Supplementary Information (SI) for Food & Function.
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Supplementary File S1

S2.1.1 Using response surface method (RSM) to optimize the extraction of SFE

It is generally known that the extraction yield of target analytes is affected by multiple parameters, including the interactions of various factors in the extraction process. Based on various single-factor assays shown in Figure S1, four single factors (steaming time, moistening time, quantity of wine addition, and drying time) were selected. To ascertain the optimum conditions for the extraction of SFE, the Box–Behnken experiment was designed with four factors and three levels, using Design-Expert (DE) 8.0.6.1 software. Experimental factors and level design are shown in Table S1. To determine the impact of various factors and their interactions on the extraction process, the data were fitted with multiple regression software to obtain the following quadratic multiple regression equation: $Y = 0.45 + 3.109\text{E-}003A + 5.053\text{E-}004B + 0.017C + 6.929\text{E-}003D - 2.785\text{E-}004AB - 3.982\text{E-}003AC - 0.011AD + 3.031\text{E-}003BC - 4.136\text{E-}003BD - 8.327\text{E-}003CD - 0.029A^2 - 0.016B^2 - 0.038C^2 - 5.880\text{E-}003D^2$. In the equation, Y was the content of schisantherin A; and A, B, C, and D were the concentration of steaming time (A), moistening time (B), quantity of wine addition (C), and drying time (D), respectively.

The analysis of variance for the fitted quadratic polynomial model for optimization of extraction parameters was presented in Table S2. The P-value for the model was $P < 0.0001$, which indicated that the model was feasible. With very small P-values ($P < 0.001$), the results revealed that C, A^2, B^2, and C^2 were significantly correlated with the content of schisantherin A. The "lack of fit" was not significant relative to the pure error ($P > 0.05$), suggesting that this model could be used to predict the outcome of the experiment. By comparing the F-value and P-value, it was found that the effect size of a single factor on the content of schisantherin A was C > D > A > B (Quantity of wine addition > Drying time > Steaming time > Moistening time).

The 3D response surfaces were shown in Figures S2 and S3, which showed the interactions of the

four independent variables and the effect on extraction yield. Using Design-Expert 12 software, combined with the regression model and interaction graphs to analyze the data further to obtain the optimal conditions for the extraction of schisantherin A: content of schisantherin A 0.455 %, steaming 2.94 h, moistening 1.98 h, 31.7 % of wine addition, and 50 ℃ drying for 4.53 h. To facilitate the operation, the final process conditions were determined: steaming for 3 h, smothering for 2 h, 32 % wine addition, and 50 ℃ drying for 4.5 h. Three parallel experiments were carried out, and the actual ester-metal yield was 0.455 ± 0.003 %, which was close to the predicted value of the model, indicating that the prediction of this model was reliable and of great significance for the actual production.

Table S1

The optimization design and result of response surface analysis experiments of wine-steamed from the fruit of *S. sphenanthera.*

No.	A Steaming time (h)	B Moistening time (h)	C Quantity of wine addition (g)	D Drying time (h)	Content of schisantherin A (%)
1	2	1.5	3	4	0.406251
2	4	1.5	3	4	0.407442
3	2	2.5	3	4	0.409379
4	4	2.5	3	4	0.409456
5	3	2	2	3	0.375371
6	3	2	4	3	0.422105
7	3	2	2	5	0.411659
8	3	2	4	5	0.425086
9	2	2	3	3	0.393113
10	4	2	3	3	0.432452
11	2	2	3	5	0.426446
12	4	2	3	5	0.422707
13	3	1.5	2	4	0.379393
14	3	2.5	2	4	0.376472
15	3	1.5	4	4	0.41513
16	3	2.5	4	4	0.424333
17	2	2	2	4	0.363125
18	4	2	2	4	0.371309
19	2	2	4	4	0.403468
20	4	2	4	4	0.395723
21	3	1.5	3	3	0.42072
22	3	2.5	3	3	0.426312
23	3	1.5	3	5	0.439137
24	3	2.5	3	5	0.428185
25	3	2	3	4	0.453358

Table S2

Variance analysis table of the regression equation for the wine-steamed from the fruit of *S. sphenanthera.*

Source	Sum of squares	Degree of freedom	Mean	F value	P value	Significance
model	0.018	14	1.306E-003	41.86	< 0.0001	**
A	1.160E-004	1	1.160E-004	3.72	0.0744	
B	3.064E-006	1	3.064E-006	0.098	0.7586	
C	3.623E-003	1	3.623E-003	116.09	< 0.0001	**
D	5.761E-004	1	5.761E-004	18.46	0.0007	*
AB	3.102E-007	1	3.102E-007	9.941E-003	0.9220	
AC	6.343E-005	1	6.343E-005	2.03	0.1759	
AD	4.639E-004	1	4.639E-004	14.86	0.0017	*
BC	3.675E-005	1	3.675E-005	1.18	0.2962	
BD	6.843E-005	1	6.843E-005	2.19	0.1608	
CD	2.773E-004	1	2.773E-004	8.89	0.0099	*
A^2	5.337E-003	1	5.337E-003	170.99	< 0.0001	**
B^2	1.664E-003	1	1.664E-003	53.31	< 0.0001	**
C^2	9.562E-003	1	9.562E-003	306.39	< 0.0001	**
D^2	2.243E-004	1	2.243E-004	7.19	0.0179	*
Residual	4.369E-004	14	3.121E-005			
Lack of fit	3.859E-004	10	3.859E-005	3.02	0.1490	-
Pure error	5.108E-005	4	1.277E-005			
Cor total	0.019	28				

Note: "**" means extremely significant ($P < 0.01$); "*" means significant ($P < 0.05$); "-" means not significant

Table S3

Quantitative real-time PCR primer sequence.

