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*Research Article*

# Phenolic Composition, Antioxidant, and α-Glucosidase Inhibitory Activities of Extracts from *Polygonatum sibiricum*

**Rujie Shi, Min Gong, Yanxin Li, and Nong Zhou**

*College of Biology and Food Engineering, Chongqing Three Gorges University, Chongqing, China*

Correspondence should be addressed to Nong Zhou; erhaizn@126.com

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As a traditional Chinese medicine and functional food, *Polygonatum sibiricum* contains many bioactive compounds with multiple biological activities. Presently, we obtained three extracts from *Polygonatum sibiricum* by different solvents and comparably analyzed their total phenols, flavonoids, and chemical compositions. Meanwhile, the antioxidant activity and the attenuation of palmitic acid (PA)-induced hepatic oxidative stress of these extracts were further investigated. The results showed that the active substances in *Polygonatum sibiricum* were inclined to dissolve in ethanol (EE), and twenty compounds were identified. EE exhibited the best antioxidant activity to scavenging DPPH and ABTS free radicals, as well as reducing power. Meanwhile, extracts from *Polygonatum sibiricum*, especially EE, could significantly increase the activity of SOD and HO-1 and ameliorate PA-induced oxidative stress in HepG2 cells through Nrf2-related pathways. Furthermore, EE could significantly inhibit the activity of α-glucosidase and effectively reduce the rapid rise of blood glucose induced by sucrose loading.

## 1. Introduction

*Polygonatum sibiricum* is the rhizome of *Liliaceae* plant, including *Polygonatum kingianum* Coll. et Hemsl., *Polygonatum sibiricum* Red., *and Polygonatum cyrtonema* Hua, which are widely distributed in China, Japan, Korea, India, Russia, and North America [1, 2]. *Polygonatum sibiricum* is often used as the traditional Chinese medicine and a functional food with plenty of active compounds [2]. Previous reports have identified various components, such as polysaccharides [3], alkaloids [4], and saponins [5, 6], in *Polygonatum sibiricum*. Recently, Tang et al. have isolated and identified six homoisoflavonoids, all of which were first discovered in *Polygonatum sibiricum* [7]. Additionally, amounts of sterols, amino acid, and volatile oil were also found [2].

*Polygonatum sibiricum* exhibited various biological and pharmacological functions due to the plenty of active ingredients, of which, polysaccharides have been widely studied [1]. Many previous research studies indicated those polysaccharides from *Polygonatum sibiricum* revealed antioxidant, anti-inflammation, anticancer, and antidiabetes activities, as well as promotion of osteoblastic differentiation and attenuation of aging [8–14]. Recently, Zhang et al. analyzed the structures of fructan and galactan from *Polygonatum* and investigated their utilization by probiotic bacteria [15]. The results showed that fructan and galactan were consisted of a (2⟶6) linked β-D-Fruf residues backbone and a (1⟶4) -β-d-galactan, respectively [15]. Meanwhile, the two new polysaccharides could remarkably promote the growth of *Bifidobacterium* and *Lactobacillus* strains [15]. What's more, *Polygonatum sibiricum* polysaccharides were verified to inhibit cyclophosphamide-induced immunosuppression in chickens, which could be used as a potential immunostimulant agent [16]. Besides polysaccharides, some other active compounds, such as saponin, steroidal glycosides, and methyl protodioscin have been researched to show hypoglycemic effects, antiproliferative, and inhibition of cervical cancer activities [17–19]. However, the polyphenols and their chemical constitution in *Polygonatum sibiricum* were not paid attention to be fully explored. Meanwhile, there have few reports about the antioxidant and antihyperglycemic activities of polyphenols from *Polygonatum sibiricum*.

Presently, HPLC-MS/MS analysis was employed to investigate the chemical constitution of different extracts from *Polygonatum sibiricum*, and the antioxidant and anti-hyperglycemic activities were evaluated in vitro and in vivo. Furthermore, the underlying mechanisms of improving palmitic acid (PA)-induced oxidative stress in HepG2 cells of these extracts were also explored.

## 2. Materials and Methods

*2.1. Reagents and Chemicals.* 1-diphenyl-2-trinitrophenylhydrazine (DPPH), 2, 2′-diazobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and Trolox were purchased from Sigma (St. Louis, MO, USA). Gallic acid and rutin were obtained from Yuanye Bio-Technology (Shanghai, China). The antibodies including anti-Nrf2, antiphospho-Nrf2, anti-HO-1, and anti-SOD2 were purchased from Cell Signaling Technology (Shanghai, China), and anti-GAPDH was obtained from Bioss (Beijing, China).

*2.2. Samples and Extract Preparation.* The fresh rhizome of *Polygonatum sibiricum* was cut into pieces and dried at 60°C for 24 h before grinded (portable pulverizer, Troody Analytical Instrument, Shanghai, China), after that the powder was sieved to pass 100 meshes for use. The extraction process was performed according to the report of Wang et al. with slight modifications [20]. Briefly, the powder of *Polygonatum sibiricum* (1 g) was mixed with 20 mL of water, 80% ethanol (v/v), and ethyl acetate, respectively, to extract in thermostat water bath at 80°C for 1 h. After being filtered, the residues were extracted twice with the corresponding solvent. The filter liquor was collected and concentrated before freeze drying to obtain water extract (WE), ethanol extract (EE), and ethyl acetate extract (EAE), respectively (Figure 1).

*2.3. Determination of Total Phenols and Total Flavonoids.* To determine the total phenols of extracts from *Polygonatum sibiricum*, Folin–Ciocalteu reagent (125 $\mu$L) was added to each extract (125 $\mu$L), which was resolved by the corresponding solution, and stored in the dark for 6 min. After that, 1.25 mL $NaCO_3$ (7%) was mixed to react for 90 min at room temperature, following the measurement of absorbance at 760 nm. Gallic acid was employed as the standard, and the content of total phenols was expressed as mg/g DW (dry weight) [21].

For the total flavonoids assay, 125 $\mu$L of different extracts were mixed with $NaNO_2$ (200 $\mu$L, 5%) for 6 min. Subsequently, 200 $\mu$L Al $(NO_3)_3$ (10%) was added to react for another 6 min, following the supply of 2 mL NaOH (1 M) and adjusting to 5 mL with deionized water. After 15 min, the absorbance was recorded at 510 nm and the results were calculated to that of rutin standard and expressed as mg/g DW (dry weight) [22].

