Shaoyao Gancao Decoction (SGD) is a classic prescription in Zhang Zhongjing’s Treatise on Febrile Diseases [1]. It uses Paeonia lactiflora and licorice to nourish yin and blood and to pass through the meridians to treat blood deficiency and body pain, including abdominal pain due to qi and blood deficiency [2–5]. Chemical composition studies show that SGD mainly contains flavonoids, triterpenoid saponins, monoterpenoid glycosides, phenolic acids, tannins, and some other compounds [6–11]. Modern pharmacological studies show that SGD has the functions of relieving spasmolysis and analgesia, protecting the liver, anti-inflammatory, relieving cough and asthma, anti-allergy, and immune regulation, etc. It is clinically applied to spastic pain, liver injury, inflammatory pain, asthma, intestinal ulcers, uterine fibroids, hyperandrogenism, fatty liver, Parkinson’s disease, etc. [12–14].

Our previous study found that SGD had an obvious liver-protecting effect [15]. After 14 days of oral
administration of SGD in model group rats, the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TBIL) were significantly decreased, and the histopathological structure of the liver was significantly improved. SGD has been widely used in the treatment of viral hepatitis, cholestatic hepatitis, and acute and chronic hepatitis B [16]. The Chinese patent medicine Jianganle Granules prepared from SGD has been used in the clinical treatment of cholestatic hepatitis, and acute and chronic hepatitis B [16]. The logical structure of the liver was significantly improved. SGD (TBIL) were significantly decreased, and the histopathological parameters of the liver were provided with therapeutic effects [17]. However, the active ingredients and mechanism of SGD against liver injury are still unclear.

This research established a new approach for finding biomarkers for the quality control of SGD. 19 chemical components were selected as the biomarkers of SGD against liver injury based on UPLC-Q-TOF-MS/MS combined with network pharmacology, and molecular docking. And the 19 components were detected in the rat serum, which also proved that they could be biomarkers. In the end, a UPLC-QQQ-MS/MS method for simultaneously determining of the contents of the 19 compounds was constructed. To the best of our knowledge, this is the first report using UPLC-MS/MS, network pharmacology, and molecular docking approaches to find the biomarkers for the quality control of SGD. This study clarified the active components, key targets, and pathways of SGD against liver injury and provided a new idea for the selection of quality control indicators in traditional Chinese medicine. The method developed in our study also provides a scientific foundation for the study of anti-liver injury effective substances in SGD.

2. Materials and Methods

2.1. Materials and Reagents. Reference substance: 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (PS020247, purity: 98.0%), coniferiferumulate (PS011045, purity: 95.0%), benzyloxyacetofuran (PS000157, purity: 98.0%), methyl gallate (PS020247, purity: 98.0%), isoliquiritigenin apiose (PS020153, purity: 98.0%), isoliquiritigenin (PS012517, purity: 98.0%) were purchased from Chengdu Pusi Biotechnology Co., LTD; ferulic acid (110773–201313, purity: 99.6%), hesperidin (110721–201617, purity: 96.1%), liquiritin (111610–201908, purity: 95.0%), caffeic acid (110773–201614, purity: 99.0%), rutin (100080–201811, purity: 91.7%), chlorogenic acid (110753–201817, purity: 96.8%), gallic acid (110831–201204, purity: 89.9%), and narirutin (112007–201817, purity: 96.1%) were purchased from the China National Institute for Food and Drug Control; liquiritigenin (MUST-17022104, purity: 99.07%), glycyrrhizic acid (MUST-17022104, purity: 99.65%), albizofuran (MUST-18041601, purity: 99.14%), neochlorogenic acid (MUST-12113001, purity: 98.00%), and naringenin (MUST-16032406, purity: 99.18%) purchased from Chengdu MUST Biotechnology Co., Ltd. Methanol, formic acid, and acetonitrile (LC-MS grade) were purchased from Merck (Merck & Co., Inc.). Distilled water is supplied by Watsons (A. S. Watson TM Limited).

2.2. Sample Preparation. Bai Shao (BS) and Gan Cao (GC) were identified by Sun Baohui, chief pharmacist at the Hebei Drug Inspection Institute. BS is the dry root of Paeonia lactiflora Pall., and GC is the dry root of Glycyrrhiza uralensis Fisch. Refer to the original record of “Treatise on Febrile Diseases,” the decocting method of water extract of SGD was determined as follows: take 12 g each of BS and GC, add 600 mL of water, boil to 300 mL, filter, cool the filtrate, concentrate to about 50 mL (1 g: 2 mL) of extract, freeze-dry, and get SGD freeze-dried powder, the average yield of freeze-dried powder is 23%. Precisely weigh 0.1 g of freeze-dried powder, put it in a 25 mL volumetric flask, add 50% methanol to dissolve, sonicate for 10 min, and dilute to the mark with 50% methanol, shake well, and filter with a 0.22 μm filter membrane.

2.3. Apparatus and Parameters

2.3.1. Optimization of UPLC-Q-TOF-MS/MS Detection Conditions. A Waters ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm) was used to separate the aqueous extract of SGD. Take acetonitrile as mobile phase A, and 0.1% aqueous acetic acid as mobile phase B. The gradient elution was as follows: 0 3 min, 5% 15% A; 3 5 min, 15% 18% A; 5 10 min, 18% 24% A; 10 14 min, 24% 26% A; 14 25 min, 26% 45% A; 25 29 min, 45% 100% A; 29 30 min, 100% 5% A; 30 35 min, 5% A. The flow rate was 0.3 mL·min⁻¹, the column temperature was 35°C, and the injection volume was 1 μL. Mass analysis was performed by ‘Triple TOF TM 6600’ (AB SCIEX, Foster City, CA, USA) equipped with an electrospray ionization (ESI) source. MS analysis was performed in both positive and negative ionization modes by full scan mode. The optimized parameters are as follows: ion spray voltage (ISV), 5.5 kV (ESI+) or −4.5 kV (ESI−); ion source temperature (TEM) 550°C; atomized gas 50 psi; auxiliary gas, 50 psi; curtain gas (CUR), 35 psi. The cluster removal potential (DP) is 80 V. Collision energies (CE) are 40 ± 20 eV (MS mode). TOF-MS scan range: m/z 100-2000. Daughter ion and scanning range: m/z 50-1500. In addition, in order to reduce systematic errors and improve the accuracy of mass spectrometry detection, the CDS system was used to calibrate the quality accuracy before each sample injection. When setting up sample batch processing, insert a quality control sample between every three samples to be tested to ensure the accuracy of the test results. The SCIEX OS-Q 2.0 software (AB SCIEX, Foster City, CA, USA) was used for data collection, and the PeakView®2.2 software (AB SCIEX, Foster City, CA, USA) was used for processing and analyzing mass spectrum data.

2.3.2. Optimization of UPLC-QQQ-MS/MS Detection Conditions. UPLC-QQQ-MS/MS analysis was performed on the LC-30A UPLC system (Shimadzu, Kyoto, Japan), including a DGU-30A3 type online vacuum degasser, LC-30AD-type binary pump, SIL-30AC-type automatic sampler, and CTO-30A-type column incubator. The
experimental conditions were as follows: Shim-pack GIST C<sub>18</sub> (100 mm × 2.1 mm, 2 μm) chromatographic column; column temperature, 35°C; flow rate, 0.3 mL·min<sup>−1</sup>; and injection volume, 1 μL. The mobile phase and gradient elution are the same with "2.3.1. Optimization of UPLC-Q-TOF-MS/MS detection conditions." In terms of mass spectrometry, we chose a QTRAP 4500 (AB SCIEX, Foster City, CA, USA) coupled with an electrospray ionization (ESI) source [18, 19]. The negative was monitored in the scanning mode of multiple reaction monitoring (MRM), the ion source was electrospray ionization (ESI), the ionization voltage (IS) was −4500 V, and the ion source temperature (TEM) was 550°C. The spray gas (GS1, N2) is 345 kPa, the auxiliary gas (GS2, N2) is 345 kPa, the interface is continuously heated, nitrogen gas is introduced throughout the whole process, the curtain gas (CUR, N2) is 207 kPa, and the collision gas (CAD) pressure is medium. The residence time (dwell time) of the ion pair is 50 ms. To optimize transitions, declustering potential (DP), and collision energy (CE) for each component compound, mass spectrometry parameters are shown in Table 1.

2.4. Structure Analysis Procedure. By searching the related literature of BS, GC, and SGD, a local database of SGD covering 690 compounds was established, including compound names, molecular formulas, precise relative molecular weights, and mol structures. For the components that could find the reference, we analyzed the structure by comparing the retention time and the secondary mass spectrometry data of the components both in the reference solution and the sample solution. For unknown components, we used OS software to confirm the structure of compounds by comparing the local database of SGD, the TCM MS/MS database, and the online ChemSpider database. Generally, ppm less than 5 is taken as a necessary judgment index. The fragmentation pattern of representative components was analyzed by the MS/MS spectrogram of the compound. The above matching compounds were classified, and the compounds contained in the sample were determined according to the typical MS/MS cracking laws of each type of compound.

2.5. Active Ingredient Identification Strategy. Referring to our previous research on the single medicine Phyllanthus emblica L. [19], the overall idea was as follows: based on the compounds identified by UPLC-Q-TOF-MS/MS, network pharmacology was used to find the active components of SGD against liver injury. Then molecular docking was used to verify the efficacy of the compounds. In order to further verify the screened biomarkers, we analyzed the chemical components that were absorbed into the rat's blood by SGD. All animal experiments meet the requirements of the Ethics Committee of the Hebei University of Chinese Medicine.

3. Results and Discussion

3.1. Identification of the Chemical Components in SGD by UPLC-ESI-Q-TOF-MS. UPLC-Q-TOF-MS was used to detect the samples in both positive and negative modes. According to reference substance comparison, literature study, and MS/MS information, choose acetonitrile 0.1% acetic acid water as the mobile phase system of good appraisal and concluded that for the 110 chemical elements, the typical total ion chromatograms for positive and negative ion modes are shown in Figure 1. All compounds and related information are shown in Tables 2 and 3.

