Analysis of Isoflavones in Pueraria by UHPLC-Q-Orbitrap HRMS and Study on α-Glucosidase Inhibitory Activity

Yan Yang 1,2, Hui Zhao 3, Furong Zhu 3, Xiaoyan Liu 4, Yu Liu 1, Feng Zeng 1,* and Bin Liu 1,3,*

1 College of Food Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China
2 Department of Physical and Chemical Analysis, Fujian Center for Disease Control and Prevention, Fuzhou 350001, China
3 College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China
4 Beijing Engineering and Technology Research Center of Food Additives, School of Food and Health, Beijing Technology and Business University, Beijing 100048, China
* Correspondence: fengzeng@fafu.edu.cn (F.Z.); 000q080003@fafu.edu.cn (B.L.)

Abstract: Pueraria is a rich source of bioactive compounds, but there is a lack of comprehensive information concerning its composition. Therefore, a UHPLC-Q-Orbitrap HRMS method was developed to identify and quantify bioactive compounds in pueraria. Twelve isoflavones were quantified, with puerarin being the most abundant, followed by Puerarin 6′′-O-xyloside, 3′-methoxy puerarin, and 3′-hydroxy puerarin. A further 88 bioactive components in eight categories were also tentatively identified. The 12 isoflavones, except for genistein, exhibited α-glucosidase inhibitory activity. The binding of these compounds to the active site of α-glucosidase was confirmed via molecular docking analysis. These findings provide a basis for identifying pueraria as a promising functional food ingredient.

Keywords: Pueraria lobata; UHPLC-Q-Orbitrap HRMS; isoflavone; molecular docking; α-glucosidase

1. Introduction

Pueraria is the dried root of Pueraria lobata (Willd.) Ohwi and is used as a functional food in countries in East Asia [1]. It contains several bioactive components (such as puerarin, daidzein, genistein, and various steroids) that possess an extensive range of pharmacological properties including cardioprotective, neuroprotective, antioxidative, anti-inflammatory, antidiabetic, hepatoprotective, hypolipidemic, and antiosteoporosis effects [2,3]. Many studies show that pueraria extracts have antidiabetic activity [4–9], however, the active ingredients in these extracts are unknown.

Pueraria research has focused on determining its puerarin content and pharmacological properties [10,11]. Some studies have demonstrated that the antidiabetic properties of pueraria are dependent not only on puerarin but also on minor isoflavonoids [1,12] including 3′-hydroxy puerarin, 3′-methoxy puerarin, puerarin 6′′-O-xyloside, formononetin, and ononin [13].

The inhibition of α-glucosidase activity can decrease the rate of blood sugar absorption and improve insulin sensitivity, thus affecting glucose levels [14,15]. α-Glucosidase inhibition also has significant effects on polysaccharide metabolism and glycoprotein processing [16]. Many flavones, such as apigenin-7-O-glucoside, calycosin, and isoorientin [17], have recently been identified as potential α-glucosidase inhibitors, and considerable effort has been applied to isolate useful inhibitors from natural food sources to develop functional foods against diabetes [18].

Further research is needed into the composition and α-glucosidase inhibition mechanisms of the active ingredients of pueraria, and a method for rapidly identifying and quantifying these ingredients is required. Due to complex active substance in pueraria, it is still a great challenge to establish a sensitive and accurate method of simultaneous
quantification of compound in pueraria. Several methods have previously been developed for the analysis of pueraria components. These include enzyme-linked immunosorbent assay (ELISA) [19,20], high-performance liquid chromatography (HPLC) [21,22], and liquid chromatography-mass spectrometry (LC-MS/MS) [23,24]. However, most of these methods are limited to the determination of only 3 to 6 compounds. In recent years, UHPLC coupled with hybrid quadrupole-Orbitrap high resolution tandem mass spectrometry (UHPLC-Q-Orbitrap HRMS) has emerged as a new technology capable of analyzing the active ingredients in food products [25,26]. The UHPLC-Q-Orbitrap HRMS system can simultaneously analyze a potentially unlimited number of compounds because its full MS-ddMS2 scan mode allows for the screening and quantifying of analytes and the retrospective analysis of unknown compounds [27]. Furthermore, this system provides accurate, exact mass measurements and improved selectivity and sensitivity for the detection and identification of low concentration analytes [28].

This study aims to elucidate the bioactive compounds and mechanisms of α-glucosidase inhibition found in pueraria. A method is developed to identify and quantify the active ingredients in pueraria using UHPLC-Q-Orbitrap HRMS. The contents of 12 isoflavones in pueraria were determined. The chemical structures of the 12 isoflavone compounds are shown in Figure 1. The α-glucosidase inhibitory activity of 12 active substances is evaluated and validated by molecular docking to provide an in-depth understanding of the antidiabetic properties of pueraria.

Figure 1. Chemical structures of the 12 isoflavone compounds.

2. Materials and Methods
2.1. Chemicals and Reagents

The standards daidzein (PubChem CID: 5281708), daidzin (PubChem CID:107971), puerarin (PubChem CID: 5281807), glycitin (PubChem CID: 187808), 3′-methoxy puerarin (PubChem CID: 5319485), genistin (PubChem CID: 5281377), genistein (PubChem CID: 5280961), formononetin (PubChem CID: 5280378), 3′-hydroxy puerarin (PubChem CID: 5748205), glycine (PubChem CID: 5317750), Puerarin 6′-O-xyloside (PubChem
CID: 100990912), and puerarin apioside (PubChem CID: 21676217), with purity ≥ 98%, were purchased from ANPEL Laboratory Technologies Inc. (Shanghai, China). HPLC-grade methanol and acetonitrile were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA) and formic acid (HPLC grade) from ANPEL. α-Glucosidase was purchased from Sigma-Aldrich, and acarbose and p-nitrophenyl-α-D-glucopyranoside (pNPG) from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific Inc. (Pittsburgh, PA, USA). Ultra-pure water with resistivity < 18.2 MΩ cm was prepared in a Millipore Direct-Q water system (Millipore, Bedford, MA, USA). Nylon syringe filters (13 mm diameter, 0.22 µm) were purchased from ANPEL.

Twelve 1.0 mg/mL stock standard solutions were prepared in DMSO and stored at −20 °C in the dark for up to six months. A 10 µg/mL mixed working standard solution was prepared by mixing 100 µL of each stock standard solution in a final volume of 10 mL methanol/water (50:50, v/v). The working standard was stored at 4 °C in a brown glass bottle for up to one month.

2.2. Sample Preparation

Pueraria samples were purchased from Baicao Pharmaceutical Co. Ltd. (Xuancheng, China) and were identified by Professor Hong Qiu of Fujian Health College. Pueraria samples were oven-dried at 40 °C for 12 h, ground to a fine powder, and passed through a 180 µm sieve. Approximately 0.1 g of powder was accurately weighed and dissolved in 50 mL 90% ethanol. Extraction was performed in an ultrasonic bath (300 W, 40 kHz) at 50 °C for 60 min followed by centrifugation at 10,000 × g for 5 min. The supernatant was diluted ten-fold with water and filtered through a 0.22 µm nylon membrane filter before analysis on the UHPLC-Q-Exactive Orbitrap HRMS.