Genes	Base sequences
β-actin	F: CTACCTCATGAAGATCCTGACC
	R: CACAGCTTCTCTTTGATGTCAC
Mapk3	F: AGTCTCTGCCCTCGAAAACCA
	R: CTTCCTCTACTGTGATGCGCTTG
Mtor	F: CAAGGCCGAATCGTCTCCA
	R: ATTTCACAATCGGAGGCAACAA
Hsp90aa1	F: TAACTCCGCCTTTGTGGAACGTC
	R: AAATTCCTTCAGCTGTTGCACAC
Casp3	F: TCTGACTGGAAAGCCGAAACT
	R: GTCCCACTGTCTGTCTCAATGC
Stat3	F: ACGAAAGTCAGGTTGCTGGT
	R: TGTGTTCGTGCCCAGAATGT
Jun	F: ACGACCTTCTACGACGATGC
	R: GCCAGGTTCAAGGTCATGCT
Hif1a	F: GGACGATGAACATCAAGTCAGCA
	R: AGGAATGGGTTCACAAATCAGCA
Egfr	F: TGAGTTCTCTGAGTGCAACTAG
	R: GAATGCGTCATCTATGTTGTCC
Src	F: CTATGTGGAGCGGATGAACTAT
	R: ATTCGTTGTCTTCTATGAGCCG
IL-6	F: CTAGTGCGTTATGCCTAAGC
	R: ATAGTGTCCCAACATTCATATTGTC
TNF-α	F: CGCCTTGGATTGACAAAC
	R: CCTTCCGTGTTCCTACCC

Table S4

Bioactive components of SFE.

Name	Formula	Type	Name	Formula	Type
(-)Gomisin L1	$C_{22}H_{28}O_6$	Lignan	Kadangustin C	$C_{28}H_{34}O_9$	Lignan
Gomisin M2	$C_{28}H_{38}O_7$	Lignan	Tigloylgomisin P	$C_{31}H_{38}O_9$	Lignan
Binankadsurin A	$C_{24}H_{32}O_8$	Lignan	Schisandrer B	$C_{22}H_{28}O_6$	Lignan
Gomisin H	$C_{30}H_{32}O_9$	Lignan	(+)-Anwulignan	$C_{20}H_{20}O_6$	Lignan
Gomisin S	$C_{28}H_{36}O_9$	Lignan	Benzoylgomisin O	$C_{34}H_{34}O_{10}$	Lignan
Gomisin T	$C_{28}H_{38}O_8$	Lignan	Patchouli alcohol	$C_{15}H_{26}O$	Terpenoid
Schisandrin B	$C_{24}H_{32}O_7$	Lignan	Aristolochic acid	$C_{17}H_{11}NO_7$	Terpenoid
Schisandrone	$C_{20}H_{24}O_5$	Lignan	Phytolaccagenin	$C_{30}H_{46}O_6$	Terpenoid
Heteroclitin B	$C_{28}H_{34}O_8$	Lignan	Glycyrrhetinic acid	$C_{30}H_{46}O_4$	Triterpenoid
Heteroclitin C	$C_{29}H_{34}O_9$	Lignan	Neokadsuranic acid C	$C_{23}H_{30}O_4$	Triterpenoid
Schizandrol A	$C_{24}H_{32}O_6$	Lignan	Ganoderic acid A	$C_{30}H_{46}O_7$	Triterpenoid
Myrislignan	$C_{20}H_{22}O_6$	Lignan	Tripterine	$C_{30}H_{46}O_4$	Triterpenoid
Schisantherin O	$C_{23}H_{28}O_9$	Lignan	Ursolic acid	$C_{30}H_{48}O_3$	Triterpenoid
Schizandrol B	$C_{24}H_{32}O_6$	Lignan	Qingyangshengenin	$C_{22}H_{30}O_4$	Triterpenoid
Gomisin O	$C_{28}H_{34}O_8$	Lignan	β-chamigrenic acid	$C_{30}H_{48}O_3$	Sesquiterpenoid
Schisandrin C	$C_{23}H_{30}O_7$	Lignan	Curcumol	$C_{15}H_{22}O_3$	Sesquiterpenoid
Gomisin J	$C_{29}H_{32}O_9$	Lignan	Isolancilactone	$C_{20}H_{26}O_5$	Sesquiterpenoid
Schisandrin A	$C_{24}H_{32}O_7$	Lignan	Quercetin	$C_{15}H_{10}O_7$	Flavonoid
Gomisin E	$C_{28}H_{32}O_9$	Lignan	Scutellarein	$C_{15}H_{10}O_6$	Flavonoid
Heteroclitin D	$C_{28}H_{36}O_9$	Lignan	Isorhamnetin	$C_{16}H_{12}O_7$	Flavonoid
Pregomisin	$C_{22}H_{26}O_6$	Lignan	Diosmetin	$C_{16}H_{12}O_6$	Flavonoid
Gomisin R	$C_{28}H_{34}O_9$	Lignan	Eupatilin	$C_{18}H_{16}O_7$	Flavonoid
Tigloylgomisin H	$C_{31}H_{38}O_9$	Lignan	Icarrin	$C_{33}H_{40}O_{15}$	Flavonoid
Gomisin F	$C_{28}H_{32}O_8$	Lignan	Neferine	$C_{39}H_{42}N_2O_6$	AlKaloid
Heteroclitin A	$C_{28}H_{36}O_9$	Lignan	L-Malic acid	$C_4H_6O_5$	Organic acid
Benzoylgomisin H	$C_{35}H_{38}O_9$	Lignan	Citric acid	$C_6H_8O_7$	Organic acid
Lancilactone A	$C_{22}H_{28}O_4$	Lignan	3.4-Dihydroxybenzoic acid	$C_7H_6O_4$	Phenolic acid
Lancilactone B	$C_{22}H_{28}O_5$	Lignan	Cinnamic acid	$C_9H_8O_2$	Phenolic acid
Schisantherin A	$C_{30}H_{36}O_{11}$	Lignan	Isoleucine	$C_6H_{13}NO_2$	Amino acid
Gomisin G	$C_{30}H_{32}O_9$	Lignan	Phenylalanine	$C_9H_{11}NO_2$	Amino acid
Schisanthenol	$C_{23}H_{28}O_4$	Lignan	Linoleic acid	$C_{18}H_{32}O_2$	Fatty acid
Schisantherin D	$C_{23}H_{28}O_4$	Lignan			