3.1.1. Identification of Flavonoids. In this study, flavonoids were detected in positive and negative ion modes, respectively, and it was found that the absorption intensity of the tested products was similar in the two modes. 28 compounds were identified in the positive mode, and 26 compounds were identified in the negative mode. It was found that the glycosidic bond was first broken by the cleavage of flavonoid glycosides, and then the chemical bond between sugars was broken. The C-4 of the C ring easily lost CO, while the hydroxyl groups of the A ring, B ring, and C ring usually lost H₂O. CH₂, CH₄, or whole branch chains (such as licorice flavone C) were occasionally dropped when there were carbon chains on the ring A. CH₃ and H₂O are easily lost when methoxyl and hydroxyl groups replace the B ring. B ring single drop is more common. Cleavage mostly occurs on the C ring, probably due to the carbonyl group on the C ring. The density of the electron cloud is large, easy to occur RDA cleavage. The characteristic ion fragment mass numbers 119 and 137 were found, which can be used as a reference for the identification of flavonoids in the future.

Compound 12<sup>α</sup> is taken as an example; it shows that the retention time of compound 12 is 9.36 min and the excimer ion peak m/z 257.0824 [M + H]<sup>+</sup> is formed in the positive mode, and its molecular formula is C₁₅H₁₂O₄. With the loss of H₂O and CO, the fragment ions m/z 239.0732 and 211.0744 were obtained. RDA cleavage produces two ionic fragments, 119.0496 and 137.0238. The secondary mass spectrometry of liquiritigenin and its pyrolysis process are shown in Figure 2.
| No. | Rt (min) | Formula          | Mass (Da) | Ppm  | MS/MS fragments                                                                 | Identification  | Source |
|-----|----------|------------------|-----------|------|---------------------------------------------------------------------------------|----------------|--------|
| 1d  | 1.48     | C₁₀H₈O₅          | 170.0215  | 1.9  | 107.0118[M + HCH₂O₃]⁺, 125.0223[M + HCH₂O₃]⁺, 153.0175[M + H₂O]⁺                | Gallic acid     | BS     |
| 2c  | 4.46     | C₂₃H₂₈O₁₂        | 496.1581  | 0.8  | 197.0824[M + HC₁₃H₁₆O₇]⁺; 133.0640[M + HC₁₃H₂₀O₈]⁺; 121.0261[M + HC₁₆H₂₀O₁₀]⁺ | Oxypaeoniforin  | BS     |
| 3a  | 4.69     | C₁₅H₁₄O₆         | 290.079   | 0.1  | 291.0863[M + H]⁺; 207.0648[M + HC₁₄H₂₀O₇]⁺; 163.0431[M + HC₁₅H₂₂O₉]⁺; 123.0432[M + HC₁₆H₂₄O₁₀]⁺ | Catechin        | BS     |
| 4d  | 6.62     | C₁₀H₁₀O₄          | 194.0579  | 0.6  | 163.0401[M + HCH₂O₃]⁺, 135.0441[M + HC₁₄H₂₀O₈]⁺ | Ferulic acid    | BS     |
| 5c  | 7.14     | C₂₃H₂₈O₁₁        | 480.1632  | 0    | 197.0808[M + HC₁₃H₁₆O₇]⁺; 179.0703[M + HC₁₅H₁₈O₉]⁺; 161.0592[M + HC₁₆H₂₂O₁₀]⁺ | Paeoniforin     | BS     |
| 6c  | 7.14     | C₂₃H₂₈O₁₁        | 480.1632  | 0    | 197.0808[M + HC₁₃H₁₆O₇]⁺; 179.0703[M + HC₁₅H₁₈O₉]⁺; 161.0592[M + HC₁₆H₂₂O₁₀]⁺ | Paeoniforin     | BS     |
| 7c  | 7.14     | C₁₇H₁₈O₆         | 318.1103  | 0.7  | 121.0653[M + HC₁₃H₁₂O₄]⁺; 105.0698[M + HC₂₀H₁₄O₄]⁺ | Albitforin      | BS     |
| 8c  | 7.14     | C₂₃H₂₈O₁₁        | 480.1632  | 0    | 197.0808[M + HC₁₃H₁₆O₇]⁺; 179.0703[M + HC₁₅H₁₈O₉]⁺; 161.0592[M + HC₁₆H₂₂O₁₀]⁺ | Albitforin R1   | BS     |
| 9a  | 8.11     | C₂₆H₂₈O₁₄        | 564.1479  | 1.6  | 565.1552[M + H]⁺; 547.1428[M + HC₂₃H₂₂O₁₂]⁺; 529.1343[M + HC₂₆H₂₄O₁₄]⁺ | Schaftoside     | GC     |
| 10a | 9.08     | C₂₇H₃₂O₁₄        | 580.1792  | 0.8  | 147.0438[M + HC₁₃H₂₀O₂]⁺; 137.0244[M + HC₁₃H₂₀O₁₀]⁺ | Glycyrrhizin-7,4′-diglucoside | GC     |
| 11a | 9.08     | C₂₇H₃₂O₁₄        | 580.1792  | 0.8  | 257.0813[M + HC₁₃H₂₀O₁₀]⁺ | Narirutin       | BS     |
| 12a | 9.36     | C₁₅H₁₂O₄         | 256.0735  | 0.7  | 257.0824[M + H]⁺; 147.0441[M + HC₁₃H₂₀O₂]⁺; 137.0238[M + HC₂₆H₂₄O₄]⁺; 119.0496[M + HC₂₆H₂₄O₄]⁺ | Liquiritigenin  | GC     |
| 13a | 9.52     | C₂₇H₃₀O₁₄        | 578.1635  | 1.8  | 579.1708[M + H]⁺; 543.1475[M + HC₂₃H₂₀O₈]⁺; 507.1297[M + HC₂₆H₂₄O₁₂]⁺ | Violanthin      | GC     |
| 14b | 10.59    | C₃₆H₃₂N₄O₂        | 565.4131  | 12   | 566.4304[M + H]⁺; 548.4196[M + HOH₂]⁺; 209.1654 | Heinsiagenin A   | GC     |
| 15d | 10.69    | C₄₁H₃₂O₂₆        | 940.1182  | 0.3  | 455.0820[M + HC₂₃H₁₂O₁₃]⁺; 345.0208[M + HC₂₃H₁₄O₁₇]⁺ | 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose | BS     |
| No. | R<sub>t</sub> (min) | Formula | Mass (Da) | Ppm | MS/MS fragments | Identification | Source |
|-----|-------------------|---------|-----------|-----|-----------------|----------------|--------|
| 16* | 11.46             | C<sub>16</sub>H<sub>12</sub>O<sub>6</sub> | 300.0634  | −2.2 | 301.0723[M + H]<sup>+</sup>; 285.0395[M + H-CH<sub>3</sub>]<sup>+</sup>; 283.0601[M + H-H<sub>2</sub>O]<sup>+</sup>; 269.0439[M + H-CH<sub>3</sub>O]<sup>+</sup> | 7,2',4'-trihydroxy-3-(5-methoxyphenyl) chromen-2-one | GC |
| 17<sup>de</sup> | 11.54             | C<sub>12</sub>H<sub>16</sub>O<sub>2</sub> | 192.115   | 2.6  | 193.0872[M + H]<sup>+</sup> | Cuminyl acetate | BS |
| 18<sup>a</sup> | 11.72             | C<sub>21</sub>H<sub>20</sub>O<sub>10</sub> | 433.2046[M + H]<sup>+</sup> | 0.38 | 149.0244[M + H-C<sub>3</sub>H<sub>6</sub>]<sup>+</sup> | Vitexin | GC |
| 19<sup>c</sup> | 11.74             | C<sub>30</sub>H<sub>32</sub>O<sub>15</sub> | 633.1793  | 0.5  | 153.0182[M + H-C<sub>23</sub>H<sub>18</sub>O<sub>11</sub>]<sup>+</sup> | Galloyl paeoniflorin | BS |
| 20<sup>a</sup> | 11.74             | C<sub>30</sub>H<sub>32</sub>O<sub>15</sub> | 633.1793  | 0.5  | 153.0182[M + H-C<sub>23</sub>H<sub>18</sub>O<sub>11</sub>]<sup>+</sup> | Galloyl paeoniflorin | BS |
| 21<sup>a</sup> | 11.75             | C<sub>15</sub>H<sub>12</sub>O<sub>5</sub> | 272.0685  | 1.8  | 153.0187[M + H-C<sub>7</sub>H<sub>5</sub>O<sub>4</sub>]<sup>+</sup> | Naringenin | GC |
| 22<sup>a</sup> | 13.26             | C<sub>26</sub>H<sub>30</sub>O<sub>13</sub> | 550.1686  | −1.4 | 551.1759[M + H]<sup>+</sup>; 257.0805[M + H-C<sub>11</sub>H<sub>18</sub>O<sub>9</sub>]<sup>+</sup> | Liquiritin apioside | GC |
| 23<sup>a</sup> | 13.26             | C<sub>26</sub>H<sub>30</sub>O<sub>13</sub> | 550.1686  | −1.4 | 551.1759[M + H]<sup>+</sup>; 257.0805[M + H-C<sub>11</sub>H<sub>18</sub>O<sub>9</sub>]<sup>+</sup> | Isoliquiritin apioside | GC |
| 24<sup>a</sup> | 13.56             | C<sub>21</sub>H<sub>22</sub>O<sub>9</sub> | 418.1264  | −0.5 | 419.1337[M + H]<sup>+</sup>; 257.0813[M + H-C<sub>11</sub>H<sub>16</sub>O<sub>7</sub>]<sup>+</sup> | Licochalcone B | GC |
| 25<sup>a</sup> | 13.56             | C<sub>21</sub>H<sub>22</sub>O<sub>9</sub> | 418.1264  | −0.5 | 419.1337[M + H]<sup>+</sup>; 257.0813[M + H-C<sub>11</sub>H<sub>16</sub>O<sub>7</sub>]<sup>+</sup> | Licochalcone B | GC |
| 26<sup>a</sup> | 13.76             | C<sub>22</sub>H<sub>20</sub>O<sub>9</sub> | 430.1264  | −0.6 | 431.1337[M + H]<sup>+</sup>; 269.0899[M + H-C<sub>13</sub>H<sub>19</sub>O<sub>13</sub>]<sup>+</sup> | Ononin | GC |
| 27<sup>a</sup> | 13.86             | C<sub>16</sub>H<sub>14</sub>O<sub>5</sub> | 286.0841  | −0.7 | 287.0925[M + H]<sup>+</sup>; 245.0782[M + H-C<sub>9</sub>H<sub>10</sub>O<sub>5</sub>]<sup>+</sup> | Licochalone B | GC |
| 28<sup>a</sup> | 14.96             | C<sub>16</sub>H<sub>12</sub>O<sub>5</sub> | 284.0685  | −0.1 | 285.0765[M + H]<sup>+</sup>; 269.0433[M + H-CH<sub>4</sub>]<sup>+</sup>; 253.0504[M + H-CH<sub>3</sub>O]<sup>+</sup> | Calycosin | GC |
| 29<sup>a</sup> | 16.83             | C<sub>14</sub>H<sub>10</sub>O<sub>4</sub> | 270.0892  | −0.8 | 271.0765[M + H]<sup>+</sup>; 229.0868[M + H-CH<sub>4</sub>]<sup>+</sup>; 177.0548[M + H-CH<sub>3</sub>O]<sup>+</sup> | Retro chalcone | GC |
| 30<sup>e</sup> | 17.11             | C<sub>21</sub>H<sub>20</sub>O<sub>7</sub> | 384.1209  | −0.2 | 385.1291[M + H]<sup>+</sup>; 367.1194[M + H-CH<sub>2</sub>O]<sup>+</sup>; 339.0833[M + H-CH<sub>3</sub>O]<sup>+</sup>; 329.1375[M + H-CH<sub>3</sub>]<sup>+</sup>; 283.0630[M + H-CH<sub>4</sub>O]<sup>+</sup> | Licopyranocoumarin | BS |
Table 2: Continued.