2.3. UHPLC-Q-Exactive Orbitrap HRMS Analysis

A Q-Exactive Orbitrap high resolution tandem mass spectrometer coupled to a Dionex Ultimate 3000 UHPLC system (Thermo Fisher, MA, USA) equipped with a Waters Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 µm) maintained at 40 °C was used for analysis of pueraria extracts. The flow rate was 0.3 mL/min, injection volume was 2 µL, and the mobile phase consisted of (A) acetonitrile and (B) water containing 0.2% formic acid. Electrospray ionization parameters were as follows: auxiliary gas flow 10 arb, sheath gas flow 35 arb, spray voltage 3.5 kV (positive mode) and 3.2 kV (negative mode), capillary temperature 320 °C, probe heater temperature 350 °C, and S-lens 60. Mass scan parameters were as follows: scan mode Full ms-ddms2, Full MS scan range 100–1500 m/z, resolution Full MS 70000 FWHM and MS/MS 17500 FWHM, AGC target Full MS 1e6 and MS/MS 2e5, maximum IT Full MS 100 ms and MS/MS 50 ms, Loop count 3, MSX count 1, Isolation width 1.5 m/z, NCE (Stepped) 10/30/40, minimum AGC target 8e3, Intensity Threshold 1.6e5, Dynamic exclusion 5 s.

2.3.1. Quantitative Method

The UPLC gradient was as follows: 0–7.5 min 95–60% B, 7.5–11 min 60–5% B, 11–12 min 5% B, 12.1 min 5–95% B, and 12.1–15 min 95% B. Full MS-ddMS2 scanning was performed in positive ionization mode.

2.3.2. Screening Method

The UPLC gradient was as follows: 0–2 min 95% B, 2–42 min 95–5% B, 42–47 min 5% B, 47.1 min 5–95% B, and 47.1–50 min 95% B. Full MS-ddMS2 scanning was performed in both positive and negative ionization modes. The Thermo Trace Finder data analysis “Screening Method” was used to search for analytes by comparison to a compound database using exact mass measurements of precursor ions (<5 ppm), MS/MS fragmentation, LC retention time, and the chemical compounds database query.
2.4. Method Validation

The optimized method was validated according to AOAC International [29] for the following parameters: linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy (recovery), and precision (relative standard deviation, RSD). Linearity was evaluated for 12 compounds using eight-point calibration curves (0.1, 5, 10, 20, 50, 100, 200, and 300 µg/L), plotting peak area (y) against concentration (x), with 1/x weighting. Recovery was determined by quantifying samples fortified with known concentrations of standards, corresponding to approximately 50%, 100%, and 150% of the concentrations expected in pueraria samples (n = 6). Intra-day (repeatability) and inter-day precision (reproducibility) were determined by the repeated analysis of samples (n = 6) fortified at three concentrations and tested on the same day (intra-day) or on three consecutive days (inter-day). Precision was expressed as the relative standard deviation (RSD) for each compound at each concentration. LOD and LOQ were estimated by diluting standard solutions to the lowest detectable concentrations and calculating three and ten times the signal-to-noise ratio, respectively.

2.5. α-Glucosidase Inhibitory Activity

α-Glucosidase inhibitory activity was assessed as previously described [15,30], with minor modifications. Twelve different isoflavone standards (500, 250, 125, 62.5, and 31.25 mg/L), α-glucosidase (0.2 U/mL), and 4-nitrophenol-α-D-glucopyranoside (pNPG, 50 µM) were each dissolved in phosphate buffer (PBS, pH 6.8). Acarbose was used as a positive control. Test solution (30 µL) and α-glucosidase (30 µL) were placed to a 96-well plate and incubated at 37 °C for 15 min. pNPG (30 µL) was then added to the wells and incubated at 37 °C for a further 20 min before quenching the reaction with 100 µL of 0.1 mol/L Na₂CO₃ and measuring the absorbance at 405 nm. Samples were analyzed in triplicate. The inhibition rate (%) was defined as \( \frac{(A_0 - A_s)}{A_0} \times 100 \), where \( A_0 \) is the absorbance of a negative control where the test solution was replaced by 30 µL PBS, and \( A_s \) is the absorbance of test samples. IC₅₀ was defined as the half-maximal inhibitory concentration of the inhibiting compound.

2.6. Molecular Docking

AutoDock 4.2 Tools were used to simulate the interaction sites between the isoflavones and α-glucosidase. The 3D protein structure of α-glucosidase (PDB code: 2qmj) was used as the molecular target and downloaded from the protein database (https://rcsb.org/, accessed on 5 July 2022). Water and ligand were removed from the receptor to obtain a stable structure. The 2D structures of the 12 isoflavones were downloaded from PubChem and configured to minimize energy using Chem3D 19.0 software. PyMol software was applied to visualize the interaction processes between receptor and ligands.

3. Results and Discussion

3.1. Optimization of Chromatographic Separation and Mass Spectrometric Detection

This study analyzed full MS-ddMS₂ scan in both positive and negative ionization modes to produce a sensitive and reliable quantitative technique. The responses of the 12 isoflavones were higher in positive mode than in negative mode. All the compounds generated a molecular ion \([M + H]^+\). The product ion \([M + H - 120]^+\) was observed with the C-glycosidic isoflavones (3′-hydroxy puerarin, puerarin, and 3′-methoxy puerarin) and was attributed to the loss of \( C_4H_8O_4 \). The O-glycosidic isoflavones (glycitin, daidzin, and genistin) generated the prominent characteristic ion \([M + H - 162]^+\) (attributed to loss of a glucose chain). The oxygen-carbon link between the glucose chain and the aglycones in O-glycosidic isoflavones was easier to break than the carbon-carbon link between glucose and aglycones in C-glycosidic isoflavones. Puerarin 6′′-O-xyloside and puerarin apioside produced similar characteristic ions due to their structural similarity. Several of the isoflavones yielded the product ions \([M + H - 28]^+\), \([M + H - 44]^+\), \([M + H - 18]^+\), or
[M + H − 15]+ due to the loss of CO, CO₂, H₂O, or CH₃. Detailed MS parameters for the 12 isoflavones are listed in Table 1.

**Table 1.** The detailed MS parameters of 12 compounds.

| No. | Compound                          | CAS          | Retention Time (min) | Adduct Ion        | Precursor Ion (m/z) | Delta (ppm) | Product Ion (m/z)                  |
|-----|-----------------------------------|--------------|----------------------|-------------------|--------------------|-------------|-----------------------------------|
| 1   | 3′-Hydroxy puerarin                | 117076-54-5  | 5.50                 | [M + H]+          | 433.11365          | 0.169       | 313.07080, 283.06024, 415.10297   |
| 2   | Puerarin                           | 3681-99-0    | 6.50                 | [M + H]+          | 417.11819          | 0.043       | 297.07581, 321.07520, 399.10709   |
| 3   | 3′-Methoxy puerarin                | 117047-07-1  | 6.88                 | [M + H]+          | 447.12888          | 0.069       | 327.08643, 429.11844, 297.07590   |
| 4   | Daidzin                            | 552-66-9     | 7.53                 | [M + H]+          | 417.11811          | 0.024       | 255.06538, 199.07590, 227.07002   |
| 5   | Glycitin                           | 40246-10-4   | 7.83                 | [M + H]+          | 447.12851          | −0.013      | 285.07599, 270.05240, 229.08578   |
| 6   | Glycitein                          | 40957-83-3   | 10.15                | [M + H]+          | 285.07571          | −0.014      | 270.05215, 242.05724, 225.05472   |
| 7   | Genistin                           | 529-59-9     | 9.09                 | [M + H]+          | 433.11301          | 0.021       | 271.06015, 128.06224, 153.01804   |
| 8   | Daidzein                           | 486-66-8     | 10.07                | [M + H]+          | 255.06535          | 0.063       | 227.07027, 199.07556, 137.02327   |
| 9   | Genistein                          | 446-72-0     | 10.51                | [M + H]+          | 271.06033          | 0.085       | 243.06522, 215.07027, 153.01839   |
| 10  | Formononetin                       | 485-72-3     | 10.89                | [M + H]+          | 269.08060          | −0.089      | 254.05652, 213.09102, 197.05974   |
| 11  | Puerarin 6′-O-xyloside             | 114240-18-5  | 6.86                 | [M + H]+          | 549.16083          | 0.102       | 297.07562, 417.11765, 399.10779   |
| 12  | Puerarin apioside                  | 103654-50-8  | 6.63                 | [M + H]+          | 549.16052          | 0.046       | 297.07556, 417.11746, 399.10742   |