Table S5

Molecular docking binding energy between six key components and six proteins.

Targets	Compound name	Vina score
AKT	Gomisin S	-7.9
JAK	Gomisin F	-7.4
JAK	Schisandrol B	-7.7
JAK	Gomisin S	-7.2
JAK	Tigloylgomisin P	-8.6
JAK	Schisantherin D	-8.4
JAK	(+)-Anwulignan	-8.8
SYK	Gomisin F	-5.7
SYK	Schisandrol B	-5.5
SYK	Gomisin S	-6.2
SYK	Tigloylgomisin P	-6.1
SYK	Schisantherin D	-7.3
GSK3	Schisandrol B	-5.5
GSK3	Gomisin S	-5.7
GSK3	Tigloylgomisin P	-6
GSK3	Schisantherin D	-7.2
MDM2	Schisantherin D	-7.9
CCND1	(+)-Anwulignan	-6.7
CCND1	schisandrol B	-7.8
CCND1	Gomisin S	-6.3
CCND1	Tigloylgomisin P	-6.2

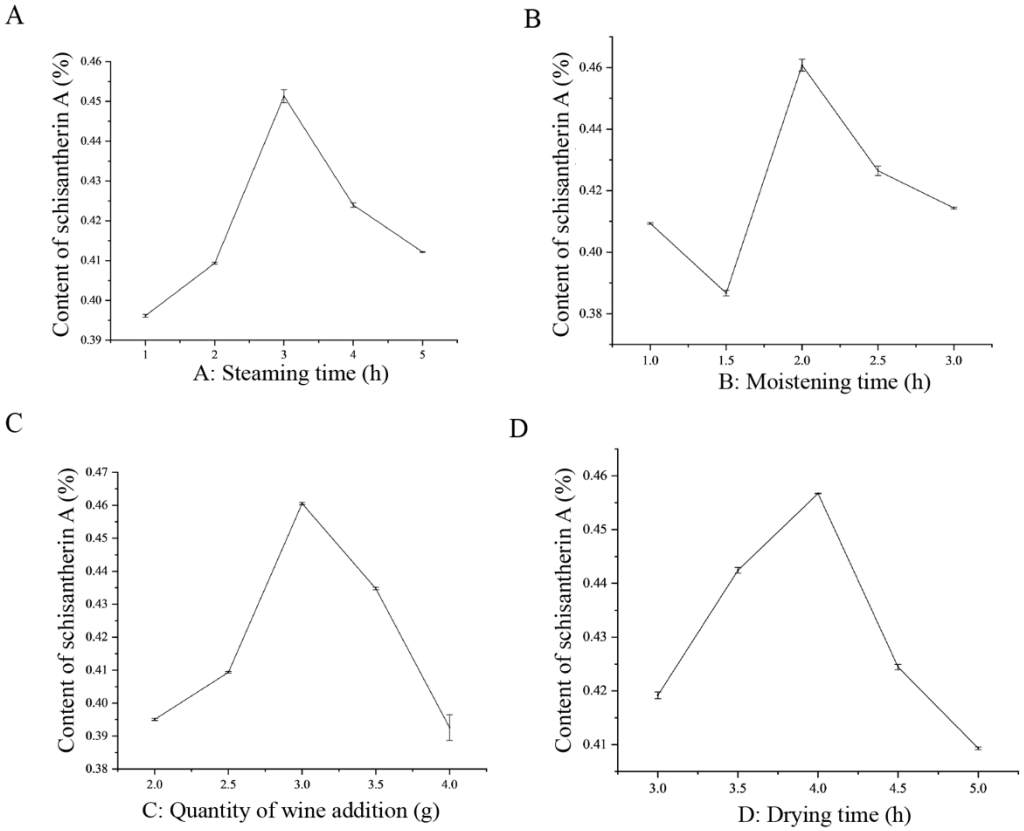

Fig. S1. Content of schisantherin A affected by (**A**) Steaming time (h); (**B**) Moistening time (h); (**C**) Quantity of wine addition (g); (**D**) Drying time (h).