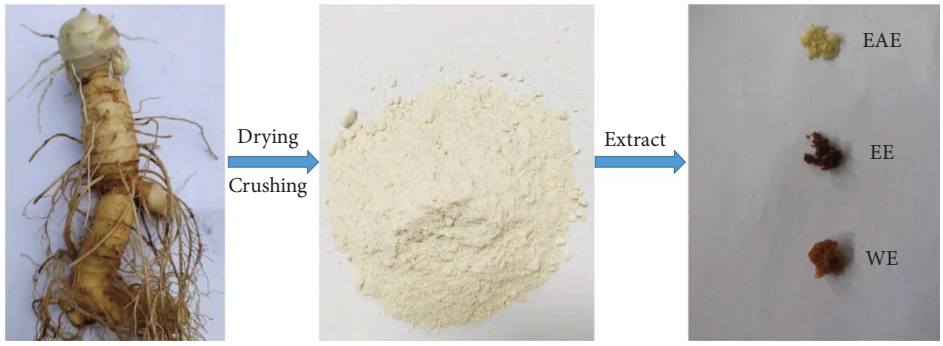
*2.4. HPLC-MS/MS Analysis of the Extracts from Polygonatum sibiricum.* High-resolution ion mobility liquid chromatography-mass spectrometry (HPLC-MS/MS, Triple Quad 5500 + QTRAP Ready, AB SCIEX, Singapore), equipped with a Zorbax Eclipse Plus $C_{18}$ column (250 mm × 4.6 mm, 5 $\mu$m) and high-resolution tandem quadrupole time of flight mass spectrometry, was employed to separate and identify the main components in the extracts. The conditions are as follows: mobile phase A was consisted with 0.1% formic acid-water (V/V), and mobile phase *B* was acetonitrile. The procedures of elution were 0–5 min, 15% *B*; 5–10 min, 15–20% *B*; 10–20 min, 20–25% *B*; 20–30 min, 25–35% *B*; 30–40 min, 35–50% *B*; 40–45 min, 80% *B*; and 45–50 min, 15% *B*. The flow rate was 0.8 mL/min with the injection volume of 20 $\mu$L. The mass spectrometry conditions are as follows: IDA acquisition mode; scanning range is 100–2000 (MS), 50–2000 (MS/MS); ESI (negative); temperature of ion source, 550°C; and spray voltage, −4500 V [23]. The ESI-mass spectrometry library, provided by the device of AB SCIEX Co. (Singapore), was used to identify the compound through comparing with the standards.

*2.5. DPPH and ABTS Scavenging Activity.* The method described by our previous report was employed to investigate the DPPH and ABTS scavenging activities [22]. Briefly, the dried samples were re-dissolved in the corresponding extraction solvents. For the DPPH assay, 1 mL extracts (40 mg/mL) were reacted with 1 mL DPPH (0.13 mM) in the dark for 30 min. After that, the absorbance was recorded at 517 nm. For ABTS assay, 100 $\mu$L extracts (40 mg/mL) were mixed with 2.9 mL ABTS (7.0 mM) in the dark for 20 min, and then the absorbance was measured at 734 nm. The results were expressed as Trolox equivalents ($\mu$mol TE/g DW).

*2.6. Reducing Power Assay.* The reducing power of the extracts was determined by the method of Guo et al. [24]. Briefly, FRAP solution was prepared with sodium acetate (300 mM), TPTZ (10 mM), and ferric chloride (20 mM) (10 : 1 : 1, v : v : v), and the dried samples were re-dissolved in the corresponding extraction solvents. After that, extracts (100 $\mu$L, 40 mg/mL) were added to FRAP (3.9 mL) and reacted in the dark for 30 min. Subsequently, the absorbance was determined at 593 nm, and the results were revealed as $FeSO_4$ equivalent ($\mu$mol FE/g DW).

*2.7. Attenuation of PA-Induced Oxidative Stress in HepG2 Cells*

*2.7.1. Methylthiazolydiphenyl-Tetrazolium Bromide (MTT) Assay.* The MTT assay was performed according to the instructions of the MTT Cell Proliferation and Cytotoxicity Assay Kit (Beyotime Institute of Biotechnology, Shanghai, China). Briefly, HepG2 cells grown in a culture dish (6 mm) were transplanted in 96-well plates and incubated at 37°C in a humidified 5% $CO_2$ atmosphere for 24 h. Subsequently, cells were treated with different concentrations (0.1 mg/mL to 4.0 mg/mL, dissolved in DMSO) of EAE, EE, and WE for another 24 h. After that, 10 $\mu$L MTT (5 mg/mL, 25 mg MTT was dissolved in 5 mL MTT solvent) and 100 $\mu$L formanzan




*Polygonatum sibiricum*

Figure 1: The preparation process of different extracts from *Polygonatum sibiricum*.

were added to incubate 4 h at 37°C, respectively. Finally, the absorbance was determined at 570 nm by a microplate reader (Perkin Elmer), and the cell viability was calculated as a relative percentage to the control group.

*2.7.2. Determination of Reactive Oxygen Species (ROS) Level.* Intracellular ROS in HepG2 cells were measured according to the instructions of the ROS Assay Kit (Beyotime Biotechnology, Shanghai, China). After being co-treated with 1.00 mg/mL of EAE, EE, and WE and stimulated by PA (100 $\mu$M) for 24 h, HepG2 cells were incubated with DCFH-DA (10 $\mu$M) for 30 min at 37°C in the dark. Subsequently, fluorescence was recorded by a multimode microplate reader (Perkin Elmer), and the ROS level was expressed as fold changes in fluorescence intensity to control.

*2.7.3. Analysis of Malondialdehyde (MDA) Content and SOD Activity.* After being preprotected by 1.00 mg/mL of EAE, EE, and WE, HepG2 cells were co-incubated with 100 $\mu$M of PA for 24 h. Subsequently, cells were collected by adding ice-cold radio-immunoprecipitation assay (RIPA) buffer (consist of 1 mM phenylmethylsulfonyl fluoride, PMSF), and the MDA content and SOD activity were investigated by the instructions of the MDA and SOD Activity Assay Kits, respectively (Beyotime Biotechnology, Shanghai, China). Additionally, the protein concentration was determined by the BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China).