| No. | Rf (min) | Formula | Mass (Da) | Ppm | MS/MS fragments | Identification | Source |
|-----|----------|---------|-----------|------|-----------------|----------------|--------|
| 31c | 17.52    | C30H32O12 | 584.1894  | −1.2 | 585.2905[M + H]+; 319.1181[M + H-C4H12O6]+; 301.0343[M + H-C6H14O7]+; 267.0852[M + H-C8H16O8]+; 249.0768[M + H-C10H18O9]+ | Benzoyl paeoniflorin | GC     |
| 32b | 17.58    | C48H72O21 | 985.4566  | 0.2  | 985.4652[M + H]+; 809.4326[M + H-C6H8O6]+; 647.3772[M + H-C12H18O13]+; 471.3455[M + H-C18H26O17]+; 453.3566[M + H-C12H18O13]+ | Licoricesaponin A3 | GC     |
| 33a | 19.25    | C16H12O4  | 268.0735  | 0.7  | 269.0826[M + H]+; 253.0506[M + H-CH4]+; 237.0555[M + H-CH4O]+; 225.0555[M + H-CH2O]+; 137.0238[M + H-9H8O]+ | Formononetin | GC     |
| 34b | 20.06    | C42H62O17 | 838.3987  | −0.9 | 484.3436[M + H-C12H16O12]+; 469.3328[M + H-C12H18O13]+; 451.3219[M + H-C12H18O13]+ | Licoricesaponin G2 | GC     |
| 35b | 20.07    | C30H46O5  | 486.3345  | −0.9 | 487.3432[M + H]+; 317.2118[M + H-C10H18O]+; 235.1689[M + H-C15H24O2]+ | Echinic acid | GC     |
| 36b | 20.07    | C30H46O5  | 486.3345  | −0.9 | 487.3432[M + H]+; 317.2118[M + H-C10H18O]+; 235.1689[M + H-C15H24O2]+ | Isoechinic acid | GC     |
| 37b | 20.07    | C30H46O5  | 486.3345  | −0.9 | 487.3432[M + H]+; 317.2118[M + H-C10H18O]+; 235.1689[M + H-C15H24O2]+ | Triphyllic acid | GC     |
| 38b | 20.07    | C30H46O4  | 468.3239  | 0     | 469.3324[M + H]+; 451.3214[M + H-C12H16O12]+; 439.3210[M + H-C18H26O17]+ | Glabrolide | GC     |
| 39b | 21.23    | C30H46O4  | 470.3396  | −0.9 | 471.3480[M + H]+; 431.3214[M + H-C12H16O12]+; 393.3210[M + H-C18H26O17]+ | Glycyrrhetinic acid | GC     |
| 40b | 21.23    | C30H46O4  | 470.3396  | −0.9 | 471.3480[M + H]+; 431.3214[M + H-C12H16O12]+; 393.3210[M + H-C18H26O17]+ | Liciritic acid | GC     |
| 41b | 21.23    | C30H46O4  | 470.3396  | −0.9 | 471.3480[M + H]+; 431.3214[M + H-C12H16O12]+; 393.3210[M + H-C18H26O17]+ | Macedonic acid | GC     |
| 42b | 21.23    | C36H54O10 | 646.3717  | −0.2 | 647.3801[M + H]+; 453.3328[M + H-C16H16O13]+ | Glycyrrhetinic acid 3-O-β-D-glucuronide | GC     |
| 43b | 21.23    | C36H54O10 | 646.3717  | −0.2 | 647.3801[M + H]+; 453.3328[M + H-C16H16O13]+ | Isomacedonic acid | GC     |
| 44b | 22.16    | C30H48O3  | 456.3604  | −1.5 | 457.3671[M + H]+; 303.2310[M + H-C10H18O]+ | Oleanolic acid | GC     |
| 45b | 22.16    | C30H48O3  | 456.3604  | −1.5 | 457.3671[M + H]+; 303.2310[M + H-C10H18O]+ | Betulinic acid | GC     |
| 46b | 22.16    | C30H48O3  | 456.3604  | −1.5 | 457.3671[M + H]+; 303.2310[M + H-C10H18O]+ | 11-Deoxyglycyrrhetinic acid | GC     |
| 47b | 22.16    | C30H48O3  | 456.3604  | −1.5 | 457.3671[M + H]+; 303.2310[M + H-C10H18O]+ | Glycyrrhetol | GC     |
| 48a | 23.21    | C20H26O6  | 356.12599 | 0.9  | 357.1333[M + H]+; 301.0710[M + H-CH4]+; 283.0605[M + H-C4H12O6]+ | Sigmoidin B | GC     |
| 49b | 23.21    | C20H26O6  | 356.1259  | 0.9  | 357.1333[M + H]+; 301.0710[M + H-CH4]+; 283.0605[M + H-C4H12O6]+ | Piperitol | GC     |
| 50a | 23.21    | C20H26O6  | 356.1259  | 0.9  | 357.1333[M + H]+; 301.0710[M + H-CH4]+; 283.0605[M + H-C4H12O6]+ | Coniferyl ferulate | GC     |
| 51a | 23.26    | C17H12O6  | 314.079   | 0.3  | 315.0872[M + H]+; 299.0583[M + H-C4H12O6]+ | Kumatakenin | GC     |
| No. | \( R_t \) (min) | Formula | Mass (Da) | Ppm | MS/MS fragments | Identification | Source |
|-----|-----------------|---------|-----------|-----|----------------|----------------|--------|
| 52a | 23.29           | C<sub>21</sub>H<sub>20</sub>O<sub>6</sub> | 368.1259  | 0   | 369.1341[M + H]+; 313.0710[M + H-C<sub>4</sub>H<sub>8</sub>]+; 285.0763[M + H-C<sub>5</sub>H<sub>8</sub>O]+; 271.0600[M + H-C<sub>6</sub>H<sub>10</sub>O]+; 243.0656[M + H-C<sub>7</sub>H<sub>10</sub>O<sub>2</sub>]+; 227.0700[M + H-C<sub>8</sub>H<sub>12</sub>O<sub>3</sub>]+ | Licoarylcoumarin | GC |
| 53e | 23.29           | C<sub>21</sub>H<sub>20</sub>O<sub>6</sub> | 368.1259  | 0   | 369.1341[M + H]+; 313.0710[M + H-C<sub>4</sub>H<sub>8</sub>]+; 285.0763[M + H-C<sub>5</sub>H<sub>8</sub>O]+; 271.0600[M + H-C<sub>6</sub>H<sub>10</sub>O]+; 243.0656[M + H-C<sub>7</sub>H<sub>10</sub>O<sub>2</sub>]+; 227.0700[M + H-C<sub>8</sub>H<sub>12</sub>O<sub>3</sub>]+ | Glycycoumarin | GC |
| 54a | 24.14           | C<sub>20</sub>H<sub>18</sub>O<sub>6</sub> | 354.1103  | −0.3| 355.1184[M + H]+; 337.1076[M + H-H<sub>2</sub>O]+; 245.0454[M + H-C<sub>6</sub>H<sub>6</sub>O<sub>2</sub>]+; 243.0454[M + H-C<sub>6</sub>H<sub>8</sub>O<sub>2</sub>]+; 229.0862[M + H-C<sub>7</sub>H<sub>10</sub>O<sub>2</sub>]+; 189.0905[M + H-C<sub>10</sub>H<sub>14</sub>O<sub>2</sub>]+; 179.0386[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>]+; 163.0384[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>]+ | Gancaonin C | GC |
| 55a | 24.14           | C<sub>20</sub>H<sub>18</sub>O<sub>6</sub> | 354.1103  | −0.3| 355.1184[M + H]+; 337.1076[M + H-H<sub>2</sub>O]+; 299.0556[M + H-C<sub>3</sub>H<sub>4</sub>O]+; 217.0500[M + H-C<sub>7</sub>H<sub>6</sub>O<sub>3</sub>]+; 201.0910[M + H-C<sub>7</sub>H<sub>6</sub>O<sub>4</sub>]+; 189.0905[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>]+; 153.0182[M + H-C<sub>13</sub>H<sub>14</sub>O<sub>2</sub>]+; 151.0386[M + H-C<sub>13</sub>H<sub>14</sub>O<sub>3</sub>]+ | Licoisofavanone | GC |
| 56a | 24.31           | C<sub>21</sub>H<sub>22</sub>O<sub>4</sub> | 338.1518  | −0.8| 339.1577[M + H]+; 297.1528[M + H-C<sub>4</sub>H<sub>8</sub>O]+; 245.1195[M + H-C<sub>6</sub>H<sub>6</sub>O<sub>2</sub>]+; 243.0654[M + H-C<sub>6</sub>H<sub>8</sub>O<sub>2</sub>]+; 229.0851[M + H-C<sub>7</sub>H<sub>10</sub>O<sub>2</sub>]+; 187.0744[M + H-C<sub>8</sub>H<sub>8</sub>O<sub>3</sub>]+; 121.0287[M + H-C<sub>14</sub>H<sub>18</sub>O<sub>2</sub>]+; 107.0498[M + H-C<sub>14</sub>H<sub>16</sub>O<sub>3</sub>]+ | Licochalcone A | GC |
| 57e | 24.4            | C<sub>22</sub>H<sub>22</sub>O<sub>6</sub> | 382.1416  | −0.1| 367.1178[M + H]+; 339.1264[M + H-C<sub>2</sub>H<sub>4</sub>O]+; 283.0605[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>]+; 281.0395[M + H-C<sub>13</sub>H<sub>14</sub>O<sub>2</sub>]+; 271.0609[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>]+; 255.0595[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>4</sub>]+; 237.0656[M + H-C<sub>11</sub>H<sub>14</sub>O<sub>2</sub>]+; 227.0700[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>]+ | Glucyrin | GC |
| 58e | 24.57           | C<sub>21</sub>H<sub>18</sub>O<sub>6</sub> | 366.1103  | −1  | 339.1264[M + H-C<sub>2</sub>H<sub>4</sub>O]+; 283.0615[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>]+; 281.0395[M + H-C<sub>13</sub>H<sub>14</sub>O<sub>2</sub>]+; 271.0609[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>]+; 255.0595[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>4</sub>]+ | Neoglycyrol | GC |
| 59a | 24.58           | C<sub>20</sub>H<sub>18</sub>O<sub>5</sub> | 338.1154  | −1  | 339.1273[M + H]+; 297.0762[M + H-C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>]+; 283.0604[M + H-C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>]+; 271.0609[M + H-C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>]+; 255.0595[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>]+; 243.0456[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>4</sub>]+; 227.0678[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>]+ | Licoflavone C | GC |
| 60a | 24.6            | C<sub>21</sub>H<sub>22</sub>O<sub>5</sub> | 354.1467  | −0.7| 355.1467[M + H]+; 299.0934[M + H-C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>]+; 215.1055[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>]+; 191.1081[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>]+; 173.0974[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>]+; 153.0550[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>4</sub>]+ | 3'-methoxyglabridin | GC |
| 61a | 25.17           | C<sub>20</sub>H<sub>20</sub>O<sub>4</sub> | 324.1361  | −0.6| 325.1362[M + H]+; 297.0609[M + H-C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>]+; 283.0604[M + H-C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>]+; 271.0609[M + H-C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>]+; 255.0595[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>]+; 243.0456[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>4</sub>]+; 227.0678[M + H-C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>]+ | Bavachin | GC |
| No. | \(R_t\) (min) | Formula | Mass (Da) | Ppm | MS/MS fragments | Identification | Source |
|-----|----------------|---------|-----------|-----|----------------|---------------|--------|
| 62a | 25.38 | \(C_{21}H_{26}O_{5}\) | 352.131 | −0.9 | 353.1399\([M+H]^+\); 325.1398\([M+H-CO]^+\); 323.0944\([M+H-CH_2O]^+\); 193.0485\([M+H-C_{12}H_{26}O_4]^+\); 189.0923\([M+H-C_{19}H_{20}O_4]^+\); 165.0548\([M+H-C_{12}H_{12}O_2]^+\); 147.0799\([M+H-C_{13}H_{14}O_2]^+\); 135.0463\([M+H-C_{13}H_{14}O_3]^+\); 123.0438\([M+H-C_{13}H_{16}O_4]^+\); 119.0494\([M+H-C_{13}H_{14}O_4]^+\) | Gancaonin G | GC |
| 63a | 25.55 | \(C_{20}H_{16}O_6\) | 352.0947 | −1.3 | 353.1023\([M+H]^+\); 335.0916\([M+H-H_2O]^+\); 325.1064\([M+H-CO]^+\); 311.0553\([M+H-C_3H_6]^+\); 295.0596\([M+H-C_3H_5O]^+\); 283.0596\([M+H-C_5H_6O]^+\); 217.0489\([M+H-C_7H_4O_3]^+\); 153.0176\([M+H-C_{13}H_{12}O_2]^+\) | Licoisoflavone B | GC |
| 64a | 26.03 | \(C_{20}H_{16}O_5\) | 336.0997 | −0.5 | 309.1153\([M+H-CO]^+\); 295.0589\([M+H-C_2H_4]^+\); 271.0615\([M+H-C_2H_4]^+\); 267.0670\([M+H-C_2H_4]^+\); 253.0488\([M+H-C_2H_4]^+\) | Glabrone | GC |
| 65a* | 26.48 | \(C_{22}H_{26}O_5\) | 370.178 | 1 | 371.1853\([M+H]^+\); 315.1234\([M+H-C_2H_4]^+\); 303.1230\([M+H-C_2H_4]^+\); 235.1331\([M+H-C_2H_4]^+\); 193.0861\([M+H-C_2H_4]^+\); 171.0703\([M+H-C_2H_4]^+\); 149.0596\([M+H-C_2H_4]^+\); 137.0602\([M+H-C_2H_4]^+\); 123.0442\([M+H-C_2H_4]^+\) | Glyasperin D | BS |
| 66d | 28.01 | \(C_{16}H_{22}O_4\) | 278.1518 | −1 | 149.0237\([M+H-C_2H_4]^+\); 121.0290\([M+H-C_2H_4]^+\) | Disobutyl phthalate | BS |
| 67d | 28.01 | \(C_{16}H_{22}O_4\) | 278.1518 | −1 | 149.0237\([M+H-C_2H_4]^+\); 121.0290\([M+H-C_2H_4]^+\) | Dibutyl phthalate | BS |
| 68d* | 28.53 | \(C_{18}H_{22}O_2\) | 280.2402 | −1.4 | 178.9986\([M+H-C_2H_4]^+\); 151.0303\([M+H-C_2H_4]^+\); 149.1343\([M+H-C_2H_4]^+\); 141.0669\([M+H-C_2H_4]^+\); 129.0689\([M+H-C_2H_4]^+\); 115.0550\([M+H-C_2H_4]^+\) | Linoleic acid | BS |
| 69d* | 31.53 | \(C_{19}H_{32}O_2\) | 242.2246 | 0 | 243.1155\([M+H]^+\); 173.0611\([M+H-C_2H_4]^+\); 143.0849\([M+H-C_2H_4]^+\); 141.0683\([M+H-C_2H_4]^+\); 129.0689\([M+H-C_2H_4]^+\); 115.0550\([M+H-C_2H_4]^+\) | Pentadecanoic acid | BS |
| 70d* | 31.94 | \(C_{19}H_{32}O_2\) | 294.2559 | −1 | 295.1906\([M+H]^+\); 263.2330\([M+H-C_2H_4]^+\); 221.2332\([M+H-C_2H_4]^+\); 219.2069\([M+H-C_2H_4]^+\); 193.1241\([M+H-C_2H_4]^+\); 181.0920\([M+H-C_2H_4]^+\) | Methyl linoleate | BS |