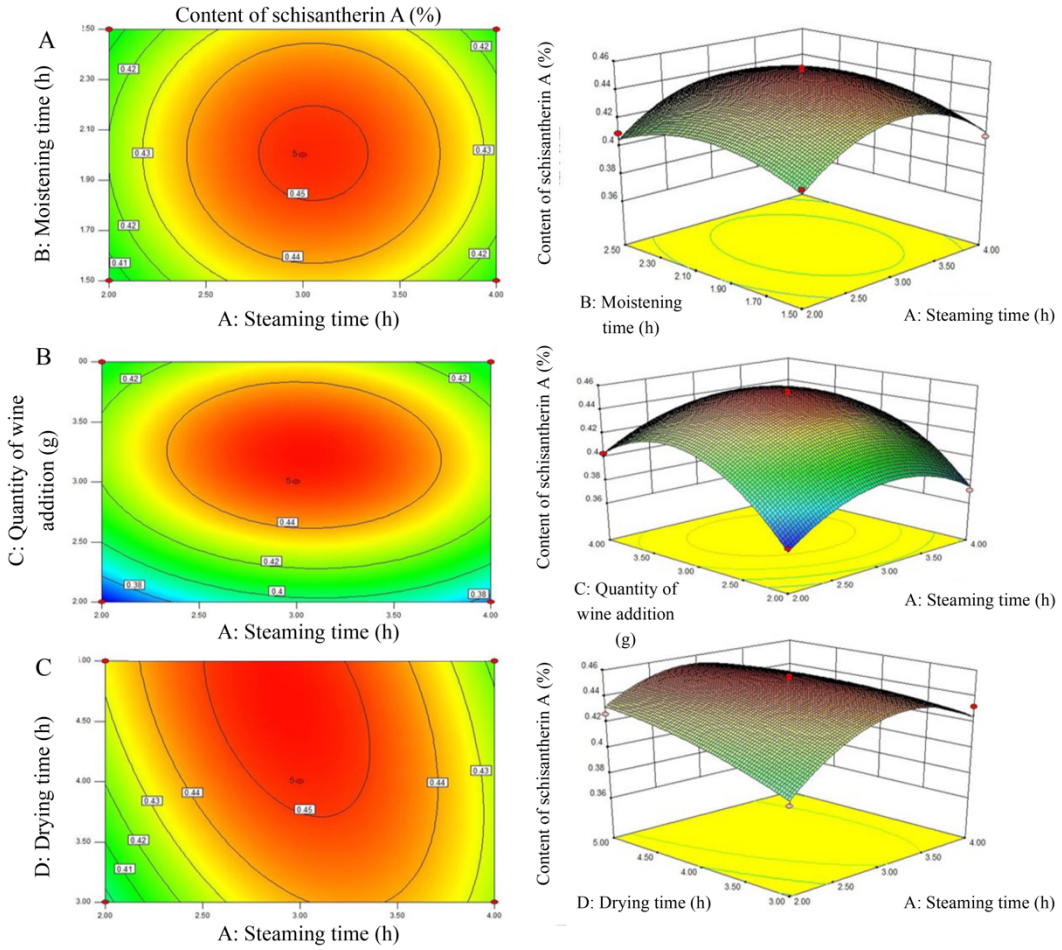

Fig. S2. The isogram and response surface of the interaction of each factor on the content of schisantherin A from the fruits of *S. sphenanthera.* A: steaming time and moistening time; B: steaming time and quantity of wine addition; C: steaming time and drying time.