Isoflavones range from non-polar to medium-polar, so an organic modifier is required to raise the polarity of the mobile phase used for isoflavone analysis [31]. Methanol and acetonitrile were assessed for the chromatographic separation of the 12 isoflavones, with acetonitrile giving the better separation and higher response. Addition of formic acid is also known to improve separation and ionization [32]. Aqueous formic acid solutions (0, 0.05, 0.1, 0.2, and 0.3%) were evaluated for the separation of the isoflavones. Peak shape and sensitivity were maximized for the positively ionized compounds in the presence of 0.2% formic acid. Extracted ion chromatograms for the 12 isoflavones in standard solution (A) and sample matrix (B) are shown in Figure 2.
3.2. Optimization of Extraction Conditions

Extraction solvent (water and 30, 50, 70, 90, and 100% methanol), time (30 to 150 min), and temperature (30 to 70 °C) were optimized (Figure 3). The concentrations of the 12 isoflavones in the extracts increased when the methanol concentration was increased from 0% to 90% but decreased with 100% methanol. Adding water to organic solvents is known to improve the solubility of polar isoflavones [1]. Isoflavone concentrations increased with longer extraction times but plateaued around 60 min. Isoflavone concentrations were not significantly different in extracts at 50 °C to 60 °C but decreased at 70 °C temperatures. The selected optimum extraction conditions were 90% methanol and ultrasonic extraction for 60 min at 50 °C.

3.3. Method Validation

The linear correlation coefficient (r²) was greater than 0.998 in all cases. The LODs ranged from 0.10 to 2.78 µg/kg, and LOQs from 0.30 to 9.26 µg/kg. Intra-day precision (RSD) ranged from 1.4 to 6.0%, and inter-day precision from 3.2 to 8.3%. Accuracy (re-
covery) ranged from 81.5 to 114.8%. These validation data (Table 2) demonstrate that this method is suitable for determining the concentration of the 12 isoflavones compounds in pueraria samples.

### Table 2. Results of Linear regression, LOD, LOQ, recovery test and precision of 12 compounds.

| Compounds                      | Linear Regression Data                  | LOD     | LOQ     | Originals (µg) | Spiked (µg) | Found (µg) | Recovery (%) | Intra-Day RSD (%) | Inter-Day RSD (%) |
|--------------------------------|----------------------------------------|---------|---------|----------------|-------------|------------|--------------|-------------------|-------------------|
| 3'-Hydroxy puerarin            | Y = 48,674.5X + 2400.93                | 0.9994  | 1.83    | 6.10           | 130.1       | 65.0       | 97.4         | 2.2               | 3.6               |
| Puerarin                       | Y = 118.793X + 339.229                 | 0.9991  | 0.57    | 1.89           | 402.8       | 130.0      | 99.4         | 2.8               | 5.2               |
| 3'-Methoxy puerarin            | Y = 110.433X + 194.781                 | 0.9989  | 0.95    | 3.16           | 158.4       | 600.0      | 93.6         | 1.4               | 4.9               |
| Daidzin                        | Y = 127.342X + 344.625                 | 0.9987  | 0.63    | 2.08           | 108.9       | 108.0      | 95.1         | 2.7               | 4.2               |
| Glycitin                       | Y = 182,859X + 539,463                 | 0.9990  | 0.57    | 1.89           | 12.4        | 6.0        | 86.2         | 3.2               | 7.3               |
| Glycitein                      | Y = 384,176X + 499,669                 | 0.9995  | 0.12    | 0.40           | 2.0         | 1.2        | 91.0         | 2.6               | 5.5               |
| Genistin                       | Y = 30,610,1X - 34,832.6               | 0.9989  | 2.78    | 9.26           | 16.6        | 12.0       | 85.2         | 3.8               | 5.1               |
| Daidzein                       | Y = 246,022X + 456,106                 | 0.9992  | 0.18    | 0.61           | 20.0        | 16.0       | 88.1         | 3.8               | 7.3               |
| Genistin                       | Y = 49,834,2X - 66,499,2               | 0.9991  | 0.95    | 3.16           | 1.4         | 12.0       | 89.9         | 3.2               | 6.7               |
| Formononetin                   | Y = 614,878X + 47,444.8               | 0.9993  | 0.10    | 0.30           | 1.1         | 1.2        | 94.4         | 3.7               | 7.0               |
| Puerarin 6'-O-xylloside        | Y = 42,977,4X + 57,239.4               | 0.996   | 1.88    | 6.25           | 226.4       | 60.0       | 89.1         | 4.0               | 6.9               |
| Puerarin apioside              | Y = 45,781,5X + 38,439.8              | 0.9994  | 1.63    | 5.43           | 60.0        | 60.0       | 97.0         | 2.8               | 6.2               |

### 3.4. Quantitative Analysis of Isoflavones in Pueraria

The developed method was applied to the analysis of 12 isoflavones in six pueraria samples. Average concentrations are summarized in Table 3. Puerarin was present at the highest concentration, followed by puerarin 6'-O-xylloside, 3'-methoxy puerarin, and 3'-hydroxy puerarin. The observed variations in isoflavone concentrations across the pueraria samples may be related to the sample origins and varieties [21].

