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Research Article

Phytochemical Compounds, Antioxidant, and Digestive Enzymes Inhibitory Activities of Different Fractions from *Ginkgo biloba L.* Nut Shells

Luo Liu [iD],[1] **Yiyi Shang** [iD],[1] **Yan Zhang** [iD],[2] **and Mingjie Pang** [iD][1,3]

[1]*The Affiliated Hospital of/College of Medical, Kunming University of Science and Technology, Department of Cardiology, The First People's Hospital of Yunnan Province, Kunming 650032, Yunnan, China*
[2]*Department of Magnetic Resonance Imaging, The First People's Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, Kunming 650032, Yunnan, China*
[3]*Department of Cardiology, The First People's Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, Kunming 650032, Yunnan, China*

Correspondence should be addressed to Mingjie Pang; mjpangkmust@163.com

This study was designed to investigate the phytochemical compounds, antioxidant, and digestive enzymes inhibitory activities of the free (F), esterified (E), and insoluble-bound (IB) fractions from *Ginkgo biloba* L. nut shells. Results showed that a total of twelve compounds were detected in *G. biloba* nut shells by using UHPLC-ESI-HRMS/MS, including two kinds of organic acids, three kinds of phenolic acids, three kinds of flavonoids, and four kinds of terpene lactones. The F fraction contained all identified compounds and had the highest contents of the total phenolics and total flavonoids. All of the three different fractions exhibited good DPPH radical and ABTS radical cation scavenging activities and strong inhibitory effects on the generation of intracellular reactive oxygen species (ROS). Moreover, these three fractions also had good inhibitory effects towards α-glucosidase and pancreatic lipase. Among the three fractions, the F fraction possessed the strongest bioactivities. The findings obtained in the current study may provide some insights and bases for the further investigation and application of *G. biloba* nut shells in clinical medicine or the nutraceutical industry.

1. Introduction

Ginkgo biloba L., a mesozoic and valuable tree species, was widely cultivated in China. Ancient people knew that ginkgo nutlets are edible and possess benefits for the heart and lungs [1]. Modern studies have shown that the main active substances of ginkgo nut include ginkgo lactone, ginkgo flavonoids, and ginkgo phenolic acids [2, 3]. Among those phytochemicals, ginkgo lactones possess the effects of preventing platelet aggregation, delaying arteriosclerosis, and anti-inflammatory [4, 5]. Ginkgo phenolic acid has anti-inflammatory, antibacterial, and antitumor activities [6]. *G. biloba* extract is also reported to have neuroprotective effects [7]. *G. biloba* and its extracts are widely used in traditional Chinese medicine, daily health care, and clinical treatment nowadays [8]. With the extensive usage of gingko nutlets, the shells are not fully exploited and utilized, resulting in a great waste. A previous study confirmed that lignin in ginkgo nut shells exhibited outstanding antioxidant activity, even much higher than commercial antioxidants [9]. However, information about the phytochemical compounds and bioactivities of different fractions from *G. biloba* nut shells is scarce.

Recently, metabolic diseases, such as obesity, hypertension, hyperlipidemia, diabetes, and coronary heart disease, have risen rapidly and been a challenge around the world, which are related to unhealthy life habits and high-fat and high-carbohydrate dietary patterns [10]. As is well known, large amounts of carbohydrate and fat intake will contribute to the formation of fat, resulting in obesity and

diabetes [11]. Besides, these metabolic diseases are related to cell oxidative stress, which is also produced by excess fat and carbohydrate to some extent [12, 13]. For instance, oxidized low-density lipoprotein (ox-LDL) plays an important role in the formation of atherosclerotic plaque, which is produced by the oxidation of excessive free radicals in the body [14, 15]. Therefore, it is vital to control high-carbohydrate and high-fat intake and free radical generation in our daily life. Dietary fat and carbohydrate could only be absorbed after being hydrolyzed by the corresponding enzyme, and pancreatic lipase and α-glucosidase are the most important enzymes in this process [16, 17]. Inhibiting the activities of these two enzymes could effectively restrain or slow down the absorption of fat and carbohydrate, which are the action mechanisms of the clinical drugs of orlistat and acarbose, respectively. However, these two drugs possess side effects, which could not be ignored. Therefore, it is necessary to exploit some natural products that possess those bioactivities with few side effects. In this study, the digestive enzyme inhibitory activities and antioxidant activities of different extracts from *G. biloba* nut shells were investigated with the aim to provide a scientific basis for further exploiting the medicinal or nutraceutical value of *G. biloba* nut shells.