*a: flavonoids, b: triterpenoids, c: monoterpenoid glycosides, d: other components, e: coumarins, * indicates the first discovered component.
| No | Rt (min) | Formula | Mass (Da) | Ppm | MS/MS fragments | Identification            | Source |
|----|----------|---------|-----------|------|-----------------|---------------------------|--------|
| 71 | 3.51     | C₇H₈O₄  | 180.0423  | 3.9  | 134.9873[M-H-C₆H₁₀O₄]⁻ | Caffeic acid               | GC     |
| 72 | 3.88     | C₇H₁₀O₅ | 198.0528  | 2.6  | 152.5018[M-H-C₆H₁₀O₄]⁻ | Ethyl gallate              | BS     |
| 73 | 4.09     | C₆H₈O₅  | 184.0372  | 3.4  | 123.0181[M-H-C₆H₈O₄]⁻ | Methyl gallate             | BS     |
| 74 | 5.45     | C₂₉H₂₈O₁₂| 496.158   | -1   | 495.1509[M-H⁻]; 465.1404[M-H-C₆H₁₀O₄]⁻; 333.1066[M-H-C₆H₈O₄]⁻; 195.0680[M-H-C₆H₈O₄]⁻; 177.0547[M-H-C₆H₈O₄]⁻; 165.0554[M-H-C₆H₈O₄]⁻; 137.0242[M-H-C₆H₈O₄]⁻ | Oxy paoniflorin | BS     |
| 75 | 7.51     | C₂₁H₂₀O₁₁| 448.1005  | -0.2 | 447.0961[M-H⁻]; 265.0405[M-H-C₆H₁₀O₄]⁻; 130.9685[M-H-C₆H₈O₄]⁻; 109.0289[M-H-C₆H₈O₄]⁻ | Kaempferol-3-O-β-D-glucoside | BS     |
| 76 | 7.51     | C₂₁H₂₀O₁₁| 448.1005  | -0.2 | 447.0961[M-H⁻]; 265.0405[M-H-C₆H₁₀O₄]⁻; 130.9685[M-H-C₆H₈O₄]⁻; 109.0289[M-H-C₆H₈O₄]⁻ | Kaempferol-7-O-β-D-glucoside | BS     |
| 77 | 8.32     | C₂₉H₂₈O₁₁| 480.1631  | -0.6 | 327.1093[M-H-C₆H₁₀O₄]⁻; 167.0652[M-H-C₆H₈O₄]⁻; 121.0299[M-H-C₆H₈O₄]⁻ | Alvimoforin                 | BS     |
| 78 | 8.32     | C₁₆H₁₈O₉  | 309.0969  | 0.2  | 309.0969[M-COOH]⁻ | Chlorogenic acid            | GC     |
| 79 | 8.32     | C₁₆H₁₈O₉  | 309.0969  | 0.2  | 309.0969[M-COOH]⁻ | Neochlorogenic acid         | BS     |
| 80 | 8.8      | C₂₅H₂₈O₁₄| 578.1635  | 0.4  | 577.1589[M-H⁻]; 487.1256[M-H-C₆H₈O₄]⁻; 437.0840[M-H-C₆H₈O₄]⁻; 395.0789[M-H-C₆H₈O₄]⁻ | Isovioliathan                 | BS     |
| 81 | 11.51    | C₁₆H₂₄O₈  | 344.357   | -2.4 | 208.8396[M-H-C₆H₈O₄]⁻; 165.0599[M-H-C₆H₈O₄]⁻; 101.0234[M-H-C₆H₈O₄]⁻ | Muranpioside F               | GC     |
| 82 | 12.21    | C₂₇H₃₀O₁₆| 609.1500  | -3.4 | 609.1500[M-H⁻]; 529.1056[M-H-C₆H₈O₄]⁻ | Rutin                       | BS     |
| 83 | 14.11    | C₂₇H₃₀O₁₆| 464.0955  | -1.3 | 463.0877[M-H⁻]; 417.0979[M-H-C₆H₈O₄]⁻; 265.1488[M-H-C₆H₈O₄]⁻; 174.9527[M-H-C₆H₈O₄]⁻; 130.9685[M-H-C₆H₈O₄]⁻ | Quercetin-3-O-β-D-glucoside | GC     |
| 84 | 14.37    | C₁₆H₁₂O₅  | 284.0685  | 2.7  | 211.0397[M-H-C₆H₈O₄]⁻; 183.0457[M-H-C₆H₈O₄]⁻; 135.0083[M-H-C₆H₈O₄]⁻ | Calycosin                    | GC     |
| 85 | 14.43    | C₂₆H₃₂O₁₄| 616.1792  | -1.7 | 615.1719[M-H⁻]; 597.1627[M-H-C₆H₈O₄]⁻; 493.1346[M-H-C₆H₈O₄]⁻; 475.1230[M-H-C₆H₈O₄]⁻; 376.8788[M-H-C₆H₈O₄]⁻; 313.0538[M-H-C₆H₈O₄]⁻ | Muranpioside H               | GC     |
| 86 | 14.82    | C₂₆H₃₂O₁₆| 726.2159  | 1    | 725.2122[M-H⁻]; 549.1637[M-H-C₆H₈O₄]⁻; 531.1518[M-H-C₆H₈O₄]⁻; 417.1194[M-H-C₆H₈O₄]⁻; 399.1085[M-H-C₆H₈O₄]⁻; 255.0668[M-H-C₆H₈O₄]⁻ | Licorice glycoside C2      | GC     |
| 87 | 15.22    | C₂₆H₁₀O₆  | 298.0477  | 1.7  | 297.0421[M-H⁻]; 197.0259[M-H-C₆H₈O₄]⁻ | Isotrifoliol                | GC     |
| 88 | 15.22    | C₂₆H₁₂O₆  | 300.0634  | -0.6 | 299.0565[M-H⁻]; 255.0305[M-H-C₆H₈O₄]⁻; 199.0415[M-H-C₆H₈O₄]⁻ | 7,2′,4′-trihydroxy-3-(5-methoxy phenyl) chromen-2-one | GC     |
| 89 | 15.83    | C₂₇H₃₂O₁₆| 562.1686  | 0.9  | 561.1641[M-H⁻]; 267.0668[M-H-C₆H₈O₄]⁻; 369.1000[M-H⁻]; 243.0681[M-H-C₆H₈O₄]⁻; 219.0674[M-H-C₆H₈O₄]⁻; 191.0724[M-H-C₆H₈O₄]⁻; 175.0753[M-H-C₆H₈O₄]⁻; 151.0048[M-H-C₆H₈O₄]⁻ | Glycyrosid              | GC     |
| 90 | 16.48    | C₂₇H₁₈O₇  | 370.1053  | 0.5  | 369.1000[M-H⁻]; 243.0681[M-H-C₆H₈O₄]⁻; 219.0674[M-H-C₆H₈O₄]⁻; 191.0724[M-H-C₆H₈O₄]⁻; 175.0753[M-H-C₆H₈O₄]⁻; 151.0048[M-H-C₆H₈O₄]⁻ | Uralenol                    | GC     |
| 91 | 17.29    | C₁₆H₁₂O₄  | 268.0735  | 0.9  | 267.0674[M-H⁻]; 251.0359[M-H-C₆H₈O₄]⁻; 223.0404[M-H-C₆H₈O₄]⁻; 135.0083[M-H-C₆H₈O₄]⁻ | Formononetin                | GC     |
| 92 | 17.92    | C₁₄H₁₄O₂₁ | 985.073   | 1.5  | 983.4542[M-H⁻]; 821.3998[M-H-C₆H₈O₄]⁻ | Licoricesaponin A3          | GC     |
Table 3: Continued.