### Table 3. The content of the 12 isoflavones compounds in 6 pueraria samples (mg/g).

| No. | Compound                     | Sample 1      | Sample 2      | Sample 3      | Sample 4      | Sample 5      | Sample 6      |
|-----|------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 1   | 3'-Hydroxy puerarin          | 10.56 ± 1.21  | 7.70 ± 2.03   | 8.52 ± 0.94   | 7.66 ± 1.79   | 10.81 ± 0.84  | 8.67 ± 2.02   |
| 2   | Puerarin                    | 27.6 ± 2.80   | 17.68 ± 1.52  | 22.42 ± 0.80  | 16.70 ± 3.80  | 19.00 ± 0.92  | 25.20 ± 1.61  |
| 3   | 3'-Methoxy-puerarin         | 9.83 ± 0.83   | 10.43 ± 2.12  | 8.10 ± 1.25   | 6.90 ± 1.45   | 9.91 ± 2.32   | 10.25 ± 3.10  |
| 4   | Daidzin                     | 6.53 ± 1.00   | 6.59 ± 0.34   | 5.31 ± 0.67   | 5.39 ± 1.21   | 5.82 ± 0.53   | 7.05 ± 0.63   |
| 5   | Glycitin                    | 0.95 ± 0.23   | 1.04 ± 0.08   | 0.83 ± 0.18   | 0.73 ± 0.04   | 1.02 ± 0.32   | 0.83 ± 0.33   |
| 6   | Glycitein                   | 0.23 ± 0.05   | 0.09 ± 0.04   | 0.30 ± 0.10   | 0.12 ± 0.04   | 0.14 ± 0.02   | 0.09 ± 0.01   |
| 7   | Genistin                    | 1.15 ± 0.03   | 1.04 ± 0.17   | 0.86 ± 0.32   | 0.92 ± 0.09   | 1.07 ± 0.04   | 1.17 ± 0.05   |
| 8   | Daidzein                    | 0.94 ± 0.26   | 0.64 ± 0.09   | 1.47 ± 0.15   | 0.80 ± 0.32   | 0.79 ± 0.08   | 1.40 ± 0.10   |
| 9   | Genistein                   | 0.05 ± 0.02   | 0.03 ± 0.01   | 0.09 ± 0.03   | 0.05 ± 0.03   | 0.05 ± 0.02   | 0.09 ± 0.01   |
| 10  | Formononetin                | 0.03 ± 0.01   | 0.04 ± 0.02   | 0.06 ± 0.02   | 0.04 ± 0.03   | 0.06 ± 0.02   | 0.07 ± 0.02   |
| 11  | Puerarin 6'-O-xylloside      | 9.07 ± 0.73   | 8.15 ± 1.54   | 7.62 ± 0.98   | 8.70 ± 2.01   | 7.47 ± 0.83   | 13.18 ± 2.39  |
| 12  | Puerarin apioside           | 1.23 ± 0.05   | 1.04 ± 0.13   | 0.93 ± 0.28   | 1.28 ± 0.16   | 0.93 ± 0.21   | 2.78 ± 0.06   |
3.5. Qualitative Analysis of Bioactive Components in Pueraria

Bioactive components in pueraria were analyzed using a UHPLC-Q-Orbitrap HRMS technique. A total of 88 compounds were identified, including flavonoids, terpenoids, alkaloids, organic acids, quinones, lipids, phenylpropanoids, and phenols, with the flavonoids being the most abundant small molecules (Table 4).

Table 4. Typical compounds identified from Pueraria by UHPLC-Q-Exactive Orbitrap/MS.

| No. | Compound | Formula | RT (min) | Adduct Ion | Precursor Ion (m/z) | Delta (ppm) | Product Ion (m/z) |
|-----|----------|---------|----------|------------|-------------------|------------|-----------------|
| 1   | Guanine  | C5H5N5O | 0.76     | [M + H]+   | 152.0567          | 0.077      | 110.0351, 135.03018 |
| 2   | L-Tyrosine | C9H11NO3 | 0.87     | [M + H]+   | 182.08121         | 0.205      | 136.07578, 123.04443 |
| 3   | Hydroxymethylfurfural | C6H6O3 | 0.87     | [M + H]+   | 127.03912         | 1.149      | 81.0342, 109.02873 |
| 4   | L-Phenylalanine | C9H11NO2 | 0.92     | [M + H]+   | 166.08615         | -0.656     | 120.08102, 149.05966 |
| 5   | Adenosine | C10H13N5O4 | 1.15    | [M + H]+   | 268.10385         | -0.666     | 136.06184, 85.02911 |
| 6   | Vitexin7-O-sulfate | C24H15O13 | 2.35    | [M − H]− | 511.05307         | 2.353      | 421.02237, 391.01184, 283.06030, 262.00897 |
| 7   | Diethyl tartrate | C8H14O6 | 4.26     | [M − H]− | 205.07042         | -0.656     | 72.99128, 89.02254 |
| 8   | 3,5,7-Trihydroxyflavone 3-glucoside | C24H15O13 | 4.73    | [M − H]− | 511.05325         | 0.548      | 81.03403, 109.02885 |
| 9   | 3′-Hydroxy Puerarin * | C21H20O10 | 5.42    | [M + H]+   | 433.11307         | -0.223     | 283.06018, 341.06555 |
| 10  | Hypaphorine | C14H18N2O2 | 5.6     | [M + H]+   | 247.1440          | 0.063      | 188.07057, 118.06542, 146.06004 |
| 11  | 7,8-Dihydroxyflavone | C15H10O4 | 6.24     | [M + H]+   | 255.06511         | -0.075     | 227.07043, 237.05447, 199.07541 |
| 12  | Apigenin-6-C-glucoside-7-O-glucosyl-Puerarin Puerarin-4′-O-β-D-glucopyranoside | C27H30O15 | 6.64    | [M − H]− | 593.14929         | -0.806     | 473.10712, 310.04730, 282.05231 |
| 13  | Puerarin xyloside | C26H28O13 | 6.71     | [M + H]+   | 569.16559         | -0.157     | 313.07053, 283.06000, 433.11307 |
| 14  | Puerarin-6′′-O-glucoside | C27H30O14 | 7.06     | [M + H]+   | 579.17059         | -0.237     | 267.06497, 297.07559, 417.11777 |
| 15  | Puerarin apioside * | C26H28O13 | 7.29     | [M + H]+   | 417.11748         | -0.609     | 297.07559, 267.06500, 399.10739 |
| 16  | Daidzin * | C21H20O9 | 7.45     | [M + H]+   | 447.12842         | -0.135     | 417.11771, 267.06500, 297.07559 |
| 17  | Puerarin-6′′-O-apisoside * | C27H30O14 | 7.97     | [M + H]+   | 549.15997         | -0.297     | 417.11771, 267.06500, 297.07559 |
| 18  | Puerarin apioside * | C27H30O14 | 8.03     | [M + H]+   | 549.15985         | -0.763     | 297.07568, 381.09705 |