*2.7.4. Western Blotting.* The process of western blotting was performed according to the method of Han et al. [25]. After treatment, cells were harvested and quantified by the BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to isolate the proteins, which were then transferred to a polyvinylidene fluoride (PVDF) membrane. After blocking with bovine serum albumin, the membrane was incubated with the specific antibodies overnight at 4°C, followed by secondary antibodies. The protein bands were visualized using chemiluminescence assay system, and the relative densities of the targeted bands were analyzed by ImageJ software (Bio-Rad Laboratories, Hercules, CA, U.S.A.).

*2.8. α-Glucosidase Inhibitory Activity.* The method reported by Han et al. was accorded to investigate the α-glucosidase inhibitory activity of extracts from *Polygonatum sibiricum* [26]. Briefly, different concentrations (1 mg/mL, 20 mg/mL, and 40 mg/mL) of extracts (10 $\mu$L) were mixed with 10 $\mu$L of PBS (pH 8.6) and α-glucosidase (0.5 U/mL) for 15 min at 37°C. After that, 20 $\mu$L *p*-nitrophenol glucopyranoside (pNPG) (3 mM) was added to react for another 10 min, and $Na_2CO_3$ (150 $\mu$L, 0.1 M) was employed to terminate the reaction before the absorbance was determined at 405 nm. Acarbose (20 mg/mL) was used as the positive control.

*2.9. Sucrose-Loading Test.* The male Kunming mice (30–35 g) were randomly divided into six groups ($n = 8$): (1) control group; (2) model group (sucrose 3 g/kg body weight); (3) EAE group (sucrose + 40 mg/kg body weight); (4) EE group (sucrose + 40 mg/kg body weight); (5) WE group (sucrose + 40 mg/kg body weight); and (6) acarbose group (sucrose + 10 mg/kg body weight). After administration with sucrose, extracts were supplied simultaneously via a gavage method. The blood glucose levels were measured using Glucose Detection Kits (Robio Co., Shanghai, China) from the tail vein at 0, 15, 30, 60, 90, and 120 min [27]. The glucose area under the curve (AUC) was calculated and compared. All the animal procedures were complied with the Guidelines for Care and Use of Laboratory Animals: 8th Edition, ISBN-10: 0-309-15396-4.

*2.10. Statistical Analysis.* The data were analyzed by GraphPad Prism 8.0 software and one-way ANOVA was employed to statistical analysis. The results were expressed as the mean ± standard deviation (SD) and $p < 0.05$ was considered statistically significant, which was exhibited with different letters.

## 3. Results

*3.1. Total Phenols and Total Flavonoids.* Three different solvents including ethyl acetate, ethanol, and water were employed to prepare the extracts from *Polygonatum*

*sibiricum*. After freeze drying, the total phenols and total flavonoids of the three samples were determined, respectively. As shown in Figure 2, the total phenols in EE reached up to $6.27 \pm 0.11$ mg/g DW, which is much higher than that of EAE and WE ($1.30 \pm 0.06$ mg/g DW and $3.76 \pm 0.12$ mg/g DW, respectively), indicating that the phenolic compounds in *Polygonatum sibiricum* are inclined to dissolve in ethanol. However, the total flavonoids in different extracts are not absolutely according with total phenols. The content of total flavonoids showed no significant differences between EE and WE, $8.24 \pm 0.17$ mg/g DW vs. $8.14 \pm 0.17$ mg/g DW, both of which were markedly higher than that of EAE ($2.26 \pm 0.08$ mg/g DW). These results revealed that ethanol is the better solvent to be used to extract phenols and flavonoids in *Polygonatum sibiricum*. Guo et al. have optimized the extraction conditions of total phenols and flavonoids in *Polygonatum sibiricum*, respectively [28, 29]. However, the content in the present study is much higher than that of the two previous reports, which might be related to the planting conditions. As the polyphenols in Titeban wild *Polygonatum sibiricum* reached up to 19.8 mg/g [30], thereby, planting location is one of the main factors that influences the generation and accumulation of total phenols and flavonoids in *Polygonatum sibiricum*.

*3.2. HPLC-MS/MS Analysis.* In order to further ensure the differences in compositions between different extracts, HPLC-MS/MS was used to separate and identify the main polyphenols in the extracts through comparing them with the standards in the ESI-MS library (AB SCIEX Co., Singapore). As shown in Table 1, twenty compounds were identified in the three extracts of *Polygonatum sibiricum*. Kaempferol and phenolic acids, including fumaric acid, malic acid, and polygalic acid, were widely existed in the three extracts. However, several compounds could not be extracted by special solvent, such as dihydrocapsaicin and sclareol glycol were not found in WE, and hydroxyecdysone, ferulic acid, catechin, *p*-coumaric acid, platycodin D, mulberroside A, and salicylic acid were only identified in EE. Therefore, the results indicated that EE obtained much more compounds from *Polygonatum sibiricum*, which is consisted with the above results. To the best of our present knowledge, many research studies have widely investigated the *Polygonatum sibiricum* polysaccharides and their biological activities, but few publications focused on the composition of polyphenols in *Polygonatum sibiricum*. Wu et al. reported the isolation and purification processing of the raw *Polygonatum* polyphenols by using the specific macroprous resin and found that the main composition of *Polygonatum* polyphenols were chlorogenic acid, ferulic acid, rutin, and ursolic acid [31]. Presently, we only discovered ferulic acid in the EE group, but some other phenolic acids, such as fumaric acid, malic acid, and polygalic acid, were abundantly identified. Meanwhile, several well-known polyphenols, including kaempferol and dihydrocapsaicin, were first found in *Polygonatum sibiricum*. Kaempferol is one of the most common flavonoids, which widely exists in fruits, vegetables, and Chinese herbal medicines. Dihydrocapsaicin is an alkaloid extracted from chili peppers with strong antibacterial activity. Additionally, saponins were widely discovered in *Polygonatum sibiricum*, which are characterized by a skeleton derived of oxidosqualene [2]. Herein, some new saponins, including baicalin, platycodin D, and mulberroside A were identified in the different extracts of *Polygonatum sibiricum*.

