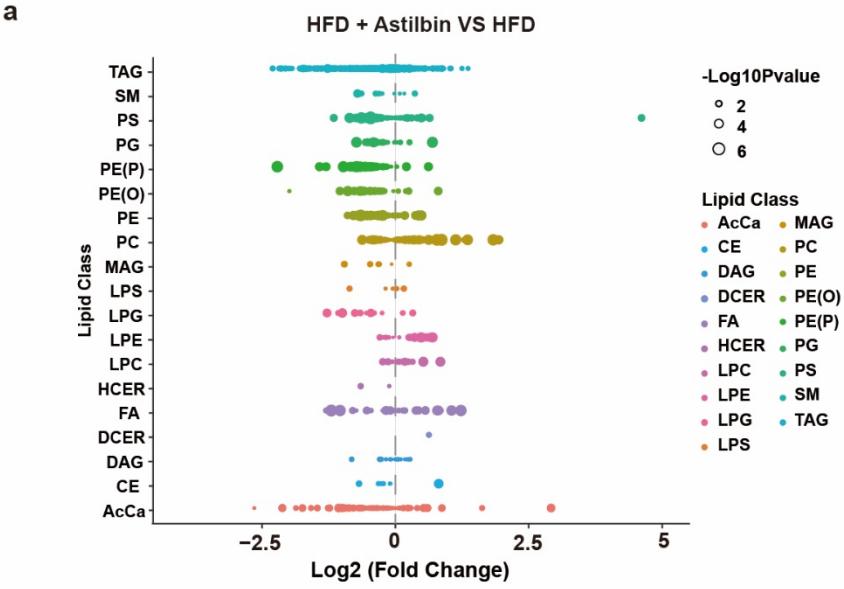
Mass spectrometry conditions: ionization mode: ESI-, capillary voltage: 3.0 k Volts, cone: 20/50 Volts, source block temperature: 100 °C, desolvation temperature: 400 °C, desolvation gas flow: 700 lit/hr, cone gas flow: 50 lit/hr, collision energy (eV) :6, detector voltage: 1800 Volts, ion pair: 449-151, 449-285,449-303. The chromatograms and MRM mode mass spectra are shown in Fig. s5

s1

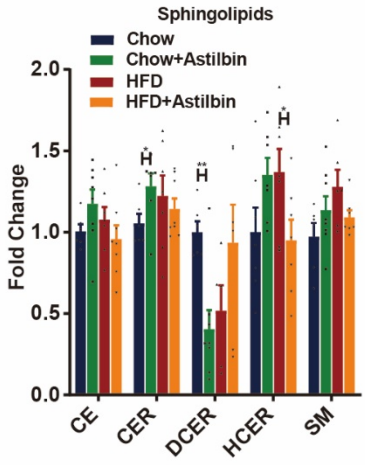

Chow
Chow + Astilbin
HFD
HFD + Astilbin
9 weeks oral gavage (20 mg astilbin/kg body weight (b.w.))
Indicator analysis
Serum inflammatory factor:
TNF-α, IL-6 ,IL-10
Fecal SCFAS
Gut microbiomic
Liver lipidomics and metabolomic
Sample collection
Phenotypic Indicators:
Basic information,
IPGTT, Tissue sections
Collect feces
Collect blood
Collect organs