| 19  | 3′-Methoxy puerarin * | C27H30O14 | 8.15     | [M + H]+   | 579.17059         | -0.242     | 327.04984, 299.05505 |
| 20  | Daidzein-6-C-(6′′-glucosyl)glucoside | C27H30O14 | 8.6      | [M + H]+   | 447.12842         | -0.135     | 327.08690, 297.07550, 429.11783 |
| 21  | Puerarin * | C21H20O9 | 11.8     | [M + H]+   | 417.11748         | -0.609     | 255.06500, 199.07533, 227.07014 |
| No. | Compound | Formula | RT (min) | Adduct Ion | Precursor Ion (m/z) | Delta (ppm) | Product Ion (m/z) |
|-----|----------|---------|----------|------------|---------------------|-------------|------------------|
| 27  | Calycosin | C16H12O5 | 8.66     | [M – H]−   | 283.06033           | −3.062      | 268.03683, 211.03873 |
| 28  | 3′,2′-Di-O-methyl flavone | C15H10O4 | 8.67     | [M – H]−   | 253.04967           | 0.135       | 224.04665, 133.02780 |
| 29  | Genistein | C21H20O10 | 9.01     | [M + H]+   | 433.11108           | −1.843      | 313.07007, 283.06003, 415.10181 |
| 30  | Genistein-8-C-glucoside | C21H20O10 | 9.09     | [M + H]+   | 433.11276           | −0.163      | 313.07047, 283.05994, 415.10199 |
| 31  | Glycitin * | C22H22O10 | 9.12     | [M + H]+   | 447.12952           | 0.947       | 285.07562, 270.05206, 253.04936 |
| 32  | 3′-O-Methyl daidzein | C16H12O5 | 9.14     | [M + H]+   | 285.07556           | −0.19       | 285.07556, 270.05200, 253.04945 |
| 33  | Genistein-8-C-(6′-O-apioside)-glucoside | C26H28O14 | 9.21     | [M + H]+   | 565.1543            | −0.877      | 433.11264, 313.07028, 283.05978 |
| 34  | Apigenin | C15H10O5 | 9.4      | [M + H]+   | 271.05966           | −1.616      | 215.07030, 153.01831, 243.06546 |
| 35  | Thermoposide | C22H22O11 | 9.62     | [M – H]−   | 461.1076            | −0.198      | 298.04742, 341.06580, 326.04242 |
| 36  | Oroxin B | C27H30O15 | 9.62     | [M – H]−   | 593.14978           | −0.866      | 269.04340, 341.06537, 298.04712 |
| 37  | Daidzein-4′-O-glycoside | C21H20O9 | 9.64     | [M + H]+   | 417.11743           | −0.579      | 255.06497, 199.07524, 255.06497 |
| 38  | Pueroside A | C29H34O14 | 9.67     | [M + H]+   | 607.2017            | −0.21       | 107.04955, 299.0919, 253.08572 |
| 39  | Genistein-8-C-apiosyl (1-6)-glucoside | C26H28O14 | 10.05    | [M + H]+   | 565.15411           | 1.072       | 271.05994, 433.11292 |
| 40  | Genistin | C21H20O10 | 10.25    | [M + H]+   | 433.1123            | −0.623      | 215.07022, 153.01828, 271.06003 |
| 41  | Emodin | C15H10O5 | 10.25    | [M-H]-     | 269.04468           | 0.23        | 224.04639, 133.02776 |
| 42  | Isoemobigenin | C23H24O10 | 10.37    | [M + H]+   | 461.144             | −0.388      | 107.04961, 299.09137, 253.08554 |
| 43  | Salicylic acid | C7H6O3 | 10.65     | [M – H]−   | 137.02267           | −0.651      | 137.02267, 93.03277 |
| 44  | 6′-O-Malonyl daidzin | C24H22O12 | 10.66    | [M + H]+   | 503.11694           | −1.462      | 255.06517, 199.07542, 284.03162, 255.02873, 299.05505 |
| 45  | Kaempferide | C16H12O6 | 10.93    | [M – H]−   | 299.0505            | 0.035       | 431.13345, 311.09109, 281.08060 |
| 46  | Formononetin-8-C-glucoside e-O-xylloside | C27H30O13 | 11       | [M + H]+   | 563.17535           | −0.567      | 268.03677, 239.03638, 211.03868 |
| 47  | Biochanin A * | C16H12O5 | 11.1     | [M – H]−   | 283.06015           | 0.05        | 268.03677, 239.03638, 211.03868 |
| 48  | 5-Hydroxy genistein-4′-O-(6′-malonyl) glucoside | C25H24O13 | 11.12    | [M + H]+   | 533.12976           | 0.793       | 285.07574, 270.05219 |
| 49  | Azelaic acid | C9H16O4 | 11.63     | [M – H]−   | 187.09612           | −0.385      | 126.09541, 187.09610 |
| 50  | 6′-O-Acetyl Daidzin | C23H22O10 | 11.85    | [M + H]+   | 459.12704           | −1.533      | 255.06511, 199.07536 |
| 51  | 13-HODE | C18H2O3 | 11.93     | [M – H]−   | 295.22656           | −0.211      | 277.21674, 195.13684, 224.59799 |
| 52  | Ferulic acid | C10H10O4 | 11.93     | [M – H]−   | 193.04945           | −0.085      | 134.03557, 178.02600 |
| 53  | Genistein-4′-O-(6′-malonyl)glucoside | C24H22O13 | 12.14    | [M + H]+   | 519.11395           | 0.633       | 215.07027, 153.01833, 271.06012 |
| 54  | Curcumenol | C15H22O2 | 12.57     | [M – H]−   | 233.15355           | −0.056      | 214.91223, 119.00460 |
| 55  | Chrysin | C15H10O4 | 12.88     | [M – H]−   | 253.04951           | −0.025      | 224.04666, 209.05945 |
| 56  | Daidzein * | C15H10O4 | 12.93     | [M + H]+   | 255.06516           | −0.025      | 199.07542, 227.07018, 137.02342 |
| 57  | Glycitein | C16H12O5 | 13.45     | [M + H]+   | 285.07574           | −0.01       | 285.07574, 270.05222, 253.04958 |
| 58  | Ononin | C16H12O5 | 13.68     | [M + H]+   | 431.1364            | −0.019      | 269.08075, 293.08151 |