*3.3. Antioxidant Activity.* Previous reports showed that *Polygonatum sibiricum* had strong antioxidant activity, which was closely associated with the polysaccharides and polyphenols. In the present study, we employed three systems to evaluate the antioxidant activity of *Polygonatum sibiricum* extracts. As shown in Figure 3, EE exhibited the strongest activity to scavenge DPPH free radical ($2.34 \pm 0.08$ TE/g DW). This phenomenon was also observed in the ABTS assay. EE and EAE showed better ABTS scavenging activity than WE ($6.21 \pm 0.22$ TE/g DW and $5.79 \pm 0.45$ TE/g DW vs. $4.14 \pm 0.57$ TE/g DW). In the FRAP assay, EE still revealed much stronger activity than that of EAE and WE. These results implied that the EE group contained more active compounds that were responsible to the antioxidant activity.

*3.4. Effect of Extracts on PA-Induced Cellular Oxidative Stress*

*3.4.1. The Effect of Extracts on Cell Viability.* The MTT assay showed that the extracts of EAE, EE, and WE revealed no significantly toxic effect against HepG2 cells when the concentrations were lower than 2.0 mg/mL (Figure 4). However, the three extracts obviously reduced the cell viability and revealed cytotoxicity at the concentration of 4.0 mg/mL. Therefore, we chose EAE, EE, and WE at a concentration up to 1.00 mg/mL in the following cellular experiments.

*3.4.2. Effect of Extracts on Intracellular ROS in HepG2 Cells.* ROS are mainly produced by the process of oxidative phosphorylation through "electron leakage" from electron transport [25]. Excess ROS could "attack" the biological macromolecules and generate various harmful ingredients, such as malondialdehyde (MDA), 3-nitrotyrosine (3-NT), and 8-hydroxy-2 deoxyguanosine (8-OHdG) [32]. As shown in Figure 5, PA (100 $\mu$M) treatment for 24 h obviously increased the accumulation of ROS in HepG2 cells, but coincubation with the extracts from *Polygonatum sibiricum* reduced the ROS level by 29.33% (EAE vs. PA), 42.89% (EE vs. PA), and 28.18% (WE vs. PA), respectively, indicating that the three extracts exhibited strong activity to attenuate PA-induced oxidative stress in HepG2 cells (Figure 5(a)). However, EE group showed much stronger activity to scavenge ROS than that of the other two extracts, which is according to the results of the DPPH, ABTS, and FRAP assay.

*3.4.3. Effect of Extracts on MDA Content in HepG2 Cells.* MDA is the end-product of lipid peroxidation, which is always used as the biomarker of oxidative stress. As seen

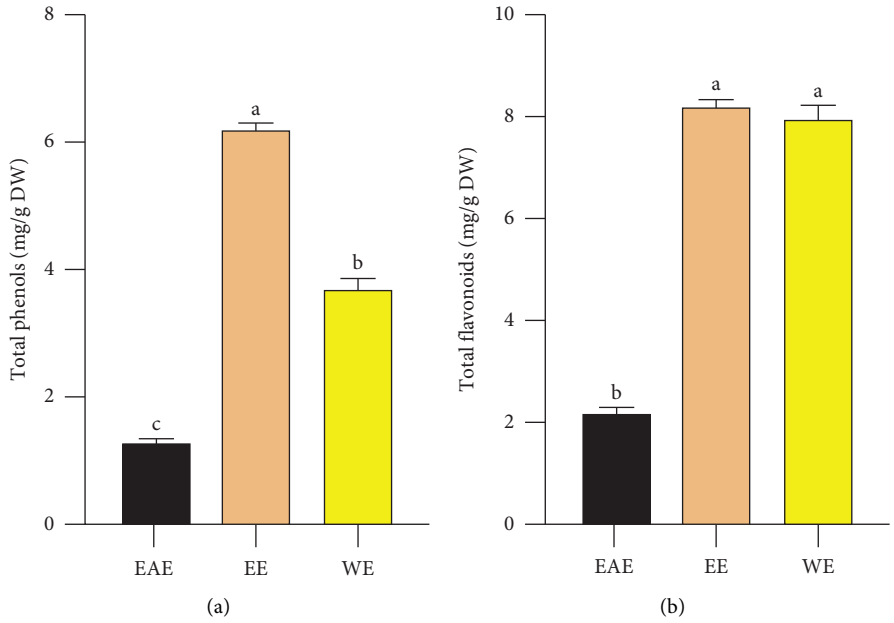



Figure 2: Total phenols and total flavonoids of different extracts from *Polygonatum sibiricum*. Different letters mean $p < 0.05$.

Table 1: HPLC-MS/MS analysis of different extracts from *Polygonatum sibiricum*.

| Compound | Formula | Expected ($m/z$) | EAE | | | EE | | | WE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Found at ($m/z$) | Intensity | RT* (min) | Found at ($m/z$) | Intensity | RT (min) | Found at ($m/z$) | Intensity | RT (min) |
| Chrysophanol | $C_{15}H_{10}O_4$ | 253.0506 | 253.0513 | 1505 | 33.00 | 253.051 | 1170 | 46.12 | 253.052 | 1378 | 33.00 |
| Quercetin dihydrate | $C_{15}H_{10}O_7$ | 301.0354 | 301.036 | 3676 | 16.78 | 301.0357 | 3657 | 16.72 | 301.0366 | 2937 | 16.79 |
| Dihydrocapsaicin | $C_{18}H_{29}NO_3$ | 306.2075 | 306.2083 | 20516 | 42.96 | 306.2079 | 111954 | 42.95 | — | — | — |
| Fumaric acid | $C_4H_4O_4$ | 115.0037 | 115.0036 | 35634 | 1.04 | 115.0037 | 122550 | 1.06 | 115.0037 | 42301 | 1.04 |
| Succinic acid | $C_4H_6O_4$ | 117.0193 | — | — | — | 117.019 | 81586 | 1.22 | 117.0193 | 4810 | 1.16 |
| Baicalin | $C_{21}H_{18}O_{11}$ | 445.0776 | — | — | — | — | — | — | 445.0765 | 8291 | 0.96 |
| Quinic acid | $C_7H_{12}O_6$ | 191.0561 | — | — | — | — | — | — | 191.0572 | 6843 | 1.02 |
| Lipoic acid | $C_8H_{14}O_2S_2$ | 205.0362 | — | — | — | — | — | — | 205.0362 | 5005 | 1.04 |
| Rosmarinic acid | $C_{18}H_{16}O_8$ | 359.0772 | 359.0768 | 8433 | 1.02 | 359.0766 | 22481 | 0.99 | 359.0771 | 33503 | 1.02 |
| Kaempferol | $C_{15}H_{10}O_6$ | 285.0405 | 285.0407 | 9066 | 23.33 | 285.0408 | 77570 | 23.31 | 285.0422 | 84317 | 23.33 |
| Malic acid | $C_4H_6O_5$ | 133.0143 | 133.0144 | 224676 | 1.04 | 133.0141 | 627573 | 1.05 | 133.0145 | 239837 | 1.05 |
| Polygalic acid | $C_{29}H_{44}O_6$ | 487.3065 | 487.3061 | 249460 | 1.02 | 487.3064 | 255013 | 1.05 | 487.3086 | 278491 | 1.02 |
| Sclareol glycol | $C_{16}H_{30}O_2$ | 253.2173 | 253.2182 | 102810 | 48.01 | 253.2175 | 101852 | 48.01 | — | — | — |
| Hydroxyecdysone | $C_{27}H_{44}O_7$ | 479.3014 | — | — | — | 479.3011 | 35149 | 42.86 | — | — | — |
| Ferulic acid | $C_{10}H_{10}O_4$ | 193.0506 | — | — | — | 193.0508 | 11900 | 5.95 | — | — | — |
| Catechin | $C_{15}H_{14}O_6$ | 289.0718 | — | — | — | 289.0718 | 9605 | 0.98 | — | — | — |
| *p*-coumaric acid | $C_9H_8O_3$ | 163.0401 | — | — | — | 163.04 | 40559 | 4.64 | — | — | — |
| Platycodin D | $C_{57}H_{92}O_{28}$ | 1223.5702 | — | — | — | 1223.5736 | 10210 | 28.25 | — | — | — |
| Mulberroside A | $C_{26}H_{32}O_{14}$ | 567.1719 | — | — | — | 567.1698 | 33833 | 0.99 | — | — | — |
| Salicylic acid | $C_7H_6O_3$ | 137.0244 | — | — | — | 137.0244 | 6239 | 8.62 | — | — | — |