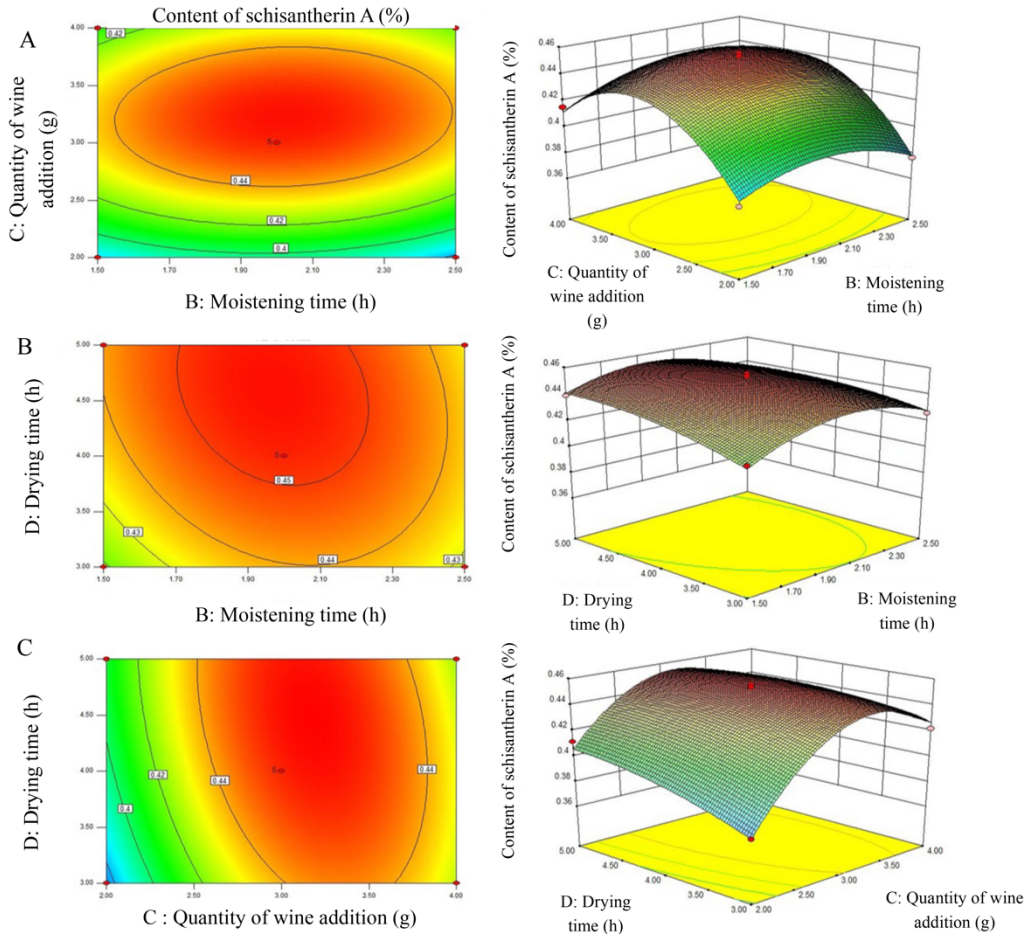

Fig. S3. The isogram and response surface of the interaction of each factor on the content of schisantherin A from the fruits of *S. sphenanthera.* A: moistening time and quantity of wine addition; B: moistening time and drying time; C: quantity of vinegar addition and drying time.

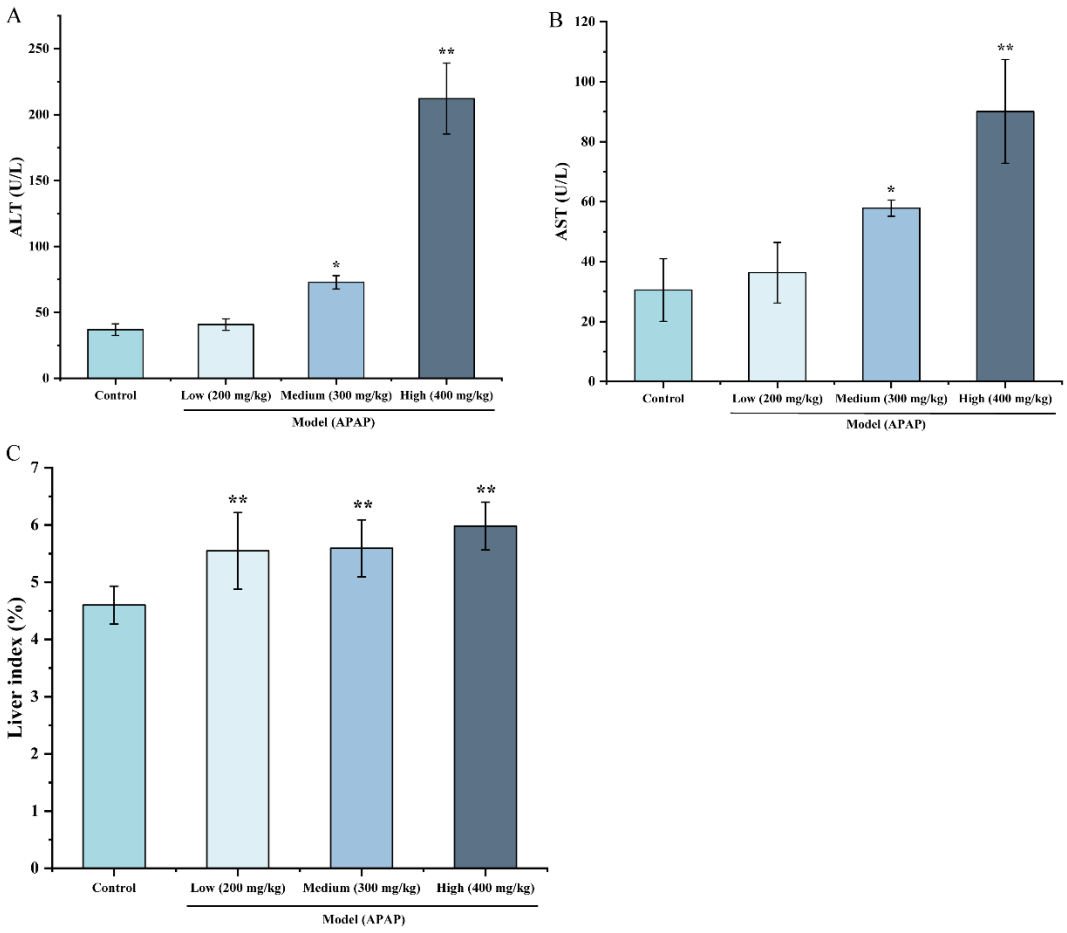

Fig. S4. Effect of APAP on traditional biochemical indexes in serum (A: ALT and B: AST) and liver index (C).