**Table 4. Cont.**
Table 4. Cont.

| No. | Compound                                      | Formula      | RT (min) | Adduct Ion | Precursor Ion (m/z) | Delta (ppm) | Product Ion (m/z) |
|-----|-----------------------------------------------|--------------|----------|------------|---------------------|-------------|------------------|
| 59  | 2",6"-Di-O-Acetyl isovitexin                  | C2H2O2      | 14.54    | [M + H]+  | 517.13501           | 0.957       | 268.08077, 254.05725 |
| 60  | Adenine                                       | C2H2N+O     | 14.54    | [M + H]+  | 136.0618            | 0.028       | 119.03558, 91.05481 |
| 61  | Tournefolal                                    | C5H5N5      | 15.17    | [M – H]– | 269.04456           | 0.11        | 133.02759, 224.04640 |
| 62  | Genistein                                      | C15H10O5    | 15.25    | [M + H]+  | 271.05997           | –0.13       | 153.01831, 215.07030, 243.06546 |
| 63  | Fraxetin                                       | C15H10O5    | 15.25    | [M + H]+  | 209.19009           | 0.098       | 167.14314, 153.12741, 111.08086 |
| 64  | D-Gluconic acid                                | C10H8O5     | 15.35    | [M + H]+  | 209.19009           | 0.098       | 167.14314, 153.12741, 111.08086 |
| 65  | Guanine                                        | C10H10O4    | 15.25    | [M + H]+  | 167.14314           | 0.0429      | 159.02824, 129.01747 |
| 66  | Tornefolal                                     | C5H5N5      | 15.17    | [M – H]– | 269.04456           | 0.11        | 133.02759, 224.04640 |
| 67  | Formononetin-7-O-(6"-acetylglucoside)          | C5H5N5      | 16.08    | [M + H]+  | 473.14249           | –1.733      | 269.08066, 254.05722 |
| 68  | Schaftoside                                    | C2H2O2      | 16.64    | [M – H]– | 563.13873           | –0.802      | 311.05511, 283.06024, 133.02768 |
| 69  | Isoferulic acid                                | C10H8O5     | 16.64    | [M – H]– | 563.13873           | –0.802      | 311.05511, 283.06024, 133.02768 |
| 70  | Formononetin *                                 | C10H8O5     | 16.64    | [M – H]– | 563.13873           | –0.802      | 311.05511, 283.06024, 133.02768 |
| 71  | Phenylnazulene-4,5-dicarboxylate               | C5H5N5      | 16.08    | [M + H]+  | 473.14249           | –1.733      | 269.08066, 254.05722 |
| 72  | 4-Hydroxybenzaldehyde                          | C2H2O2      | 16.64    | [M – H]– | 563.13873           | –0.802      | 311.05511, 283.06024, 133.02768 |
| 73  | Licoflavone A                                  | C7H6O2      | 21.06    | [M + H]+  | 323.12753           | –0.256      | 267.06509, 239.07011, 199.07059 |
| 74  | 5-Hydroxy-6,7-dimethoxyflavone                 | C2H2O2      | 21.06    | [M – H]– | 297.07553           | –0.215      | 267.06509, 239.07011, 199.07059 |
| 75  | Neobavaisoflavone                              | C17H14O5    | 21.1     | [M – H]– | 321.11197           | –0.166      | 237.05444, 265.04950, 277.04938 |
| 76  | Isoliquiritigenin                              | C20H16O4    | 21.1     | [M + H]+  | 257.08066           | –0.175      | 137.02339, 147.0402, 119.04937 |
| 77  | Glabrone                                       | C15H12O4    | 23.5     | [M – H]– | 335.09191           | 0.21        | 307.09659, 291.06564, 277.04761 |
| 78  | Oleamidale                                     | C15H12O4    | 23.5     | [M + H]+  | 335.09191           | 0.21        | 307.09659, 291.06564, 277.04761 |
| 79  | Stigmasterol                                   | C18H35NO    | 23.72    | [M + H]+  | 413.37805           | 0.257       | 265.25244, 247.24191, 69.0763, 83.08614 |
| 80  | Physcion                                       | C29H48O     | 23.72    | [M – H]– | 283.06204           | 0.14        | 109.06524, 97.05629, 395.36667 |
| 81  | β-Sitosterol                                   | C16H12O5    | 26.98    | [M + H]+  | 415.72112           | 4.522       | 91.09415, 384.09723 |
| 82  | Isoliquiritigenin                              | C16H12O5    | 26.98    | [M + H]+  | 415.72112           | 4.522       | 91.09415, 384.09723 |
| 83  | Proline                                        | C5H9NO2     | 27.56    | [M + H]+  | 116.07082           | 0.215       | 70.06588, 99.01923, 73.04050 |
| 84  | Allantoin                                      | C4H6N4O3    | 27.56    | [M – H]– | 157.03476           | –0.857      | 114.02900, 111.95873 |
| 85  | Glycinflanin G                                 | C25H24O5    | 29.59    | [M + H]+  | 405.16962           | –0.03       | 281.0444, 319.09567, 293.04517 |
| 86  | Psoralidin                                     | C20H16O5    | 29.59    | [M + H]+  | 337.10654           | –0.51       | 309.11124, 281.11731, 253.05052, 223.07600 |
| 87  | Curcumanolide A                                | C15H22O2    | 35.42    | [M + H]+  | 235.16924           | –0.016      | 179.10669, 123.04434 |
| 88  | 7-Methoxycoumarin                              | C10H8O3     | 38.04    | [M + H]+  | 177.05463           | 0.009       | 63.03896, 149.05975, 135.04413, 117.03380 |

The compounds marked with **"** were identified by the reference standards.
3.6. α-Glucosidase Inhibitory Activity and Molecular Docking

All 12 isoflavones, except for genistein, exhibited α-glucosidase inhibitory activity (Table 5). Daidzin, 3′-methoxy puerarin, and genistin demonstrated slightly lower inhibitory activity than acarbose. Studies have shown that phenolic compounds with isoflavone skeletons exhibit in vitro α-glucosidase inhibition activity [14,33]. Similar to our study, Liu et al. [34] demonstrated, using high resolution α-glucosidase and radical scavenging profiles, that a crude methanol extract of *Pueraria lobata* was a potent α-glucosidase inhibitor. Molecular docking analysis was performed to explore the structure-activity relationships of these compounds (Figure 4) and the findings were consistent with the α-glucosidase inhibition test. The method of molecular docking provides a new perspective for the study of active substances in food [35,36]. 3′-Methoxy puerarin was the most potent compound, with a docking energy of −7.9 kcal/mol (the same as the positive control acarbose). 3′-Methoxy puerarin formed nine hydrogen bonds with seven active site residues in the receptor protein. Genistin formed eight hydrogen bonds with six active site residues. However, acarbose formed ten hydrogen bonds with seven active site residues, with a shorter hydrogen bond length. Consequently, acarbose had stronger binding affinity with α-glucosidase than the 12 isoflavones. Previous research has shown that pueraria exerts its antidiabetic activity not only via suppression of α-amylase, α-glucosidase, and the sodium-dependent glucose transporter, but possibly through other means. Pueraria protects against STZ-induced diabetes via antioxidant, antiapoptotic, antihypoxic, and anti-inflammatory pathways [37]. It regulates glucose and lipid metabolism to improve insulin resistance by various means in Luo [38]. Pueraria also increases the expression of glucose transporter type 4 [5]. However, beneficial in vitro properties of compounds should be validated by in vivo studies.

Table 5. The result of α-glucosidase inhibitory activity (IC\(_{50}\)) and molecular docking.

| No. | Compound                        | IC\(_{50}\) (mg/L) | Docking Energy (kcal/mol) | Active Site Residues                  | Hydrogen Bond Number |
|-----|---------------------------------|---------------------|---------------------------|--------------------------------------|----------------------|
| 1   | 3′-Hydroxy puerarin             | 203.6 ± 4.2         | −8.6                      | GLU-767, ILE-734, ARG-653             | 4                    |
| 2   | Puerarin                       | 154.2 ± 1.7         | −8.1                      | GLU-658, ILE-734, ARG-653, TYR-733    | 4                    |
| 3   | 3′-Methoxy-puerarin             | 95.9 ± 3.8          | −7.9                      | GLU-767, GLU-658, ARG-643, ARG-647, ARG-653, TYR-660, GLU-788 | 9                    |
| 4   | Daidzin                        | 72.94 ± 2.5         | −8.3                      | GLU-658, GLN-272, ARG-653, TYR-660    | 5                    |
| 5   | Glycitin                       | 139.7 ± 5.1         | −9.0                      | GLU-658, GLU-767, TYR-660             | 3                    |
| 6   | Glycitein                      | 246.0 ± 2.6         | −8.1                      | LEU-754, LYS-817, HIS-625             | 3                    |
| 7   | Genistin                       | 101.3 ± 6.3         | −8.7                      | GLN-275, ASP-649, GLN-272, ARG-653, GLU-767, ILE-734 | 8                    |
| 8   | Daidzein                       | 114.8 ± 1.9         | −8.1                      | GLU-767                               | 2                    |
| 9   | Genistein                      | /                   | −8.1                      | /                                     | /                    |
| 10  | Formononetin                   | 128.3 ± 2.0         | −8.1                      | GLU-767                               | 2                    |
| 11  | Puerarin 6′-O-xyloside         | 169.6 ± 3.4         | −8.7                      | LYS-776, ARG-283, ALA-644             | 4                    |
| 12  | puerarin apioside              | 96.55 ± 2.6         | −9.0                      | GLU-767, TYR-660, AGR-730             | 3                    |
| 13  | Acarbose                       | 58.6 ± 4.4          | −7.9                      | GLU-275, ASP-759, GLN-272, ARG-730, GLU-767, ILE-734 | 10                   |
Figure 4. Molecular docking conformations of isoflavones with α-glucosidase (short, dotted yellow lines represent hydrogen bonds. (1). 3'-hydroxy puerarin, (2). puerarin, (3). 3'-methoxy puerarin, (4). daidzin, (5). glycitin, (6). glycitein, (7). genistin, (8). daidzein, (9). genistein, (10). formononetin, (11). Puerarin 6''-O-xyloside, (12). puerarin apioside.