*Retention time (RT).

in Figure 5(b), PA treatment for 24 h increased 2 times of MDA than that of control group in HepG2 cells. However, the three extracts from *Polygonatum sibiricum* significantly attenuated the generation of MDA, and EE treatment showed the best observed effect, which decreased the MDA content by 45.95% (compared to the PA group) (Figure 5(b)). These results implied that the extracts from *Polygonatum sibiricum*, especially that extracted by ethanol, revealed strong activity to scavenge free radicals and exhibited robust capacity to attenuate PA-induced oxidative damages in HepG2 cells.

*3.4.4. Effect of Extracts on SOD Activity in HepG2 Cells.* SOD is one of the most important antioxidant enzymes that responses for converting $O_2^{\bullet -}$ into $H_2O_2$, which is further catalyzed to nontoxic substances, $O_2$ and $H_2O$ [33].

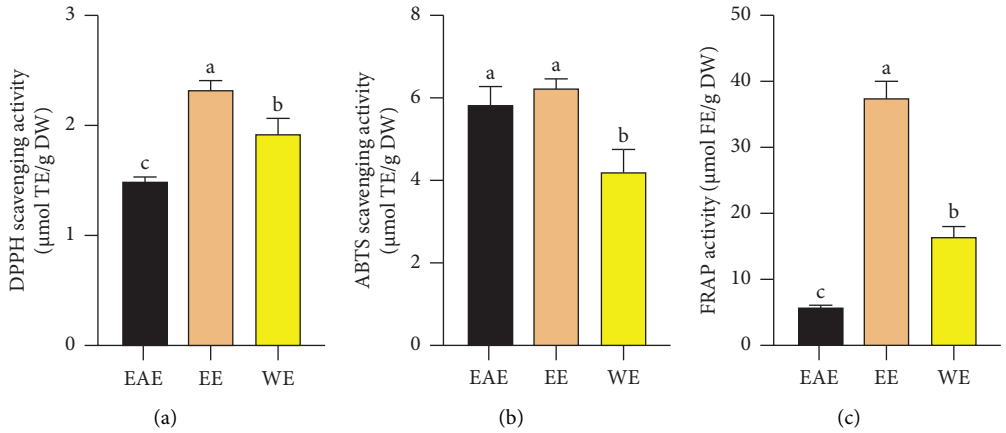



Figure 3: The antioxidant activity of different extracts from *Polygonatum sibiricum*. Different letters mean $p < 0.05$.

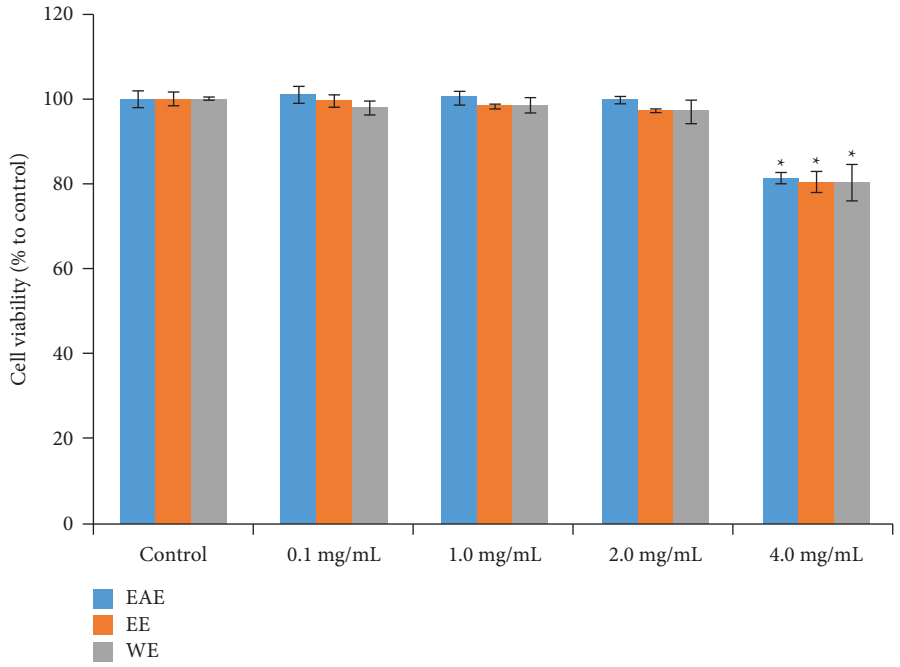



Figure 4: Effect of different concentrations of extracts on the cell viability of HepG2 cells. (*) $p < 0.05$.