| No | $R_t$ (min) | Formula | Mass (Da) | Ppm | MS/MS fragments | Identification | Source |
|---|-------------|---------|-----------|-----|-----------------|----------------|--------|
| 93<sup>a</sup> | 18.71 | C$_{12}$H$_{12}$O$_4$ | 256.0735 | 2.2 | 255.0663[M-H]$^-$; 119.0508[M-H-C$_2$H$_4$O$_2$]$^-; 135.0093[M-H-C$_4$H$_8$O]$^-$ | Pinocembrin | GC |
| 94<sup>a</sup> | 19.93 | C$_{14}$H$_{14}$O$_4$ | 270.0892 | 1.2 | [M-H-C$_4$H$_8$O]$^-$; 149.0420[M-H-C$_4$H$_8$O]$_2$; 133.0289[M-H-C$_6$H$_8$O$_2$]; 117.0352[M-H-C$_8$H$_8$O$_3$]; 105.0342[M-H-C$_8$H$_8$O] | Retrochalcone | GC |
| 95<sup>b</sup> | 20.17 | C$_{42}$H$_{60}$O$_{16}$ | 820.3881 | 0.5 | 819.3884[M-H]$^-$; 351.0593[M-H-C$_3$H$_4$O$_2$] | Licoricesaponin E2 | GC |
| 96<sup>b</sup> | 20.3 | C$_{50}$H$_{76}$O$_{21}$ | 1012.2879 | 0.3 | 1011.4853[M-H]$^-$; 497.1146[M-H-C$_3$H$_4$O$_2$] | Licoricesaponin D3 | GC |
| 97<sup>b</sup> | 20.17 | C$_{42}$H$_{62}$O$_{16}$ | 822.4038 | 2.3 | 821.4106[M-H]$^-$; 351.0577[M-H-C$_3$H$_4$O$_2$] | Glycyrrhizic acid | GC |
| 98<sup>b</sup> | 20.28 | C$_{42}$H$_{62}$O$_{16}$ | 822.5038 | 2.3 | 821.4106[M-H]$^-$; 351.0577[M-H-C$_3$H$_4$O$_2$] | Licoricesaponin H2 | BS |
| 99<sup>b</sup> | 20.28 | C$_{42}$H$_{62}$O$_{16}$ | 822.4038 | 2.3 | 821.4106[M-H]$^-$; 351.0577[M-H-C$_3$H$_4$O$_2$] | Licoricesaponin K2 | GC |
| 100<sup>a</sup> | 21.6 | C$_{21}$H$_{26}$O$_6$ | 368.1259 | -0.2 | 367.1196[M-H]$^-$; 337.0718[M-H-CH$_2$O]$^-$ | Licoarylcoumarin | GC |
| 101<sup>d</sup> | 22.79 | C$_{12}$H$_{16}$O$_2$ | 192.1150 | -3.5 | 190.8682[M-H]$^-$; 162.8911[M-H-C$_2$H$_4$] | Cuminyl acetate | GC |
| 102<sup>a</sup> | 23.02 | C$_{20}$H$_{20}$O$_4$ | 324.1362 | 2.9 | 323.1280[M-H]$^-$; 213.0929[M-H-C$_6$H$_6$O$_2$] | Isobavachalcone | GC |
| 103<sup>a</sup> | 23.02 | C$_{20}$H$_{20}$O$_4$ | 324.1362 | 2.9 | 323.1280[M-H]$^-$; 213.0929[M-H-C$_6$H$_6$O$_2$] | Glabridin | GC |
| 104<sup>a</sup> | 23.38 | C$_{20}$H$_{18}$O$_6$ | 354.1103 | 1.9 | 353.1046[M-H]$^-$; 285.1144[M-H-C$_5$H$_8$] | Licoflavonol | GC |
| 105<sup>a</sup> | 23.38 | C$_{20}$H$_{18}$O$_6$ | 354.1103 | 1.9 | 353.1046[M-H]$^-$; 285.1144[M-H-C$_5$H$_8$] | Glycyrrhizin | GC |
| 106<sup>d</sup> | 23.38 | C$_{20}$H$_{20}$O$_4$ | 356.1259 | 0 | 355.1179[M-H]$^-$; 203.1076[M-H-C$_9$H$_12$O$_2$] | Piperitol | GC |
| 107<sup>e</sup> | 23.41 | C$_{21}$H$_{26}$O$_6$ | 368.1259 | -0.2 | 367.1196[M-H]$^-$; 337.0718[M-H-CH$_2$O]$^-$ | Glycyoumarin | GC |
| 108<sup>a</sup> | 23.52 | C$_{21}$H$_{26}$O$_5$ | 352.1311 | 0.7 | 351.1245[M-H]$^-$; 321.0815[M-H-C$_6$H$_10$O$_2$] | Gancaonin M | GC |
| 109<sup>d</sup> | 23.67 | C$_{21}$H$_{22}$O$_5$ | 354.1467 | 2.5 | 353.1389[M-H]$^-$; 232.0924[M-H-C$_8$H$_8$O$_2$] | Dehydroglyasperin C | GC |
| 110<sup>e</sup> | 23.67 | C$_{21}$H$_{22}$O$_5$ | 354.1467 | 2.5 | 353.1389[M-H]$^-$; 232.0924[M-H-C$_8$H$_8$O$_2$] | Licochalcone D | GC |
| 111<sup>**</sup> | 24.27 | C$_{25}$H$_{26}$O$_5$ | 406.1787 | 0.8 | 405.1685[M-H]$^-$; 311.1714[M-H-C$_8$H$_8$O] | 6,8-Diprenylgenistein | GC |
| No  | $R_t$ (min) | Formula | Mass (Da) | $Ppm$ | MS/MS fragments | Identification | Source |
|-----|------------|---------|-----------|-------|-----------------|----------------|--------|
| 112^a | 24.27 | C_{20}H_{18}O_{6} | 352.0947 | 3.8 | 351.0887[M-H]^-; 321.0413[M-H-C_{5}H_{4}O]^-; 307.0988[M-H-C_{4}H_{4}O]^-; 283.0985[M-H-C_{4}H_{4}O]^-; 265.0872[M-H-C_{4}H_{4}O]^-; 241.0877[M-H-C_{5}H_{10}O]^-; 199.0768[M-H-C_{5}H_{10}O]^-; 199.0768[M-H-C_{5}H_{10}O]^-; 199.0768[M-H-C_{5}H_{10}O]^-; | Semilicoisofavone B | GC |
| 113^e | 24.47 | C_{22}H_{22}O_{6} | 382.1416 | 0.2 | 255.0313[M-H-C_{10}H_{12}O]^-; 255.0313[M-H-C_{10}H_{12}O]^-; 255.0313[M-H-C_{10}H_{12}O]^-; 255.0313[M-H-C_{10}H_{12}O]^-; 255.0313[M-H-C_{10}H_{12}O]^-; 255.0313[M-H-C_{10}H_{12}O]^-; 255.0313[M-H-C_{10}H_{12}O]^-; 255.0313[M-H-C_{10}H_{12}O]^-; | Glucyriin | GC |
| 114^e | 24.67 | C_{21}H_{16}O_{6} | 366.1103 | 1 | 365.0913[M-H-C_{6}H_{12}O]^-; 365.0913[M-H-C_{6}H_{12}O]^-; 365.0913[M-H-C_{6}H_{12}O]^-; 365.0913[M-H-C_{6}H_{12}O]^-; 365.0913[M-H-C_{6}H_{12}O]^-; 365.0913[M-H-C_{6}H_{12}O]^-; 365.0913[M-H-C_{6}H_{12}O]^-; 365.0913[M-H-C_{6}H_{12}O]^-; | Neoglycyrol | GC |
| 115^e | 24.69 | C_{20}H_{16}O_{5} | 336.0997 | 1.4 | 335.0928[M-H]^-; 319.0617[M-H-CH_{4}]^-; 319.0617[M-H-CH_{4}]^-; 319.0617[M-H-CH_{4}]^-; 319.0617[M-H-CH_{4}]^-; 319.0617[M-H-CH_{4}]^-; 319.0617[M-H-CH_{4}]^-; 319.0617[M-H-CH_{4}]^-; | Phaseol | GC |
| 116b | 25.09 | C_{30}H_{46}O_{5} | 486.3345 | 1.7 | 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; | Echinatic acid | GC |
| 117b | 25.09 | C_{30}H_{46}O_{5} | 486.3345 | 1.7 | 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; | Isoechinatic acid | GC |
| 118b | 25.09 | C_{30}H_{46}O_{5} | 486.683 | 1.7 | 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; 485.3281[M-H-CH_{3}]^-; | Triphyllic acid | BS |
| 119a | 25.26 | C_{20}H_{18}O_{4} | 322.1205 | 2.3 | 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; | Licoflavone A | BS |
| 120a | 25.26 | C_{20}H_{18}O_{4} | 322.1205 | 2.3 | 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; 321.1138[M-H]^-; | Isobavachromene | GC |
| 121d | 26.4 | C_{16}H_{22}O_{4} | 278.1528 | 1.5 | 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; | Diisobutyl phthalate | GC |
| 122d | 26.4 | C_{16}H_{22}O_{4} | 278.1528 | 1.5 | 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; 277.1459[M-H-CH_{4}]^-; | Dibutyl phthalate | GC |
| 123** | 26.66 | C_{23}H_{26}O_{4} | 392.1987 | 1 | 391.1933[M-H]^-; 391.1933[M-H]^-; 391.1933[M-H]^-; 391.1933[M-H]^-; 391.1933[M-H]^-; 391.1933[M-H]^-; 391.1933[M-H]^-; 391.1933[M-H]^-; | Hispaglabridin A | GC |
| 124^b | 27.78 | C_{30}H_{46}O_{4} | 470.3396 | 1.7 | 469.3332[M-H]^-; 469.3332[M-H]^-; 469.3332[M-H]^-; 469.3332[M-H]^-; 469.3332[M-H]^-; 469.3332[M-H]^-; 469.3332[M-H]^-; 469.3332[M-H]^-; | Liquiritic acid | GC |
| 125** | 28.79 | C_{30}H_{44}O_{4} | 468.3249 | 2.6 | 467.3189[M-H]^-; 467.3189[M-H]^-; 467.3189[M-H]^-; 467.3189[M-H]^-; 467.3189[M-H]^-; 467.3189[M-H]^-; 467.3189[M-H]^-; 467.3189[M-H]^-; | Glabrolide | GC |