4. Conclusions

A rapid and sensitive quantitative method was developed and validated for the simultaneous determination of 12 isoflavones in pueraria using UHPLC-Q-Orbitrap HRMS. The method showed good linearity, accuracy, and precision. In 12 isoflavones, the content of puerarin was the highest. Qualitative analysis also identified 88 small molecule components, with flavonoids being the most abundant. α-Glucosidase inhibition testing and molecular docking elucidated the interactions between the isoflavones and α-glucosidase, confirming the inhibitory activity of these bioactive components. Molecular docking showed that 3'-methoxy puerarin had the highest α-glucosidase inhibitory activity. Pueraria is a promising functional food that could potentially be used as an α-glucosidase inhibitor to control postprandial hyperglycemia. Further in vivo studies are needed to explore the α-glucosidase inhibitory effects of pueraria.

Author Contributions: Writing—original draft, Y.Y.; methodology, H.Z.; data curation, F.Z. (Furong Zhu); software, Y.L.; validation, X.L.; writing—review & editing, F.Z. (Feng Zeng); project administration, supervision, B.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the fund of Science and Technology Planning Project of Fujian Province (No. 2021L3007), Regional Development Project of Fujian Province (No. 2020N3002), Major Special Project of Fujian Province (No. 2021NZ0101), the Natural Science Foundation of Fujian Province (No. 2020J01092), the Fujian provincial health technology project (No. 2019-ZQN-29) and Construction of Fujian Provincial Scientific and Technological Innovation Platform (No. 2019Y2001).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: All authors declare that they have no conflict of interest.
References

1. Wong, K.H.; Li, G.Q.; Li, K.M.; Razmovski-Naumovski, V.; Chan, K. Optimisation of Pueraria isolflavonoids by response surface methodology using ultrasonic-assisted extraction. *Food Chem.* 2017, 231, 231–237. [CrossRef] [PubMed]

2. Bharti, R.; Chopra, B.S.; Raut, S.; Khatri, N. Pueraria tuberosa: A Review on Traditional Uses, Pharmacology, and Phytochemistry. *Front. Pharmacol.* 2020, 11, 582506. [CrossRef] [PubMed]

3. Wang, S.; Zhang, S.; Wang, S.; Gao, P.; Dai, L. A comprehensive review on Pueraria: Insights on its chemistry and medicinal value. *Biomed. Pharmacother.* 2020, 131, 110734. [CrossRef] [PubMed]

4. Li, Y.; Luo, X.X.; Yan, F.D.; Wei, Z.B.; Tu, J. Puerariae Lobatae Radix elevated expression levels of OB-R, IRS2, GLUT1 and GLUT2 to regulate glucose metabolism in insulin-resistance HepG2 cells. *Zhongguo Zhong Yao Za Zhi* 2012, 42, 1399–1944.

5. Lertpatipanpong, P.; Janpajit, S.; Park, E.Y.; Kim, C.T.; Baek, S.J. Potential Anti-Diabetic Activity of Pueraria lobata Flower (Flos Puerariae) Extracts. *Molecules* 2020, 25, 3970. [CrossRef]

6. Sun, R.; Deng, X.; Zhang, D.; Xie, F.; Wang, D.; Wang, J.; Ravallie, M.S.; Jiang, F.; Fu, L. Anti-diabetic potential of *Pueraria lobata* root extract through promoting insulin signaling by PTP1B inhibition. *Bioorg. Chem.* 2019, 87, 12–15. [CrossRef]

7. Shukla, R.; Banerjee, S.; Tripathi, Y.B. Pueraria tuberosa extract inhibits iNOS and IL-6 through suppression of PKC-alpha and NF-kB pathway in diabetes-induced nephropathy. *J. Pharm. Pharmacol.* 2018, 70, 1102–1112. [CrossRef]

8. Liu, J.; Shi, Y.C.; Lee, D.Y. Applications of Pueraria lobata in treating diabetics and reducing alcohol drinking. *Chin. Herb. Med.* 2019, 11, 141–149. [CrossRef]

9. Zhang, Z.T.; Guo, N.; Zhuang, G.D.; Deng, S.M.; He, W.J.; Chen, Z.Q.; Xu, Y.H.; Tang, D.; Wang, S.M. Metabolic Profiling of Carbonyl Compounds for Unveiling Protective Mechanisms of Pueraria lobata against Diabetic Nephropathy by UPLC-Q-Orbitrap HRMS/MS Analysis. *J. Agric. Food Chem.* 2021, 69, 10943–10951. [CrossRef]

10. Wang, Q.S.; Wang, Y.L.; Zhang, W.Y.; Li, K.D.; Luo, X.F.; Cui, Y.L. Puerarin from Pueraria lobata alleviates the symptoms of irritable bowel syndrome-diarrhea. *Food Funct.* 2021, 12, 2211–2224. [CrossRef]

11. Liu, Y.; Qu, Y.; Chen, Q.; Han, X.; Cai, M.; Hao, L. Puerarin suppresses the hepatic glucoseogenesis via activation of P3K/Akt signaling pathway in diabetic rats and HepG2 cells. *Biomed. Pharmacother.* 2021, 137, 111325. [CrossRef] [PubMed]

12. Wong, K.H.; Razmovski-Naumovski, V.; Li, K.M.; Li, G.Q.; Chan, K. Comparing morphological, chemical and anti-diabetic characteristics of Puerariae Lobatae Radix and Puerariae Thomsonii Radix. *J. Ethnopharmacol.* 2015, 164, 53–63. [CrossRef]

13. Zhu, W.F.; Li, J.L.; Meng, X.W.; Zhang, P.Z.; Wu, W.T.; Liu, R.H. Research advances in chemical constituents and pharmacological activities of Pueraria genus. *Zhongguo Zhong Yao Za Zhi* 2021, 46, 1311–1331.

14. Papoutsis, K.; Zhang, J.; Bowyer, M.C.; Brunton, N.; Gibney, E.R.; Lyng, J. Fruit, vegetables, and mushrooms for the preparation of extracts with alpha-amylase and alpha-glucosidase inhibition properties: A review. *Food Chem.* 2021, 338, 128119. [CrossRef] [PubMed]

15. Hou, Z.W.; Chen, C.H.; Ke, J.P.; Zhang, Y.Y.; Qi, Y.; Liu, S.Y.; Yang, Z.; Ning, J.M.; Bao, G.H. alpha-Glucosidase Inhibitory Activities of Puerariae) Extracts. *Molecules* 2019, 25, 344. [CrossRef] [PubMed]

16. Assefa, S.T.; Yang, E.Y.; Chae, S.Y.; Song, M.; Lee, J.; Cho, M.C.; Jang, S. Alpha Glucosidase Inhibitory Activities of Plants with Focus on Common Vegetables. *Plants* 2019, 8, 2. [CrossRef]

17. Zhu, J.; Chen, C.; Zhang, B.; Huang, Q. The inhibitory effects of flavonoids on alpha-amylase and alpha-glucosidase. *Crit. Rev. Food Sci. Nutr.* 2020, 60, 695–708. [CrossRef]