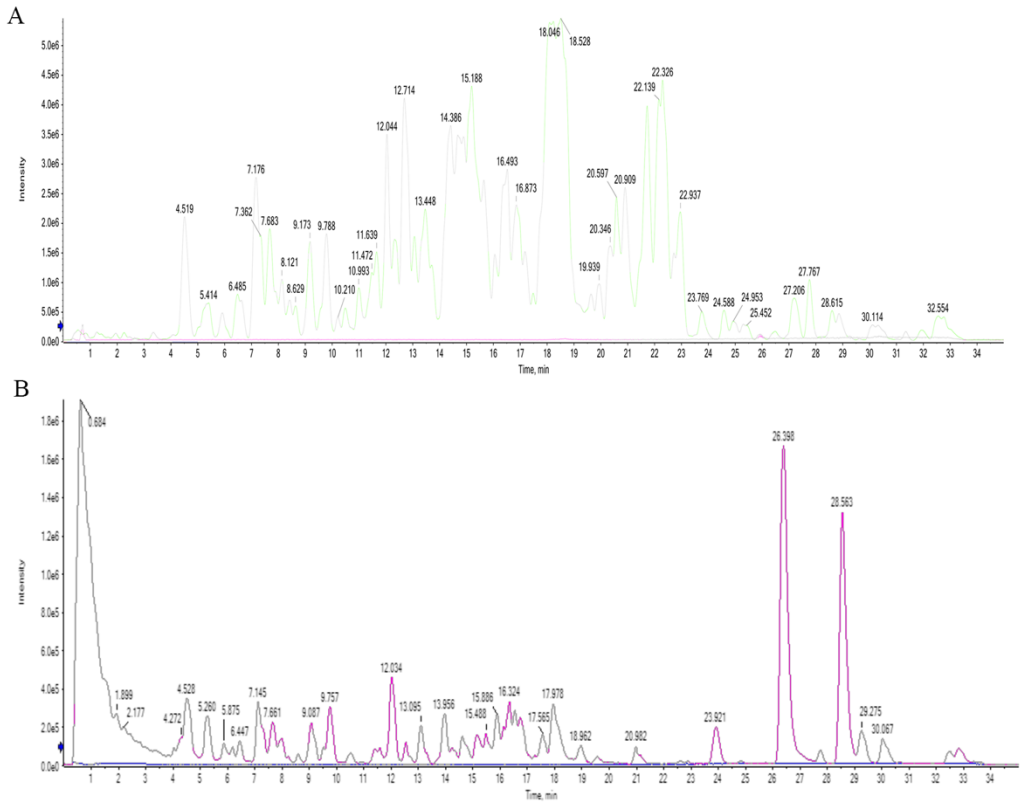

Fig. S5. Typical total ion flow diagram of extract of wine-steamed *S. sphenanthera* fruit (SFE) in UPLC-ESI-QTOF-MS/MS: positive ion mode (A), and negative ion mode (B).

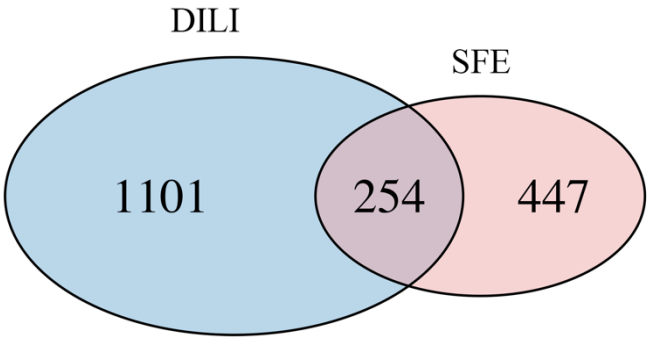

Fig. S6. Overlapping molecular targets between DILI and SFE bioactive compounds. DILI, drug-induced liver injury; SFE, the extract of wine-steamed *S. sphenanthera* fruit.