a: flavonoids, b: triterpenoids, c: monoterpenoid glycosides, d: other components, e: coumarins, * indicates the first discovered component.
3.1.2. Identification of Triterpenoids. In this study, a total of 13 triterpenoids were identified in positive mode and 13 in negative mode, mainly pentacyclic triterpenoids. For the pentacyclic triterpenes in positive mode, the A and B rings are easy to break off, followed by the C ring. D and E rings are not easy to break off. However, when the E ring is
connected with glucose and other sugars through the glycosidic bond, the glycosidic bond is easy to break off, and the glycosidic bond becomes an aglycone, or two sugars can be directly broken off. In addition, the cleavage of most pentacyclic triterpenoids is confined to the cleavage of the A and B rings. In the negative mode, the glycosidic bond in triterpenoid saponins is basically broken, while the others are similar to the positive mode.

Taking compound 32b as an example, the retention time was 17.58 min, and the excimer ion peak m/z 985.4652 [M+H]+ was determined by first-order full-scan mass spectrometry in positive mode. The fragment ions m/z 809.4326, 615.3883, 647.3772, 471.3455, and 453.3356 were obtained by the loss of C₆H₉O₆, C₆H₈O₇, C₁₂H₁₂O₁₁, C₁₂H₁₉O₁₁, and C₁₂H₂₀O₁₁. The specific process is shown in Figure 3. The compound was identified as licoricesaponin A3.

3.1.3. Identification of Monoterpene. Six monoterpene glycosides were identified in positive mode, and four were identified in negative mode. In the positive mode, it was found that the ester bond and glycosidic bond of the monoterpene glycosides were broken, the glucosidic and benzyl methyl benzene ketone may continue to dehydroxylation or –O or –CHO cracking for small molecule compounds. Monoterpene glycosides are more difficult to dissociate in the negative mode, and only a few have secondary mass spectrometry, the reason is unclear.

Taking compound 31c as an example, the retention time for compound 31c was 17.52 min. In the positive ion mode, first-order full-scan mass spectrometry showed the excimer ion peak m/z 585.2905 [M+H]+, and second-order scanning mass spectrometry showed that there are fragment ions m/z 319.1181, 301.1034, 197.0796, 267.0852, 249.0768, 133.0646,
179.0700, 151.0752, and 105.0332, as shown in Figure 4. The compound can be identified as benzoyl paeoniflorin.

3.1.4. Identification of Coumarin. In this study, six coumarin compounds were detected in SGD. In the positive mode, according to the secondary fragmentation, the substituents on the pyrone ring of the basic nucleus are very easy to fall off, especially the small groups such as hydroxyl and methoxy, followed by the chain alkane substituents. However, if there is a closed ring substituent on the pyran ring, the stability is increased because the closed ring substituent and the basic core of the compound are coplanar, so it is not easy to crack and fall off. The substituents on the benzene ring of the basic core nucleus are relatively stable. The ring-opening substituents are easier to fall off than the closed-ring substituents. When the benzene ring is connected with an aromatic ring, the aromatic ring may fall off as a whole.

For compound 58*, the retention time was 24.57 min. In positive mode, the first-order full-scan mass spectrometry analysis showed that the excimer ion peak m/z 367.1178 [M + H]+, and the second-order scanning mass spectrometry analysis showed that there are fragment ions m/z 339.1264, 337.0716, 309.0405, 281.0453, 253.0506, 197.0595, 153.0680. They are formed by the removal of CO, CH₃O, C₇H₁₆, and other groups from the parent ion, and the specific process is shown in Figure 5. The compound was further identified as neoglycyrol by reference compound alignment.