18. Kumar, S.; Narwal, S.; Kumar, V.; Prakash, O. alpha-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacogn. Rev.* 2011, 5, 19–29. [CrossRef]

19. Juengsanguanpornsk, W.; Yusakul, G.; Kittisiripanya, T.; Krittanai, S.; Juengwatanantrakul, T.; Sakamoto, S.; Putalun, W. Quantification of methylisomiroestrol, a phytoestrogen of *Pueraria candollei*, by enzyme-linked immunosorbent assay in comparison with high-performance liquid chromatography. *J. Pharm. Biomed. Anal.* 2021, 192, 113674. [CrossRef]

20. Krittanai, S.; Kittisiripanya, T.; Udomsin, O.; Tanaka, H.; Sakamoto, S.; Juengwatanantrakul, T.; Putalun, W. Development of a colloidal gold nanoparticle-based immunochromatographic strip for the one-step detection of miroestrol and puerarin. *Biomed. Chromatogr.* 2018, 32, e4330. [CrossRef]

21. Huang, Z.Y.; Shen, Q.N.; Li, P.; Su, L.L.; Chen, L.H.; Lu, T.L.; Mao, C.Q. Quality research of *Puerariae Lobatae Radix* from different habitats with UPLC fingerprint and determination of multi-component content. *Zhongguo Zhong Yao Za Zhi* 2019, 44, 2051–2058. [PubMed]

22. Qu, L.; Song, K.; Zhang, Q.; Guo, J.; Huang, J. Simultaneous Determination of Six Isolavones from *Puerariae Lobatae Radix* by CPE-HPLC and Effect of Puerarin on Tyrosinase Activity. *Molecules* 2020, 25, 344. [CrossRef] [PubMed]

23. Ahn, S.J.; Kim, H.J.; Lee, A.; Min, S.S.; In, S.; Kim, E. Determination of 12 herbal compounds for estimating the presence of Angelica Gigas Root, Corins Fruit, Licorice Root, Pueraria Root, and Schisandra Fruit in foods by LC-MS/MS. *Food Addit. Contam. Part A Chem.* 2020, 37, 1437–1448. [CrossRef] [PubMed]

24. Masada, S.; Hosoe, J.; Arai, R.; Demizu, Y.; Hakamatsu, T.; Goda, Y.; Uchiyama, N. Miroestrol Quantification in *Pueraria mirifica* Crude Drugs and Products by Single-Reference UPLC/PDA/MS Using Relative Molar Sensitivities to Kwakhurin. *Chem. Pharm. Bull.* 2021, 69, 573–580. [CrossRef] [PubMed]
25. Ma, T.; Fu, Q.; Mei, Q.; Tu, Z.; Zhang, L. Extraction optimization and screening of angiotensin-converting enzyme inhibitory peptides from Channa striatus through bioaffinity ultrafiltration coupled with LC-Orbitrap-MS/MS and molecular docking. *Food Chem.* 2021, 354, 129589. [CrossRef] [PubMed]

26. Zhu, L.; Ma, S.; Li, K.; Xiong, P.; Qin, S.; Cai, W. Systematic Screening of Chemical Constituents in the Traditional Chinese Medicine Arnebiae Radix by UHPLC-Q-Exactive Orbitrap Mass Spectrometry. *Molecules* 2022, 27, 2631. [CrossRef] [PubMed]

27. Yu, Y.; Yao, C.; Guo, D.A. Insight into chemical basis of traditional Chinese medicine based on the state-of-the-art techniques of liquid chromatography-mass spectrometry. *Acta Pharm. Sin. B* 2021, 11, 1469–1492. [CrossRef]

28. Ricciutelli, M.; Moretti, S.; Galarini, R.; Sagratini, G.; Mari, M.; Lucarini, S.; Vittori, S.; Caprioli, G. Identification and quantification of new isomers of isopropyl-malic acid in wine by LC-IT and LC-Q-Orbitrap. *Food Chem.* 2019, 294, 390–396. [CrossRef]

29. AOAC International. Guidelines for validation of botanical identification methods. *J. AOAC Int.* 2012, 95, 268–272. [CrossRef]

30. Hu, G.; Peng, X.; Dong, D.; Nian, Y.; Gao, Y.; Wang, X.; Hong, D.; Qiu, M. New ent-kaurane diterpenes from the roasted arabica coffee beans and molecular docking to alpha-glucosidase. *Food Chem.* 2021, 345, 128823. [CrossRef]

31. Wu, W.; Zhang, Y.; Zhang, F.; Liu, J.; Ren, Z.; Xu, Y.; Liu, T.; Zhou, W.; Li, H.; Zhang, C. An analytical strategy for accurate, rapid and sensitive quantitative analysis of isoflavones in traditional Chinese medicines using ultra-high performance supercritical fluid chromatography: Take Radix Puerariae as an example. *J. Chromatogr. A* 2019, 1606, 460385. [CrossRef] [PubMed]

32. Du, G.; Zhao, H.; Song, Y.; Zhang, Q.; Wang, Y. Rapid simultaneous determination of isoflavones in Radix puerariae using high-performance liquid chromatography-triple quadrupole mass spectrometry with novel shell-type column. *J. Sep. Sci.* 2011, 34, 2576–2585. [CrossRef] [PubMed]

33. di Stefano, E.; Oliviero, T.; Udenigwe, C.C. Functional significance and structure–activity relationship of food-derived α-glucosidase inhibitors. *Curr. Opin. Food Sci.* 2018, 20, 7–12. [CrossRef]

34. Liu, B.; Kongstad, K.T.; Qinglei, S.; Nyberg, N.T.; Jager, A.K.; Staerk, D. Dual high-resolution alpha-glucosidase and radical scavenging profiling combined with HPLC-HRMS-SPE-NMR for identification of minor and major constituents directly from the crude extract of Pueraria lobata. *J. Nat. Prod.* 2015, 78, 294–300. [CrossRef] [PubMed]

35. Shen, B.; Shangguan, X.; Yin, Z.; Wu, S.; Zhang, Q.; Peng, W.; Li, J.; Zhang, L.; Chen, J. Inhibitory Effect of Fisetin on alpha-Glucosidase Activity: Kinetic and Molecular Docking Studies. *Molecules* 2021, 26, 5306. [CrossRef] [PubMed]

36. Liu, J.L.; Kong, Y.C.; Miao, J.Y.; Mei, X.Y.; Wu, S.Y.; Yan, Y.C.; Cao, X.Y. Spectroscopy and molecular docking analysis reveal structural specificity of flavonoids in the inhibition of alpha-glucosidase activity. *Int. J. Biol. Macromol.* 2020, 152, 981–989. [CrossRef] [PubMed]

37. Srivastava, S.; Pandey, H.; Singh, S.K.; Tripathi, Y.B. Anti-oxidant, anti-apoptotic, anti-hypoxic and anti-inflammatory conditions induced by PTY-2 against STZ-induced stress in islets. *Biosci. Trends* 2019, 13, 382–393. [CrossRef]

38. Luo, X.X.; Xu, G.L.; Li, Y.; Li, B.T.; Tu, J. Kudzu root (Ge-Gen) regulates on glucose and lipid metabolism to ameliorating insulin resistance on 3T3-L1 adipocytes. *Zhongguo Zhong Yao Za Zhi* 2016, 41, 2687–2694.