Presently, we found that treatment with the three extracts obviously restored the activity of SOD, which was suppressed by PA (Figure 5(c)). Additionally, EE showed a much higher ability to increase SOD activity than the other two extracts. Compared to the PA group, EE treatment increased the activity of SOD by 81.71%, indicating that EE had stronger antioxidant capacity.

*3.4.5. The Extracts from Polygonatum sibiricum Promoted the Protein Expression of SOD, HO-1, and p-Nrf2.* In the western bolting analysis, we found that the extracts from *Polygonatum sibiricum* significantly promoted the protein expression of SOD, which is according to the result of SOD activity (Figure 6). Similarly, EE showed a better effect than that of EAE and WE treatments (Figure 6). HO-1 is another important antioxidant enzyme that catalyzes the rate-limiting step in heme catabolism. Increasing the expression of HO-1 could efficiently mitigate oxidative stress. As shown in Figure 6, EE treatment obviously stimulated HO-1 expression in PA co-incubated HepG2 cells. In order to further uncover the antioxidant mechanism of *Polygonatum sibiricum*, we investigated the effect of the extracts on the protein expression of p-Nrf2. Nrf2 is a nuclear transcription factor that closely relates with the expression of antioxidant enzymes, including SOD and HO-1 [34]. Under normal conditions, Nrf2 is sequestered by binding to Kelch-like ECH-associated protein 1 (Keap 1), which is kept in the cytoplasm [35]. However, upon activation, Nrf2 will dissociate from Keap 1 through phosphorylation and translocate to the nucleus to promote the expression of target genes [36]. Herein, we found that PA treatment obviously

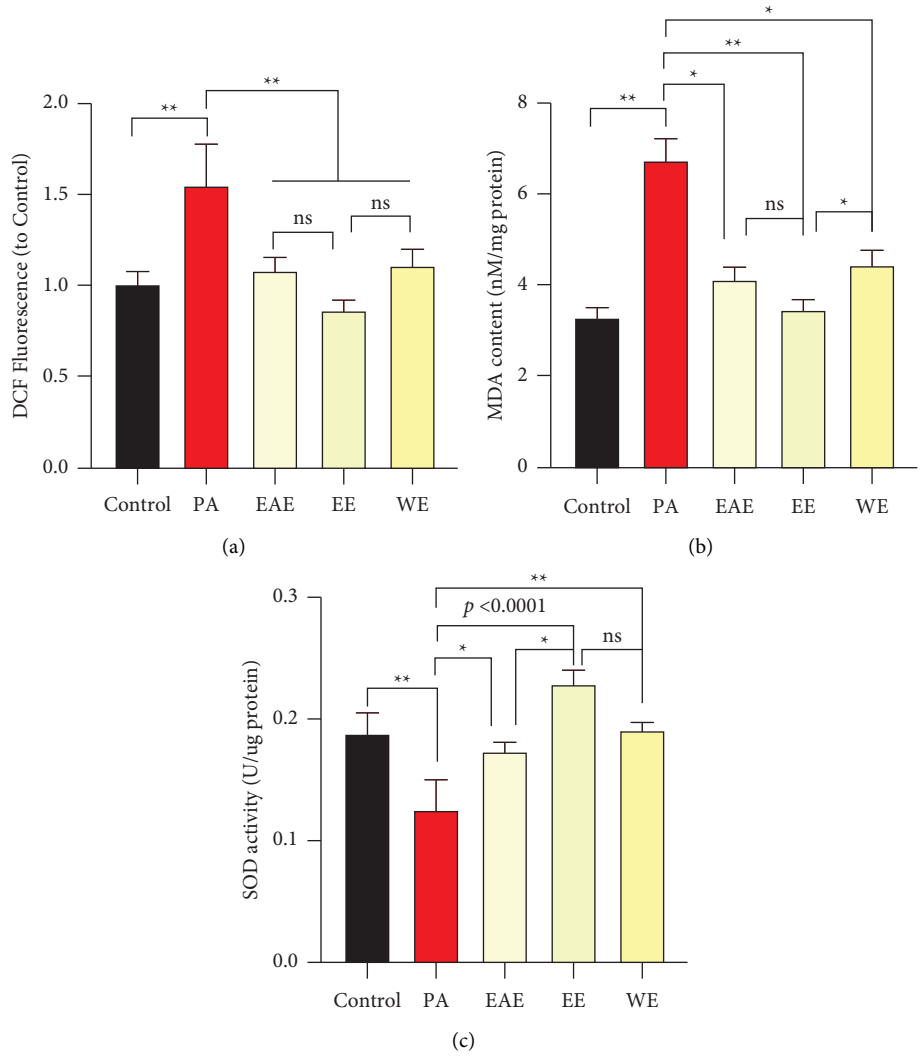



Figure 5: The antioxidant activity of different extracts from *Polygonatum sibiricum* in HepG2 cells. (a) ROS level; (b) MDA content; (c) SOD activity. (*) and (**) mean $p < 0.05$ or $p < 0.01$, ns meas no significance, respectively, compared to the PA group.

inhibited the phosphorylation of Nrf2, but co-incubated with EE markedly promoted p-Nrf2 expression, which exhibited better effect than that of WE and EAE (Figure 6). These results indicated that the extracts from *Polygonatum sibiricum* could increase the activity of antioxidant enzymes by stimulating their proteins expression via an Nrf2-dependent pathway.

*3.5. α-Glucosidase Inhibitory and Hypoglycemic Activity.* Different concentrations of extracts were prepared to investigate the α-glucosidase inhibitory activity. As shown in Figure 7, the activity of α-glucosidase was obviously inhibited by the increasing concentrations of extracts. Interestingly, EE showed the best inhibitory activity against α-glucosidase with the suppression ratio of 90.39% at 40 mg/mL, which was much higher than that of the other two groups (AEA, 78.66% and WE, 79.58%) at the same concentration. The results of HPLC-ESI-MS/MS indicated that the extracts from *Polygonatum sibiricum* contained lots of bioactive compounds, and some of them have reported to possess strong α-glucosidase inhibitory capacity. For example, kaempferol, a kind of natural flavonoids that existed in the three extracts, was reported to have notable inhibition activity on α-glucosidase in a mixed-type manner with the $IC_{50}$ of $1.16 \pm 0.04 \times 10^5$ M [37]. Meanwhile, several ingredients including ferulic acid and catechin, which were only found in EE, also strongly inhibited α-glucosidase ($IC_{50}$, 0.866 mg/mL and 1.12 μM, respectively) via noncompetitive or competitive mechanisms [38, 39]. These results implied that the strong α-glucosidase inhibitory activity of EE was probably due to the distinctive compounds that extracted from *Polygonatum sibiricum*.