3.1.5. Identification of the Other Types. In this study, 17 other compounds were identified, such as aliphatic pentadecanoic acid, and dibutyl phthalate. Aromatic compounds are benzaldehyde, cuminyl acetate, etc. Terpenoids include menthol and so on. If the chemical structure is a chain structure, only alkyl drop will occur. When the ester group is contained, the C–O bond in the ester group is more polar and therefore more likely to break, break off the hydroxyl group, or make its lower molecular weight end fall off.

Taking the compound 70d* as an example, the retention time was 31.94 min. In the positive mode, first-order full-scan mass spectrometry showed the excimer ion peak m/z 295.1906 [M + H]+, and second-order scanning mass spectrometry showed that missing CH₃OH, C₅H₁₀O, C₇H₁₆O, C = C, CH₂ = CH₂, C₅H₁₂, CH₄ fragment ions m/z 168.1140, 295.1906, 263.2330, 245.2253, 221.2332, 193.1241, 121.1007,
and 105.0670 were formed, and the specific process is shown in Figure 6. The compound was further identified as methyl linoleate.

3.2. Biomarkers Screening and Validation by Network Pharmacology and Components Absorbed into Blood. A total of 343 potential targets related to the 110 identified compounds were obtained from TCMSP (https://old.tcmsp-e.com/tcmsp.php), SWISS (http://www.swisstargetprediction.ch/), BATMAN (http://bionet.ncpsb.org.cn/batman-tcm/). Through the keyword “liver injury”, a total of 12784 disease targets were obtained from the OMIM (https://omim.org/), Gene Cards (https://www.genecards.org/), and DisGeNET (https://www.disgenet.org/) databases. The Venn diagram is shown in Figure 7. Through protein-protein interaction analysis, 276 targets with a high correlation degree were obtained, and 44 top-ranking genes were obtained after PPI result analysis and screening with a median degree greater than two times, as shown in Figure 8. The relevant parameters of the first 20 targets with a higher degree of integration are shown in Table 4. These 44 retained proteins were further imported into the Cytoscape 3.9.1 software for KEGG enrichment analysis, and association analysis with related compounds was conducted.

The KEGG pathways of these genes are shown in Figure 9 and Figure 1 in the supplementary materials. The correlation display of the top 20 biological processes (BP) by GO analysis and the GO enrichment analysis obtained the top 10 biological process (BP) items, the top 10 cellular composition (CC) items, and the top 10 molecular functions (MF) entries are shown in Figures 10 and 2 in the supplementary materials. Finally, the “component-target-function” network is visualized in Figure 11, 132 proteins and 19 compounds (including 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose, ferulic acid, coniferyl ferulate, benzoyl paeoniflorin, hesperidin, liquiritin, liquiritigenin, glycyrrhizic acid, caffeic acid, rutin, chlorogenic acid, gallic acid, methyl gallate, isoliquiritin apioside, albiflorin, neochlorogenic acid, isoliquiritin, narirutin, and naringenin) were obtained. Among the 132 proteins and 19 compounds, the EGFR pathway had the highest score and played an important role in the treatment of liver injury, as shown in Table 4. On this basis, we verified it by the molecular docking method. According to the cluster analysis of binding energy, STAT3 and CTNNB1 were clustered into one class; PIK3CA, SRC, HRAS, and HSP90AA1 were clustered into one class, and MAPK1 and EGFR were clustered into one class as shown in Figure 12 and Table 1 in the supplementary materials. The

Figure 5: The secondary mass spectrometry of neoglycyrol and its pyrolysis process.
results showed that the docking binding free energies of the 19 compounds were less than $-6.4\text{ kcal.mol}^{-1}$. The results of molecular docking between typical chemical compositions are shown in Figure 13 and Table 5. Each type exhibits a similar comprehensive binding capacity with different chemical constituents. All 19 compounds were detected in rat serum by the UPLC-QQQ-MS/MS method, further confirming that these compounds are suitable biomarkers. Detailed information about the analysis of chemical components in rat serum can be found in Table 2 in the supplementary materials.

3.3. Validation of the UPLC-QQQ-MS/MS Method. The stock solution containing the 19 components was prepared. The concentrations of hesperidin, naringenin, liquiritin, glycyrrhizic acid, liquiritigenin, isoliquiritin, isoliquiritin apioside, caffeic acid, ferulic acid, coniferyl ferulate, albiflorin, gallic acid, methyl gallate, benzoyl paoniflorin, narirutin, chlorogenic acid, neochlorogenic acid, rutin and...
### Table 4: The first 20 key targets of SGD in treating liver injury.

| No. | Target name  | Betweenness centrality | Closeness centrality | Degree |
|-----|-------------|------------------------|----------------------|--------|
| 1   | EGFR        | 0.0461                 | 0.6045               | 104    |
| 2   | CTNNB1      | 0.0527                 | 0.5978               | 101    |
| 3   | HSP90AA1    | 0.0561                 | 0.5833               | 94     |
| 4   | SRC         | 0.0239                 | 0.5821               | 93     |
| 5   | HRAS        | 0.0260                 | 0.5600               | 86     |
| 6   | STAT3       | 0.0176                 | 0.5588               | 83     |
| 7   | MAPK1       | 0.0232                 | 0.5485               | 75     |
| 8   | PIK3CA      | 0.0051                 | 0.5086               | 59     |
| 9   | FGF2        | 0.0047                 | 0.5165               | 58     |
| 10  | APP         | 0.0276                 | 0.5341               | 56     |
| 11  | BCL2L1      | 0.0050                 | 0.5236               | 54     |
| 12  | GSK3B       | 0.0059                 | 0.5175               | 51     |
| 13  | KDR         | 0.0035                 | 0.5135               | 49     |
| 14  | NRAS        | 0.0034                 | 0.4926               | 49     |
| 15  | IL2         | 0.0047                 | 0.5057               | 49     |
| 16  | ACE         | 0.0167                 | 0.5096               | 47     |
| 17  | MMP2        | 0.0020                 | 0.5076               | 45     |
| 18  | RELA        | 0.0067                 | 0.5106               | 44     |
| 19  | KIT         | 0.0033                 | 0.4953               | 43     |
| 20  | PRKCA       | 0.0078                 | 0.5076               | 43     |

**Figure 9:** KEGG analysis of potential target genes of the SGD: colored by cluster ID, top 20 clusters of KEGG.
1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose were 0.98, 0.96, 35.80, 13.90, 282.50, 5.00, 19.40, 0.09, 0.15, 0.20, 68.00, 3.30, 0.20, 10.50, 261.70, 0.20, 0.06, 0.08, 0.03, 0.025 ng·μL⁻¹. The standard curve of the reference substance solution was established, with the concentration (ng·μL⁻¹) as the X-axis and the peak area as the Y-axis. The structures of...
the 19 compounds are shown in Figure 14. Details of the reference material standard curves are listed in Table 6.

The same sample was injected 6 times continuously to test the precision of the instrument. The RSD values of the peak areas of 19 components were less than 2.05%. It indicated that the precision of the instrument was good.

Inject the same samples at 0, 2, 6, 8, 12, 16, 20, and 24 h, respectively, and determine the peak areas. The RSDs of the peak areas of 19 components were all less than 2.11%. The experimental results suggested that the stability of the sample was good within 24 h.

Six samples of the same batch were prepared in parallel, and each sample was injected twice. The RSDs of the contents of 19 components were all less than 2.61%. The experimental results showed that the stability of the sample was good within 24 h.

3.4. Contents Determination Results of 19 Components. The developed quantitative analysis method was subsequently applied to 10 batches of the SGD. The results demonstrated a successful application of this UPLC-QQQ-MS/MS assay for the quantification of 19 constituents in different samples. The contents, summarized in Table 7 and Figure 15, were calculated with the external reference compound methods. It shows that S6, S7, and S8 have the highest content, while other batches are lower than the median.

In this experiment, UPLC-Q-TOF-MS/MS was employed to analyze SGD. A total of 110 compounds, including 54 flavonoids, 23 triterpenoids, 10 monoterpenoids, 6 coumarins, and 17 other compounds. It can be seen from this result that most of the compounds in SGD are flavonoids, the number of compounds accounted for more than 50% (55/110). From the results of content determination by UPLC-QQQ-MS/MS, the contents of glycyrrhizic acid (content: 3.44%) and liquiritin (content: 1.37%) were significantly higher than those of some flavonoids (all below 1%). These two compounds should be our focus, and
Figure 13: Result of molecular docking between typical chemical composition and targets. (a) Liquiritin with CTNNB1. (b) Liquiritin with EGFR. (c) Liquiritin with MAPK1. (d) Liquiritin with STAT3. (e) Benzoyl paeoniflorin with HRAS. (f) Benzoyl paeoniflorin with PIK3CA. (g) Benzoyl paeoniflorin with SRC. (h) Benzoyl paeoniflorin with HSP90AA1.