s2

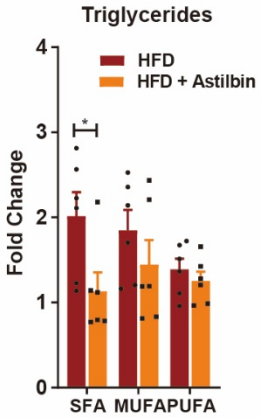
a



b



c



d

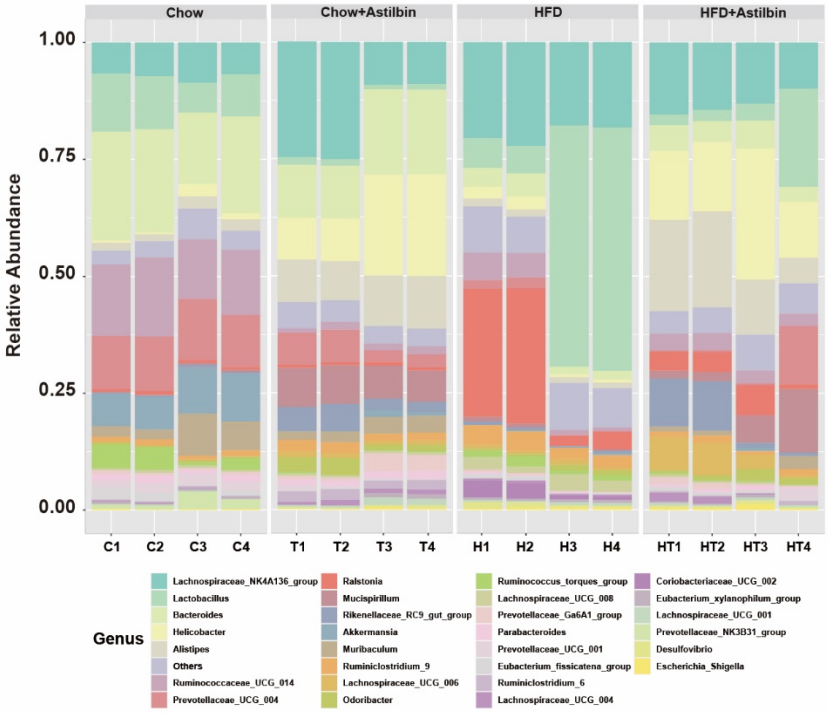
Lipidlon	Fold change	P-value
TAG(56:7/FA20:5)	0.933016	0.004501
TAG(54:6/FA18:2)	0.842621	0.006688
TAG(55:2/FA18:2)	0.411757	0.00689
TAG(52:5/FA16:0)	0.973564	0.00833
TAG(56:6/FA20:3)	0.94084	0.008978
TAG(52:6/FA16:1)	0.986643	0.011012
TAG(56:6/FA22:6)	1.029228	0.013085
TAG(56:8/FA18:1)	1.196244	0.01385
TAG(55:1/FA18:1)	1.016715	0.021164
TAG(54:5/FA20:4)	0.945089	0.024522
TAG(50:5/FA18:3)	0.838938	0.02545
TAG(54:6/FA20:4)	0.939373	0.027399
TAG(54:6/FA16:0)	0.927384	0.029162
TAG(54:4/FA20:2)	0.904416	0.029762
TAG(54:6/FA22:6)	1.121496	0.041949
TAG(54:7/FA22:6)	1.109265	0.042664
TAG(55:7/FA22:6)	0.958775	0.046767
TAG(56:8/FA16:1)	0.977744	0.047557
TAG(49:0/FA16:0)	1.057419	0.048298
TAG(56:4/FA18:1)	0.846389	0.049217

e

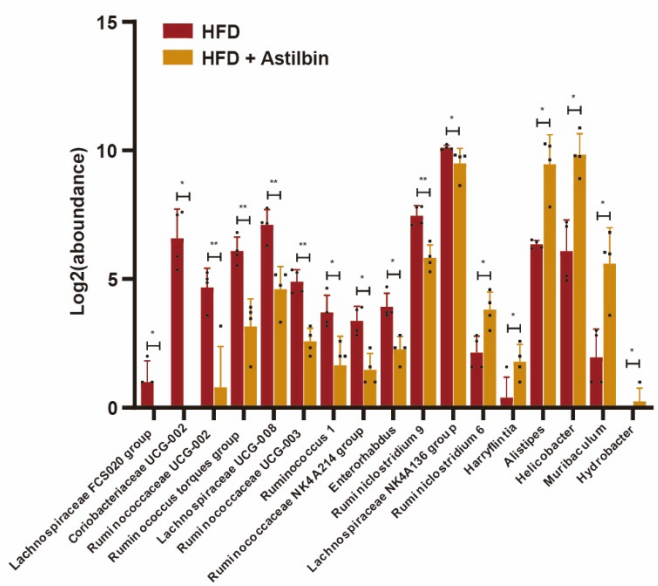
Lipidlon	Fold Change	P-value
PE(P-16:0/16:0)	0.22312	9.72E-06
PE(P-16:0/16:1)	0.38797	0.002198
PE(P-14:0/18:1)	0.42331	0.003648
LPG(20:4)	0.42832	0.003905
PS(16:0/16:1)	0.46801	0.014554
PE(O-16:0/20:4)	0.5072	0.00696
LPG(22:4)	0.52344	0.001223
PE(P-18:1/20:3)	0.53077	2.70E-05
PE(14:0/22:5)	0.56049	0.030059
PE(O-16:0/22:6)	0.56234	0.000264
PS(16:0/16:0)	0.5759	0.00025
PC(18:0/22:5)	1.812	7.74E-06
PC(18:1/20:5)	1.8245	0.004986
PE(O-18:0/18:3)	1.8441	0.006599
LPC(20:5)	1.8971	0.000547
PC(16:0/22:5)	1.9278	3.60E-06
PC(18:2/20:5)	2.713	1.00E-04
PC(18:0/20:0)	3.7944	1.89E-05
PC(20:0/20:5)	4.0803	0.001748
PS(20:0/20:5)	26.588	0.023116