In order to further confirm the hypoglycemic activity of the extracts, a sucrose-loading test was employed to investigate the time course of the blood glucose levels at 0, 15, 30, 60, 90, and 120 min. As shown in Figure 8, the level of blood glucose did not markedly changed at any time point in the control group. However, intragastric administration with 3 g/kg body weight of sucrose alone obviously increased

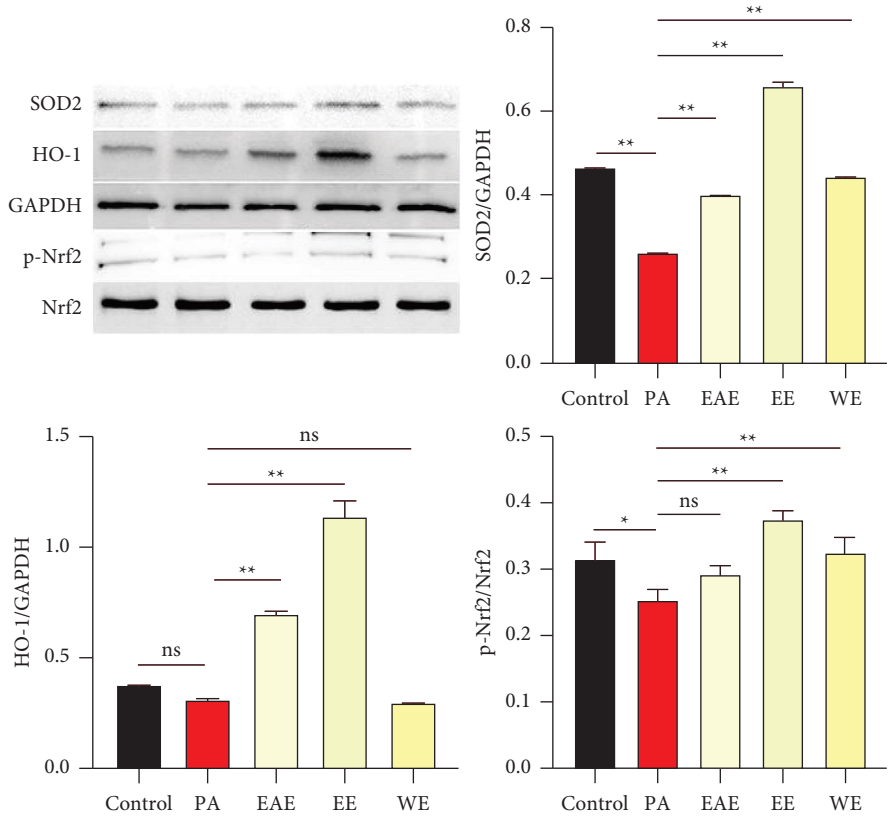



Figure 6: Effect of different extracts from *Polygonatum sibiricum* on the protein expression of HO-1, SOD2, and p-Nrf2. (*) and (**) mean $p < 0.05$ or $p < 0.01$, respectively, and ns means no significant differences, compared to the PA group.

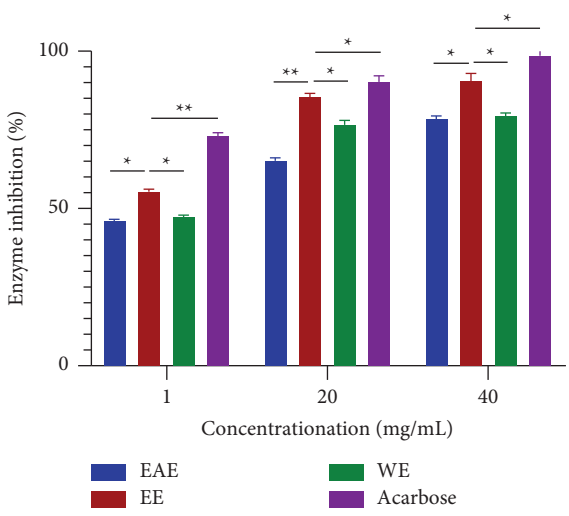



Figure 7: Inhibitory effects of different extracts on $\alpha$-glucosidase activity. (*) $p < 0.05$ and (**) $p < 0.01$.

the blood glucose and peaked at 30 min. In comparison, treatment with 40 mg/kg body weight of extracts effectively attenuated the observed increase in blood glucose levels and the EE group, as well as the acarbose group, revealed much better effects than the other two groups (Figure 8(a)). Moreover, the values of AUC also showed that the EE group had a stronger capacity to regulate the blood glucose level (Figure 8(b)), indicating that an extract from *Polygonatum sibiricum* by 80% ethanol exhibited favorable hypoglycemic activity.

## 4. Discussion

In the present study, we demonstrated that *Polygonatum sibiricum* was rich in polyphenols and flavonoids, which were inclined to dissolve in ethanol (Figure 2). Twenty compounds, including phenolic acids, kaempferol, and saponins were identified in the extracts of *Polygonatum sibiricum*, but most of them were enriched in EE (Table 1). Accordingly, EE showed the best antioxidant activity to scavenging DPPH and ABTS free radicals, as well as reducing power (Figure 3). Meanwhile, EE exhibited a better effect than the other two extracts for attenuation of PA-induced oxidative stress in HepG2 cells by increasing SOD and HO-1 activity through Nrf2-related pathways (Figures 4–6). Moreover, the extracts from *Polygonatum sibiricum*, especially EE group, revealed strong inhibitory activity against $\alpha$-glucosidase and significantly attenuated sucrose-induced rapid increase of blood glucose in vivo (Figures 7 and 8).