Table 5: The possible binding site (amino acid residue) of the target of the molecular docking results.

| Name                                    | Binding energy (kcal/mol) | Hydrogen bonding sites             | Hydrogen bond length | Hydrophobic action site |
|-----------------------------------------|---------------------------|------------------------------------|----------------------|-------------------------|
| (A) Liquiritin with CTNNB1.             | −7.5                      | Trp338(A), Asn380(A), Arg342(A), Lys345(A) | 3.28 Å, 3.07 Å, 3.19 Å, 2.84 Å, 3.12 Å | Lys312(A), Val349(A), Val346(A), Tyr306(A), Gln302(A), Pro770(A), Lys704(A), Leu768(A), Ala719(A), Lys721(A), Leu764(A), Glu738(A), Thr830(A), Leu820(A), Asp831(A), Met769(A), Gly772(A), Leu694(A) |
| (B) Liquiritin with EGFR.               | −8.9                      | Thr766(A)                           | 2.70 Å               | Leu107(A), Ala52(A), Thr110(A), Asp111(A), Gly32(A), Glu33(A), Lys114(A), Tyr113(A), Ser153(A), Val39(A), Leu156(A), Glu109(A), Lys615(A), Val563(A), Cys468(A), Pro471(A), Met470(A), Arg335(A), Ile569(A), Thr515(A), Lys573(A), Asp570(A), Asn567(A) |
| (C) Liquiritin with MAPK1.              | −9.1                      | Met108(A)                           | 2.80 Å               | Gln61(X), Asp33(X), Ile21(X), Val29(X), Glu37(X), Glu31(X), Asp30(X), Phe28(X), Lys117(X), Gly15(X), Asp119(X), Ala146(X), Ala18(X), Gly131(X), Phe666(A), Ser629(A), Ala758(A), Pro757(A), Asn170(A), His670(A), Glu259(A), Glu849(A), Arg818(A), Ile653(A), Gly837(A), Met811(A) |
| (D) Liquiritin with STAT3.              | −7.4                      | Nonexistent                         | Nonexistent          |                         |
| (E) Benzoyl paeoniflorin with HRAS.     | −10.9                     | Cys32(X), Asn116(X), Ser17(X), Lys16(X) | 2.94 Å, 2.83 Å, 3.13 Å, 3.07 Å |                         |
| (F) Benzoyl paeoniflorin with PIK3CA.   | −10.0                     | Asn756(A), Arg662(A), Cys838(A)     | 3.12 Å, 2.85 Å, 3.10 Å |                         |
Table 5: Continued.

| Name                              | Binding energy (kcal/mol) | Hydrogen bonding sites | Hydrogen bond length | Hydrophobic action site                                                                 |
|-----------------------------------|---------------------------|------------------------|----------------------|----------------------------------------------------------------------------------------|
| (G) Benzoyl paeoniflorin with SRC.| −10.1                     | ASer345(A), Thr338(A)  | 3.12 Å, 2.81 Å       | Gly344(A), Leu273(A), Tyr340(A), Leu393(A), Ala293(A), Ala403(A), Val323(A), Phe405(A), Asp404(A), Val281(A), Lys295(A), Ala390(A), Val150(A), Asp93(A), Met98(A), Val186(A), Trp162(A), Phe138(A), Leu107(A), Leu48(A), Gly135(A), Ile96(A), Ala55(A), Asn51(A), Arg58(A), Ser52(A) |
| (H) Benzoyl paeoniflorin with HSP90AA1 | −10.7                    | Thr184(A)              | 2.92 Å               |                                                                                         |

Figure 14: The structures of the 19 compounds.
Table 6: Calibration curves of the analytes.

| Analytes                      | Calibration curve | Linear range (ng/μL^-1) | r   | LOQ (ng/μL^-1) |
|-------------------------------|-------------------|--------------------------|-----|----------------|
| Hesperidin                   | Y = 2E + 06X + 6462 | 0.0392~0.98              | 0.9992 | 0.01          |
| Naringenin                   | Y = 2E + 06X – 3159 | 0.0384~0.96              | 0.9985 | 0.01          |
| Liquiritin                   | Y = 3E + 06X + 4E + 04 | 1.432~35.8              | 0.9979 | 0.04          |
| Glycyrrhizic acid            | Y = 835033X + 1E + 04 | 4.62~115.5              | 0.9825 | 1.20          |
| Liquiritigenin               | Y = 7E + 06X + 4E + 04 | 0.554~13.9              | 0.9990 | 0.12          |
| Isoliquiritin                | Y = 4E + 06X + 20213 | 0.201~5.025             | 0.9998 | 0.05          |
| Isoliquiritin apioside       | Y = 2E + 06X + 50766 | 0.776~19.4              | 1.0000 | 0.02          |
| Caffeic acid                 | Y = 1E + 07X + 27935 | 0.036~0.09              | 0.9988 | 0.01          |
| Ferulic acid                 | Y = 1E + 06X + 7406  | 0.006~0.15              | 0.9985 | 0.002         |
| Coniferyl furulate           | Y = 625840X + 3624  | 0.008~0.20              | 0.9989 | 0.002         |
| Albiflorin                   | Y = 33658X + 44760  | 2.74~68.00              | 0.9978 | 0.68          |
| Gallic acid                  | Y = 4E + 07X + 3E + 04 | 0.132~3.30             | 0.9977 | 0.33          |
| Methyl gallate               | Y = 1E + 07X + 9012  | 0.008~0.20              | 0.9999 | 0.002         |
| Benzoyl paeoniflorin         | Y = 24147X – 2883.4 | 0.42~10.50              | 0.9991 | 0.11          |
| Narirutin                    | Y = 5E + 06X + 1108  | 0.008~0.20              | 0.9999 | 0.002         |
| Chlorogenic acid             | Y = 6E + 06X + 6980  | 0.0024~0.06             | 0.9990 | 0.001         |
| Neochlorogenic acid          | Y = 5E + 06X + 2745  | 0.0032~0.08             | 0.9995 | 0.001         |
| Rutin                        | Y = 1E + 06X + 7917  | 0.0012~0.03             | 0.9994 | 0.001         |
| 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose | Y = 1E + 06X + 3214 | 0.0010~0.025            | 0.9995 | 0.001         |

Table 7: Contents of 19 components.

| Analytes               | Contents (g/g) |
|------------------------|----------------|
|                        | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10 | Mean |
| Hesperidin             | 0.35| 0.32| 0.40| 0.11| 0.24| 0.35| 0.24| 0.26| 0.25| 0.30| 0.28 |
| Naringenin             | 0.42| 0.22| 0.51| 0.22| 0.26| 0.31| 0.32| 0.19| 0.26| 0.33| 0.30 |
| Liquiritin             | 10.50| 11.41| 11.57| 11.42| 12.26| 18.91| 21.36| 13.13| 12.77| 13.97| 13.73 |
| Glycyrrhizic acid      | 31.33| 33.71| 31.61| 34.04| 33.91| 45.09| 39.31| 35.53| 31.59| 32.46| 34.36 |
| Liquiritigenin         | 1.80| 0.58| 1.28| 0.66| 0.59| 1.07| 1.11| 0.88| 1.13| 1.03| 1.01 |
| Isoliquiritin          | 1.29| 1.83| 1.28| 1.89| 1.87| 1.61| 1.71| 1.46| 1.33| 1.43| 1.57 |
| Isoliquiritin apioside | 1.49| 2.43| 1.31| 2.52| 2.98| 3.43| 3.62| 3.29| 1.64| 1.68| 2.44 |
| Caffeic acid           | 0.35| 0.68| 0.67| 0.35| 0.23| 0.44| 0.50| 0.22| 0.31| 0.34| 0.41 |
| Ferulic acid           | 0.05| 0.12| 0.13| 0.05| 0.06| 0.09| 0.04| 0.05| 0.09| 0.03| 0.07 |
| Coniferyl furulate     | 0.08| 0.10| 0.11| 0.07| 0.09| 1.00| 0.09| 0.07| 0.08| 0.09| 0.18 |
| Albiflorin             | 9.97| 8.65| 9.38| 10.32| 9.12| 7.3| 7.51| 9.62| 8.98| 7.87| 8.87 |
| Gallic acid            | 2.11| 2.46| 2.83| 2.71| 2.95| 2.01| 2.10| 2.38| 2.06| 2.40| 2.40 |
| Methyl gallate         | 0.10| 0.05| 0.09| 0.07| 0.09| 0.97| 1.97| 1.07| 0.97| 1.08| 1.09 |
| Benzoyl paeoniflorin   | 2.71| 2.09| 1.91| 1.79| 1.91| 0.79| 0.81| 0.80| 1.00| 0.92| 1.47 |
| Narirutin              | 0.08| 0.12| 0.07| 0.07| 0.09| 0.97| 1.01| 1.02| 1.02| 0.98| 0.99 |
| Chlorogenic acid       | 0.02| 0.03| 0.03| 0.02| 0.03| 0.02| 0.04| 0.07| 0.02| 0.04| 0.03 |
| Neochlorogenic acid    | 0.05| 0.07| 0.02| 0.06| 0.06| 0.10| 0.06| 0.04| 0.05| 0.04| 0.15 |
| Rutin                  | 0.02| 0.02| 0.03| 0.03| 0.02| 0.03| 0.02| 0.05| 0.04| 0.04| 0.02 |
| 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose | 0.02| 0.03| 0.08| 0.03| 0.04| 0.04| 0.05| 0.05| 0.07| 0.04| 0.05 |
furthermore the contents of paeoniflorin and benzoylpaeoniflorin are also relatively high.

4. Conclusion

This research established a new method to find biomarkers for the quality control of SGD by the combined application of UPLC-MS/MS, network pharmacology, and molecular docking. Firstly, by comparing the retention times and mass spectrometry dates of the reference database and the self-built database, 110 compounds were identified. Secondly, based on the compounds identified above, network pharmacology was used to find the active components of SGD against liver injury, and 19 chemical components were selected as biomarkers, including 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose, ferulic acid, coniferyl ferulate, benzoyl paeoniflorin, hesperidin, liquiritin, liquiritigenin, glycyrrhizic acid, caffeic acid, rutin, chlorogenic acid, gallic acid, methyl gallate, isoliquiritin apioside, albiflorin, neochlorogenic acid, isoliquiritin, narirutin, and naringenin. Thirdly, molecular docking is used to verify the efficacy of the compounds and shows that the compounds bind well to the key target. Furthermore, the 19 components were detected in the serum, which also proved that they could be biomarkers. Finally, we determined the contents of 19 key components in 10 different batches of SGD. The method has satisfactory linearity, stability, accuracy, repeatability, and recovery. This study clarified the active components, key targets, and pathways of SGD against liver injury and provided a new idea for the selection of quality control indicators of traditional Chinese medicine based on pharmacological activity.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

Ethics approval was not required for this research.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Table 1: Binding energies of representative compounds and targets. Table 2. 128 blood absorbed components. Figure 1: KEGG analysis of potential target genes of SGD, top 20 clusters of KEGG. Figure 2: GO analysis of potential target genes of SGD. (Supplementary Materials)

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