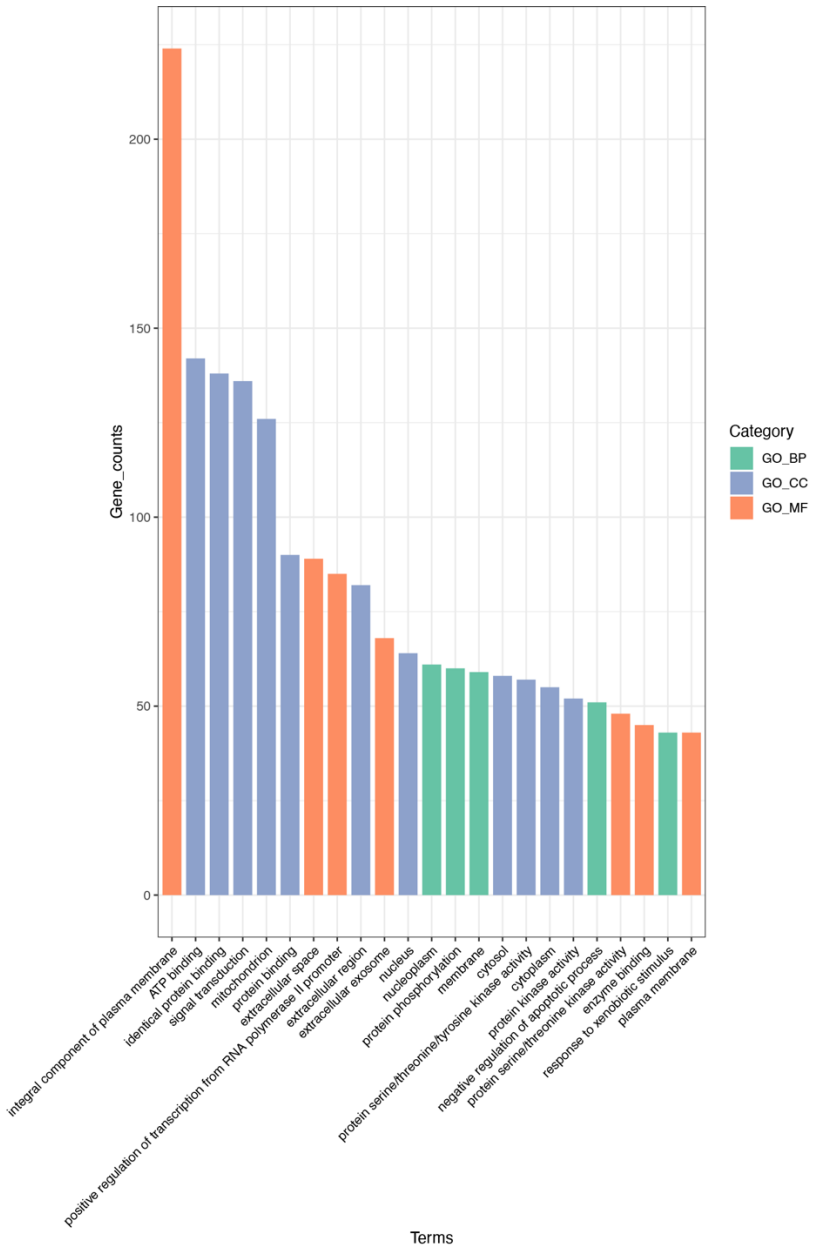

Fig. S7. GO enrichment analysis of SFE compounds-DILI interacting targets project.

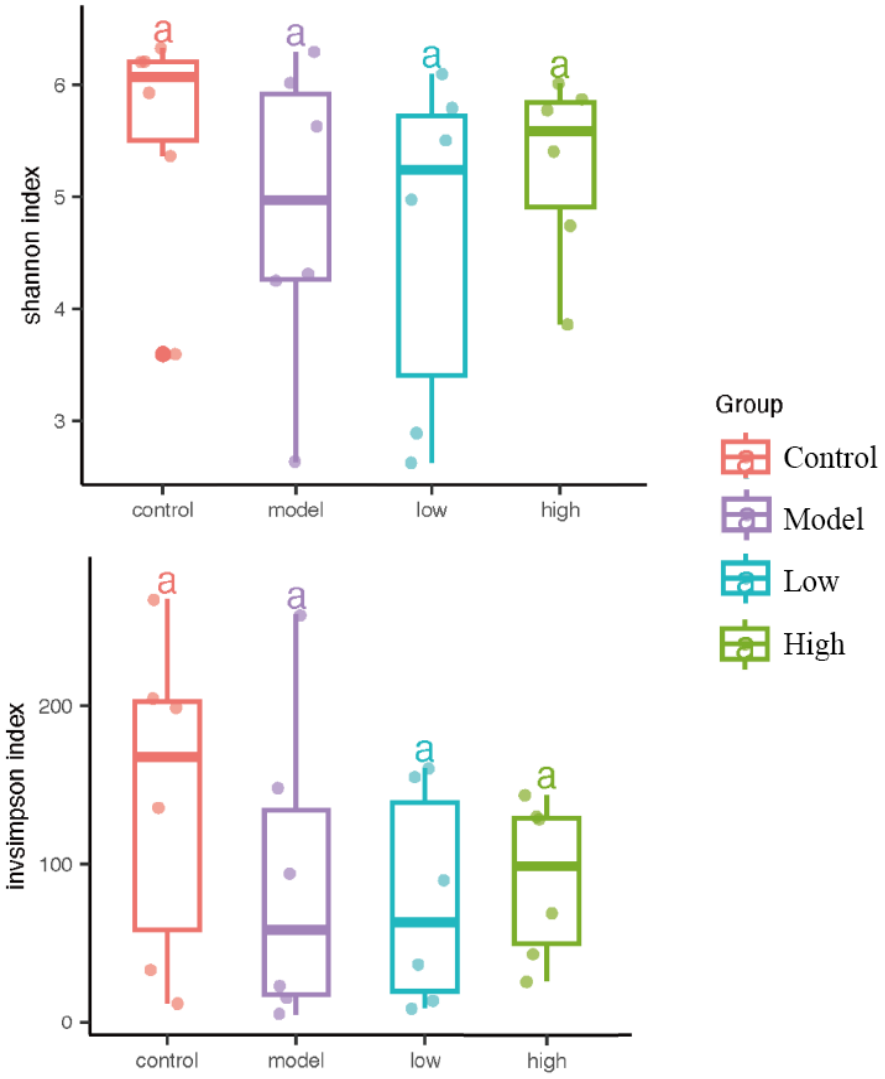

Fig. S8. Box plot of gut microbial alpha diversity index after SFE pretreatment.

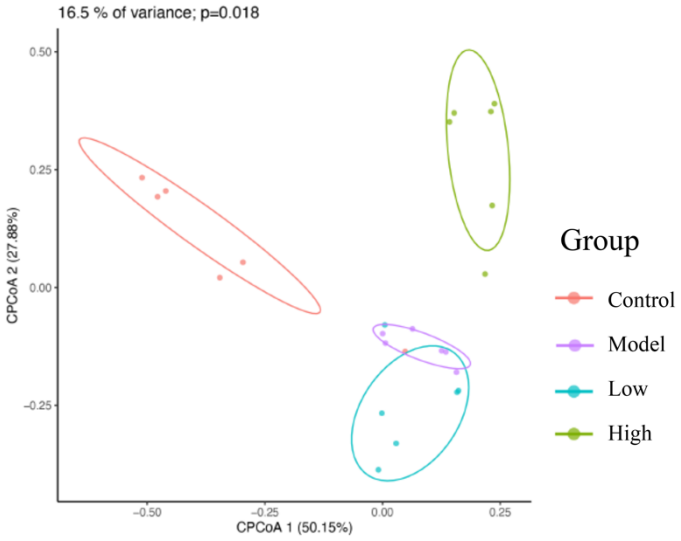

Fig. S9. PCoA analysis of gut microbe after SFE pretreatment based on unweighted UniFrac distance matrix.