s3

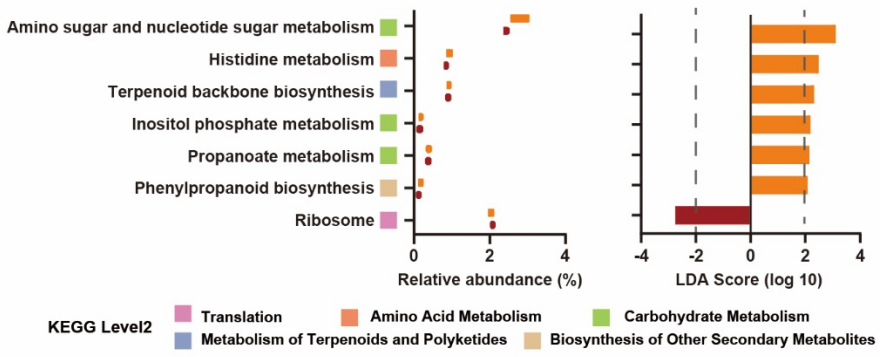
a


Chow
Chow+Astilbin
HFD
HFD+Astilbin
Relative Abundance
1.00
0.75
0.50
0.25
0.00
C1 C2 C3 C4 T1 T2 T3 T4 H1 H2 H3 H4 HT1 HT2 HT3 HT4
Genus
Lachnospiraceae_NK4A136_group
Lactobacillus
Bacteroides
Helicobacter
Alistipes
Others
Ruminococcaceae_UCG_014
Prevotellaceae_UCG_004
Ralstonia
Mucispirillum
Rikenellaceae_RC9_gut_group
Akkermansia
Muribaculum
Ruminiclostridium_9
Lachnospiraceae_UCG_006
Odoribacter
Ruminococcus_torques_group
Lachnospiraceae_UCG_008
Prevotellaceae_Ga6A1_group
Parabacteroides
Prevotellaceae_UCG_001
Eubacterium_fissicatena_group
Ruminiclostridium_6
Lachnospiraceae_UCG_004
Coriobacteriaceae_UCG_002
Eubacterium_xylanophilum_group
Lachnospiraceae_UCG_001
Prevotellaceae_NK3B31_group
Desulfovibrio
Escherichia_Shigella

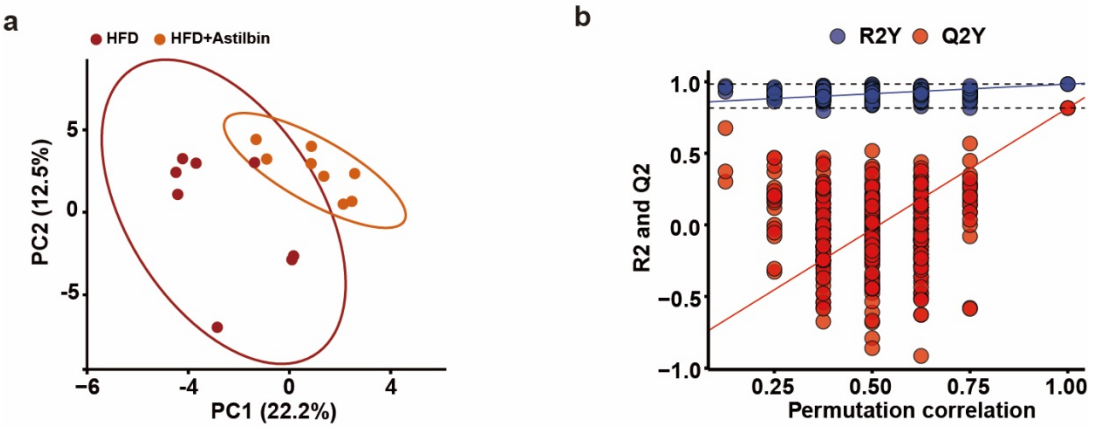
b


HFD
HFD + Astilbin
Log2(abundance)
15
10
5
0
Lachnospiraceae FCS020 group
Coriobacteriaceae UCG-002
Ruminococcaceae UCG-002
Ruminococcus torques group
Lachnospiraceae UCG-008
Ruminococcaceae UCG-003
Ruminococcus 1
Ruminococcaceae NK4A214 group
Enterorhabdus
Ruminiclostridium 9
Lachnospiraceae NK4A136 group
Ruminiclostridium 6
Harryflintia
Alistipes
Helicobacter
Muribaculum
Hydrobacter

c


Amino sugar and nucleotide sugar metabolism
Histidine metabolism
Terpenoid backbone biosynthesis
Inositol phosphate metabolism
Propanoate metabolism
Phenylpropanoid biosynthesis
Ribosome
0 2 4
Relative abundance (%)
-4 -2 0 2 4
LDA Score (log 10)
KEGG Level2
Translation
Amino Acid Metabolism
Carbohydrate Metabolism
Metabolism of Terpenoids and Polyketides
Biosynthesis of Other Secondary Metabolites