*Polygonatum sibiricum* has been used as the tonic herb and functional food for thousands of years in China, with lots of active compounds, such as polysaccharides, alkaloids, and saponins [2]. Previously, many research studies have focused on the polysaccharides of *Polygonatum sibiricum*,

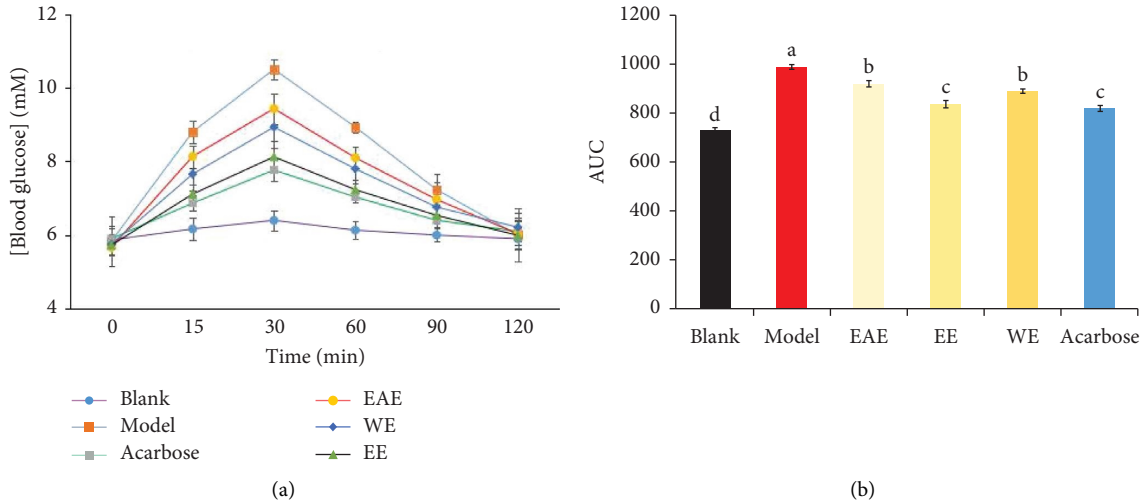



Figure 8: The effect of EA and acarbose on the blood glucose after intragastric administration of sucrose (a) and the area under curve (AUC) of blood glucose were calculated. (b) Different letters mean $p < 0.05$.

including the structures and activities. Few reports were found to explore the composition and activity of polyphenols in *Polygonatum sibiricum*. Hereon, three common used solvents, including water, 80% ethanol, and ethyl acetate, were employed to extract the polyphenols in *Polygonatum sibiricum* and the result indicated that the polyphenols were more inclined to dissolve in ethanol with the total phenols and total flavonoids of $6.27 \pm 0.11$ mg/g DW and $8.24 \pm 0.17$ mg/g DW, respectively (Figure 2), which were higher than that of the two previous reports [28, 29]. This might be closely related to the planting conditions, as the polyphenols in Titeban wild *Polygonatum sibiricum* can reached up to 19.8 mg/g [30]. These results suggested that EE group may be composed with more active ingredients, which was subsequently confirmed by HPLC-MS/MS analysis. Exactly, kaempferol, fumaric acid, malic acid, and polygalic acid were identified in all three extracts, but several active compounds, such as hydroxyecdysone, ferulic acid, catechin, *p*-coumaric acid, platycodin D, mulberroside A, and salicylic acid were only found in EE (Table 1). Compare to the previous study, we found that much more phenolic acids, including fumaric acid, malic acid, and polygalic acid were widely identified presently [28]. Moreover, saponins have been largely discovered in *Polygonatum sibiricum* with hypoglycemic effects and the activity of modulating gut microbiota [5, 6, 17]. Herein, some new saponins, including baicalin, platycodin D, and mulberroside A were first identified in the different extracts, which enriched the composition of polyphenols in *Polygonatum sibiricum*.

EE showed the strongest activity to scavenge DPPH and ABTS free radical, as well as reducing power, which was closely associated with the special compounds that were identified in EE (Table 1), such as ferulic acid, catechin, *p*-coumaric acid, mulberroside A, and salicylic acid. These compounds have been reported to possess strong free radical scavenging activity. Similarly, EE treatment exhibited much stronger activity to attenuate PA-induced hepatic oxidative stress via an Nrf2-dependent pathway due to these active compounds (Figures 4–6). Nrf2 is a nuclear transcription factor existed in the cytoplasm with Keap1 under normal conditions. However, Nrf2 responses to the oxidative stress and could be activated by phosphorylation, following dissociated from Keap1 and transferred into nucleus to promote the expression of antioxidant enzymes, such as SOD and HO-1 [35, 36]. For example, supplement with ferulic acid for 12 weeks could obviously attenuate the decrease of SOD activity, and reduce the production of ROS and MDA in the liver of Sprague–Dawley rats, which was close related to the activation of Nrf2 [40, 41]. Catechin, a well-known green tea polyphenol, was reported to significantly restore the level of cytochrome P450 and the activity of antioxidant enzymes, including SOD, catalase, glutathione reductase, and glutathione peroxidase through activating Nrf2-related pathways [42, 43]. As one of the most important carbohydrate digestive enzymes, $\alpha$-glucosidase could free the D-glucose from the nonreducing end site of substrate by destroying the bond between glucosidic oxygen and glucosyl residue. Inhibition the activity of $\alpha$-glucosidase could obviously attenuate the digestion of carbohydrate and effectively control the rapid rise of postprandial blood glucose [26, 44, 45]. Presently, we found that EE from *Polygonatum sibiricum* exhibited stronger $\alpha$-glucosidase inhibitory activity than that of the other two extracts as the contribution of the functional compounds (Figure 7). For instance, kaempferol, ferulic acid, catechin, and salicylic acid were reported to exhibit strong $\alpha$-glucosidase inhibitory activity via noncompetitive or competitive mechanisms [37–39, 46]. Moreover, in vivo experiment revealed that EE treatment could effectively attenuate the observed increase of blood glucose levels after intragastric administration with 3 g/kg body weight of sucrose (Figure 8), which further confirmed the hypoglycemic activity of EE. However, the single component that contributed to the capacity of regulating blood glucose of EE need to be further explored.

In summary, *Polygonatum sibiricum* contains plenty of polyphenols and flavonoids, which were inclined to dissolve

in ethanol. EE from *Polygonatum sibiricum* could obviously increase the expression of SOD2 and HO-1 and revealed strong antioxidant activity through Nrf2-related pathways. Moreover, EE showed significantly hyperglycemic activity via inhibiting the activity of $\alpha$-glucosidase. Our results provided much more information about the chemical constituent and bioactivity of polyphenols in *Polygonatum sibiricum*.

## Data Availability

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

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

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