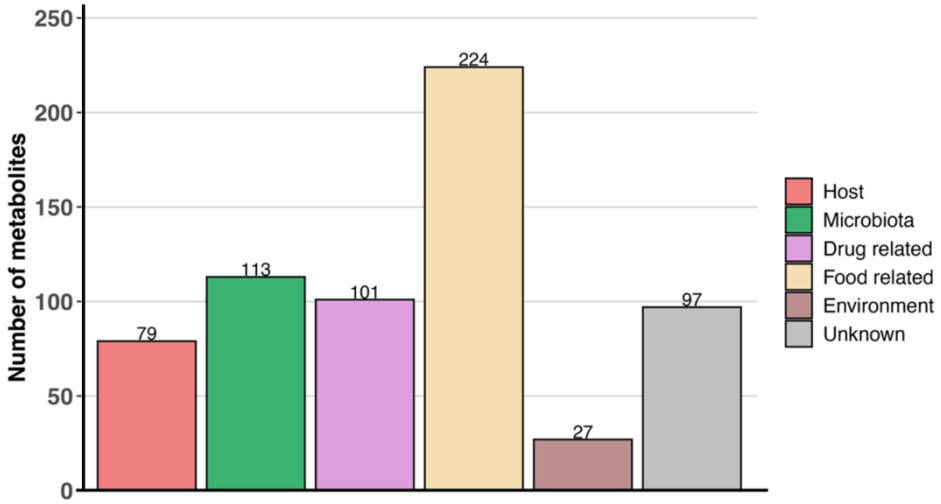

Fig. S10. Bar graph about the source of detected metabolites from all four groups (data combined from control, model, low-dose, and high-dose SFE groups).

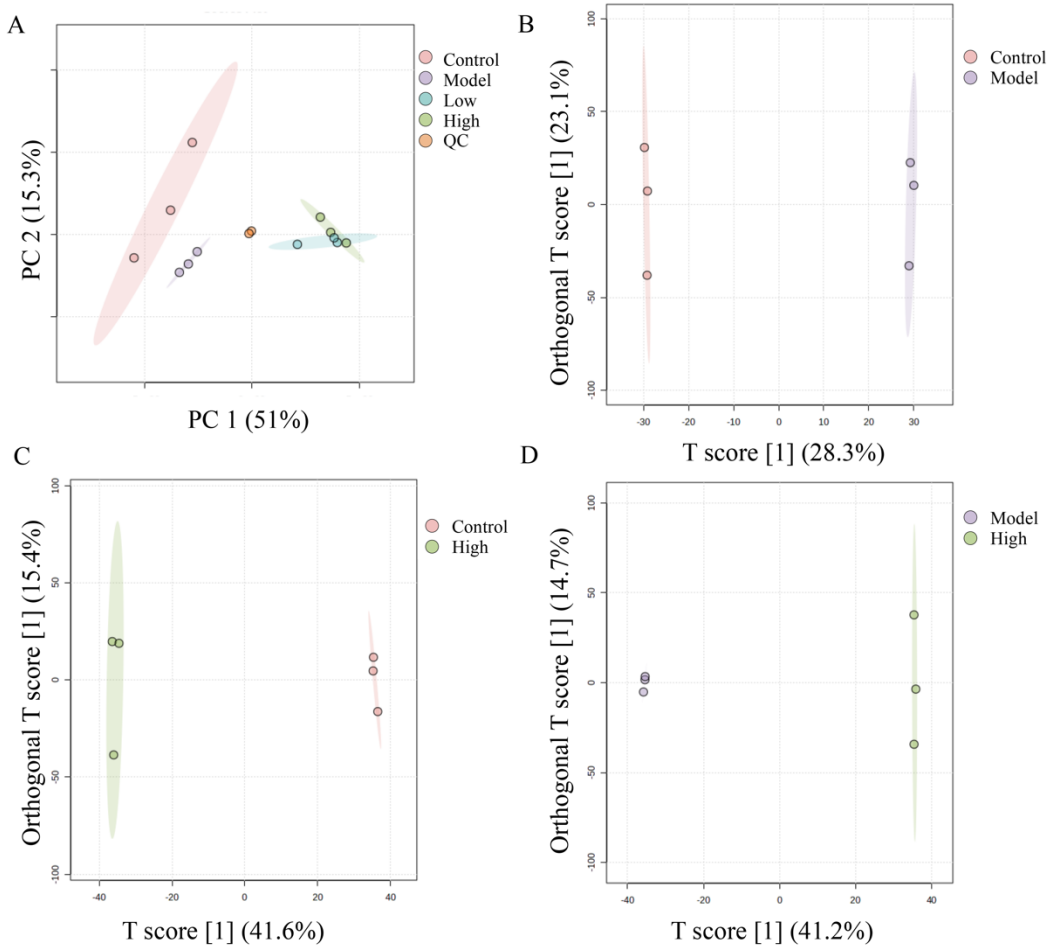

Fig. S11. Multivariate statistical analysis of untargeted metabolomics data in the fecal samples. PCA score plot of each sample (**A**), OPLS-DA analysis of three comparison groups: control vs. model (B), control vs. high (C), and model vs. high (D).