2. Materials and Methods

2.1. Materials and Chemical Reagents.
Fresh *G. biloba* nuts were purchased from a local market in Kunming city, Yunnan Province, China. Human umbilical vein cell fusion cells (EA.hy926) were supplied by the Kunming Animal Research Institute (Kunming, Yunnan, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6- sulphonic acid) (ABTS), porcine pancreatic lipase (from porcine pancreas, 163 U/mg, EC: 3.1.1.3), α-glucosidase (from Saccharomyces cerevisiae, EC: 3.2.1.20; Type I, lyophilized powder, ≥10 units/mg protein), *p*-nitrophenyl laurate (purity ≥99.0%), *p*-nitrophenyl-α-D-glucopyranoside (pNPG, purity ≥99.0%), methylthiazol-2-yl-2,5- diphenyltetrazolium bromide (MTT), and 2′,7′-dichlorofluorescein diacetate (DCFH- DA, purity ≥97%) were obtained from Sigma (Sigma-Aldrich, Shanghai, China). Fetal bovine serum (FBS), streptomycin, penicillin, and Dulbecco's modified Eagle's medium (DMEM) were supplied by Gibco (Grand Island, NY). All of the other chemicals used in this study were of analytical grade.

2.2. Sample Extraction.
Fresh *G. biloba* nuts were steamed for 30 min. The nut shells were manually gathered and lyophilized. The free (F), esterified (E), and insoluble-bound (IB) fractions of the nut shells were extracted by referring to the methods reported previously [18]. Briefly, the dried shells were powdered to pass through a 60-mesh sieve. Then, 50 g of shell powder was defatted by petroleum ether three times (1 : 5, w/v) and subsequently ultrasonically extracted with 250 mL of mixture solution of 70% acetone and 70% methanol (1 : 1, v/v) for 30 min at room temperature. After the centrifugation, the supernatant phase was used to extract F and E fractions, and the residue was used to extract the IB

fraction. For the extraction of F and E fractions, the supernatant phase was evaporated by using a rotary evaporator (Hei-VAP, Heidolph, Germany) at 40°C to get an aqueous phase. After adjusting the aqueous phase to pH 2 by 6 MHCl, the aqueous phase was extracted three times with diethyl ether-ethyl acetate (1 : 1, v/v). After being evaporated and lyophilized in the diethyl ether-ethyl acetate phase, the F fraction was obtained. The remaining aqueous phase was added 4 M of NaOH (1 : 10 v/v) to hydrolyze at room temperature for 4 h; the following operation was similar to that of F, and the E fraction was obtained. For the extraction of the IB fraction, the remained residue was hydrolyzed with 4 M of NaOH (1 : 10, v/v) for 4 h at room temperature. After centrifugation, the supernatant was adjusted to pH 2, and the following operation was the same as that of the F fraction to get the IB fraction.

2.3. Determination of the Total Phenolic and Total Flavonoid Contents.
The total phenolic contents (TPCs) and total flavonoid contents (TFCs) of different fractions from *G. biloba* nut shells were measured according to the previous methods [19]. In brief, all freeze-dried samples were dissolved separately in 80% methanol. For TPC measurement, the reaction mixture was recorded at 765 nm by using a Spectra Max M5 reader (Molecular Device, Sunnyvale, CA, USA). TPCs were expressed as mg gallic acid equivalent (GAE)/g of dry extract. When determining TFC, the absorbance of the reaction solution was measured at 510 nm. TFCs were displayed as mg rutin equivalent (RE)/g of dry extract.