s4

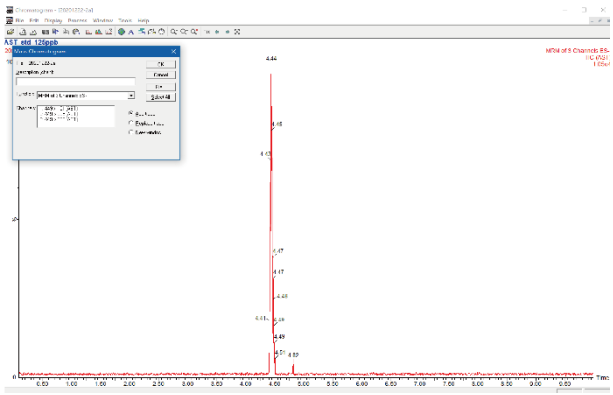


c

Number	Metabolites	Fold Change	P-value	VIP
1	L-Proline	0.352	0.017	1.458
2	D-2-Aminobutyric acid	0.409	0.013	1.434
3	N-Acetylornithine	0.431	<0.001	1.952
4	beta-Alanine	0.437	<0.001	2.133
5	4-Aminobutanoate	0.451	<0.001	1.872
6	4-Chloropyridine-2,6-Dicarboxylic Acid	0.506	0.046	1.043
7	Tryptamine	0.564	0.029	1.366
8	D-Arabitol	0.566	0.003	1.614
9	Glutamate	0.566	0.024	1.406
10	L-Ornithine hydrochloride	0.572	0.037	1.260
11	Oleamide	0.576	0.018	1.383
12	Xanthine	0.580	0.001	1.831
13	N-Acetyl-D-glucosamine	0.587	0.018	1.353
14	L-Asparagine	0.605	0.024	1.332
15	L-Cysteine	0.619	0.016	1.512
16	sn-Glycerol-1-phosphate	0.630	0.019	1.294
17	1-Monopalmitin	0.657	0.002	1.653
18	Pentitol	0.666	<0.001	1.835
19	D-Panose	1.543	0.037	1.445
20	Adenine	1.597	0.011	1.561
21	Lactitol	1.616	0.015	1.611
22	Trehalose	1.647	0.011	1.639
23	Adenosine	1.726	0.025	1.522
24	beta-Sitosterol	1.754	0.024	1.372
25	Lactobionic acid	2.089	0.011	1.379
26	Arabinose 5-phosphate	4.211	<0.001	1.869
27	Adenosine 5-Monophosphate	5.926	<0.001	2.233

s5

a



b

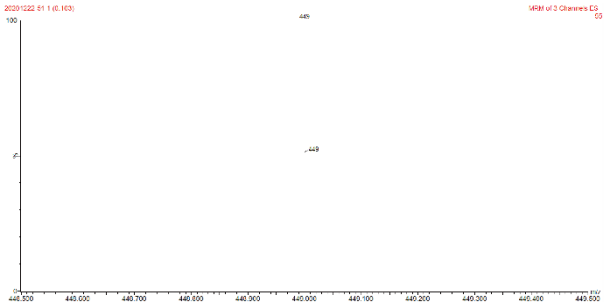


Figure Captions

Figure s1. Development of astilbin intervention models.

Figure s2. Effects of astilbin consumption on HFD-induced liver lipid metabolism disorders. (**a)** Log2 fold changes in lipid species in the HFD + Astilbin versus HFD groups and the corresponding significance values displayed as –log10 (*P* value). Each dot represents a lipid species, and the dot size indicates significance. (**b)** Intensity fold change of sphingolipids. (**c)** Levels of SFA, MUFA, and PUFA in the liver TG of mice in the HFD + Astilbin and HFD groups. (**d)** Top 20 TGs according to the *P* value, detected in livers of mice in the HFD + astilbin and HFD groups ($n = 8$). (**e)** Top 20 glycerophospholipids according to the *P* value, detected in livers of mice in the HFD + Astilbin and HFD groups ($n = 8$).

Figure s3. Effects of astilbin consumption on microbiota composition of HFD-fed mice. (**a)** Bar graph of bacterial abundance at the genus level. **(b)** Astilbin consumption induced altered flora in HFD mice. **(c)** LDA-based analysis of KEGG level 3 in the HFD + Astilbin versus HFD groups significantly altered the pathway.

Figure s4. Effect of astilbin on liver metabolism in HFD-fed mice. (**a)** PCA analysis of major sources of metabolite variability. **(b)** Validation plot for discriminating HFD + Astilbin and HFD groups. **(c)** Statistical analysis of differential metabolites in the liver to distinguish the HFD + Astilbin group from the HFD group.

Figure s5. Characterization of astilbin. (a) Chromatogram and ion pair information. (b) MRM mode mass spectra.