2.4. Phytochemical Identification with UHPLC-ESI-HRMS/MS.
The phytochemicals in the three fractions were identified by using a Thermo Fisher Ultimate 3000 system with a Thermo Fisher Scientific Q-Exactive Orbitrap mass spectrometer (Bremen, Germany) in the negative mode. Phytochemicals were separated by using an Agilent Zorbax SB-C18 column (2.1 × 100 mm, 1.7 μm). Acidified water (0.1% formic acid) and acetonitrile were used as the mobile phases A and B, respectively, and the elution gradient was as follows: 0–2 min, 5% B; 2–20 min, 5%–50% B; 20–30 min, 50%–70% B; 30–32 min, 70% B; 32–34 min, 70%–5% B; and 34–40 min, 5% B. The injection volume and the flow rate were 3.0 μL and 0.2 mL/min, respectively, and the column temperature was set at 30°C. The mass spectrometry conditions were the same as in the previous study [20].

2.5. DPPH Radical and ABTS Radical Cation Scavenging Activities.
DPPH radical and ABTS radical cation scavenging activities of all fractions were determined according to a previously reported method [21, 22]. The optical density (OD) value was measured by using a Spectra Max M5 reader at 517 nm and 745 nm, respectively. Each scavenging activity was calculated by using the following formula: DPPH radical or ABTS radical cation scavenging ratio (%) = [(OD$_{control}$-OD$_{sample}$)/OD $_{control}$] × 100%.

2.6. Inhibition on H2O2-Induced Intracellular Reactive Oxygen Species (ROS) Production in EA.hy926 Cells. The measurement of intracellular ROS in EA.hy926 triggered by H2O2 was conducted according to a method reported earlier [23] with a slight modification. Cells were seeded in a 6-well plate at 2.0×10^5 cells per well. After being cultivated for 24 hours, $200 \mu L$ of fresh DMEM containing $50 \mu g/mL$ of the sample and $10 \mu g/mL$ of vitamin C (Vc) were added into the corresponding well, and the cells in the control group (CK) and the H_2O_2 group were still cultured in fresh DMEM. All groups were incubated for another 24 hours. Then, 1.0 mM H_2O_2 was added to groups of H2O2, Vc, F, E, and IB for 5 hours. Thereafter, cells were washed, harvested, and labeled with DCFH-DA. Finally, the fluorescence of each group was detected with a flow cytometer (Guava easy Cyte 6-2L, Millipore, Billerica, USA). The content of intracellular ROS in CK was regarded as 100%.

2.7. Determination of α-Glucosidase Inhibition. The inhibitory abilities of the three fractions against α-glucosidase were evaluated as per the previously reported method [24] with a minor modification. In brief, $10 \mu L$ of α-glucosidase (200 μg/ mL) and $10 \mu L$ of sample solution were mixed with $110 \mu L$ of phosphate buffer saline (pH 6.8) and incubated for 30 min at 37°C. Then, $20 \mu L$ of pNPG (2.5 mM) was added as a substrate and incubated for another 30 min at 37°C. The reaction was stopped by adding $60 \mu L$ of Na_2CO_3 (0.2 mol/L). A reaction mixture without a sample was used as a control. The OD value of each reaction mixture was measured by using a Spectra Max M5 reader at 405 nm. The inhibition ratio (%) was calculated as follows: [(OD control-OD sample)/OD control] × 100%.

2.8. Determination of Pancreatic Lipase Inhibition. The inhibitory effects of the three fractions against pancreatic lipase were evaluated according to the previously reported method [25]. Briefly, 100 mM of Tris buffer (pH 8.2) and 0.1% of *p*-nitrophenyl laurate were used as reaction buffer and substrate, respectively. A sample ($100 \mu L$) with appropriate concentrations was mixed with reaction buffer ($200 \mu L$) and the substrate ($250 \mu L$), and then, lipase solution ($50 \mu L$) was added to start the reaction. The reaction mixture without a sample was used as a control. All reaction mixtures were incubated at 37°C for 120 minutes. The OD value of each reaction mixture was measured by using a Spectra Max M5 reader at 400 nm. The inhibition of pancreatic lipase activity (%) was calculated as follows: [(OD control-OD sample)/OD control] × 100%.

2.9. Statistical Analysis. Each experiment was carried out at least three times. The data are expressed as mean ± standard deviation (SD) and analyzed with Origin 8.5 software (OriginLab, Northampton, Massachusetts, USA). The significant differences between groups were analyzed by one-way ANOVA with the Tukey test ($p < 0.05$).

3. Results and Discussion

3.1. TPCs and TFCs of Different Fractions. TPCs and TFCs of all fractions in *G. biloba* nut shells are summarized in Table 1. For TPCs, the F fraction had the highest content, followed by the E fraction, while the IB fraction contained the lowest content of phenolic compounds ($p < 0.05$). The TPC in the F fraction was almost four times higher than that in the IB fraction. A similar phenomenon was also observed in TFCs. The F fraction contained the highest TFC, followed by the E fraction, and the IB fraction had the lowest TFC ($p < 0.05$). Moreover, the TFC in the F fraction was approximately five times higher than that in the IB fraction. A previous study also reported that the free fraction of *Rhus chinensis* Mill. fruits had the highest content of phenolic compounds among three different fractions [18]. Previous studies confirmed that the different extracts from leaves of *G. biloba* are rich in polyphenols and flavonol glycosides [26, 27]. The present results indicated that *G. biloba* nut shells may be a good source of dietary phenolic compounds.

3.2. Characterization of Phytochemical Compounds. The phytochemical compounds in each fraction of *G. biloba* nut shells were characterized by UHPLC-ESI-HRMS/MS, and the results are presented in Figure 1 and Table 2. In the current work, phytochemical compounds in each fraction were characterized by comparing their mass data with the corresponding commercial standard or with the available data reported earlier. Figure 1 and Table 2 show that a total of 12 phytochemical substances were identified, including two organic acids (peaks **1** and **2**), three kinds of phenolic acids (peaks **3**, **4**, and **5**), three kinds of flavonoids (peaks **6**, **7**, and **9**), and four kinds of terpene lactones (peaks **8**, **10**, **11**, and **12**). Among the three fractions, the F fraction was found to contain all of those 12 phytochemical compounds, while both of the E and IB fractions only had six substances. A previous study also confirmed that some flavonoid glycosides, such as derivatives of quercetin, kaempferol, and isorhamnetin, were detected in the extract of *G. biloba* leaves [27]. Ginkgolides are the characteristic phytochemical compounds of *G. biloba*, and in the current work, four different ginkgolides were detected, namely, ginkgolide C (peak **8**), bilobalide (peak **10**), ginkgolide A (peak **11**), and ginkgolide B (peak **12**). A previous study reported that ginkgolide B, isolated from the leaves and root bark of *G. biloba*, exhibited good antioxidant, anti-inflammatory, and other activities with potential capacities to prevent many diseases [28]. Ginkgolide C has been considered to possess a potential capacity to inhibit adipogenesis in adipocytes, thereby improving metabolic syndrome [29].

In addition, according to Figure 1, the F fraction almost contained the most abundant phytochemical compounds. Therefore, the relative content of each substance in *E* and IB fractions was compared with that in the F fraction (set as 100%) based on the peak area. Table 1 shows that IB possessed the highest gluconic acid content ($p < 0.05$). For the malic acid content, the *E* and IB fractions accounted for about 55.65% and 62.55% of that in the F fraction,

TABLE 1: Total phenolic contents (TPCs) and total flavonoid contents (TFCs) of different fractions from *Ginkgo biloba* L. nut shells.

	F	E	IB
Total phenolic contents (mg gallic acid/g dry extract)	443.64 ± 1.99^{a}	152.85 ± 1.03^{b}	112.86 ± 0.58^{c}
Total flavonoid contents (mg rutin/g dry extract)	190.49 ± 1.50^{a}	61.91 ± 0.02^{b}	41.98 ± 0.15^{c}

All values are expressed as mean ± S.D. ($n = 3$). Different letters in the same row indicate significant differences at the $p < 0.05$ level.
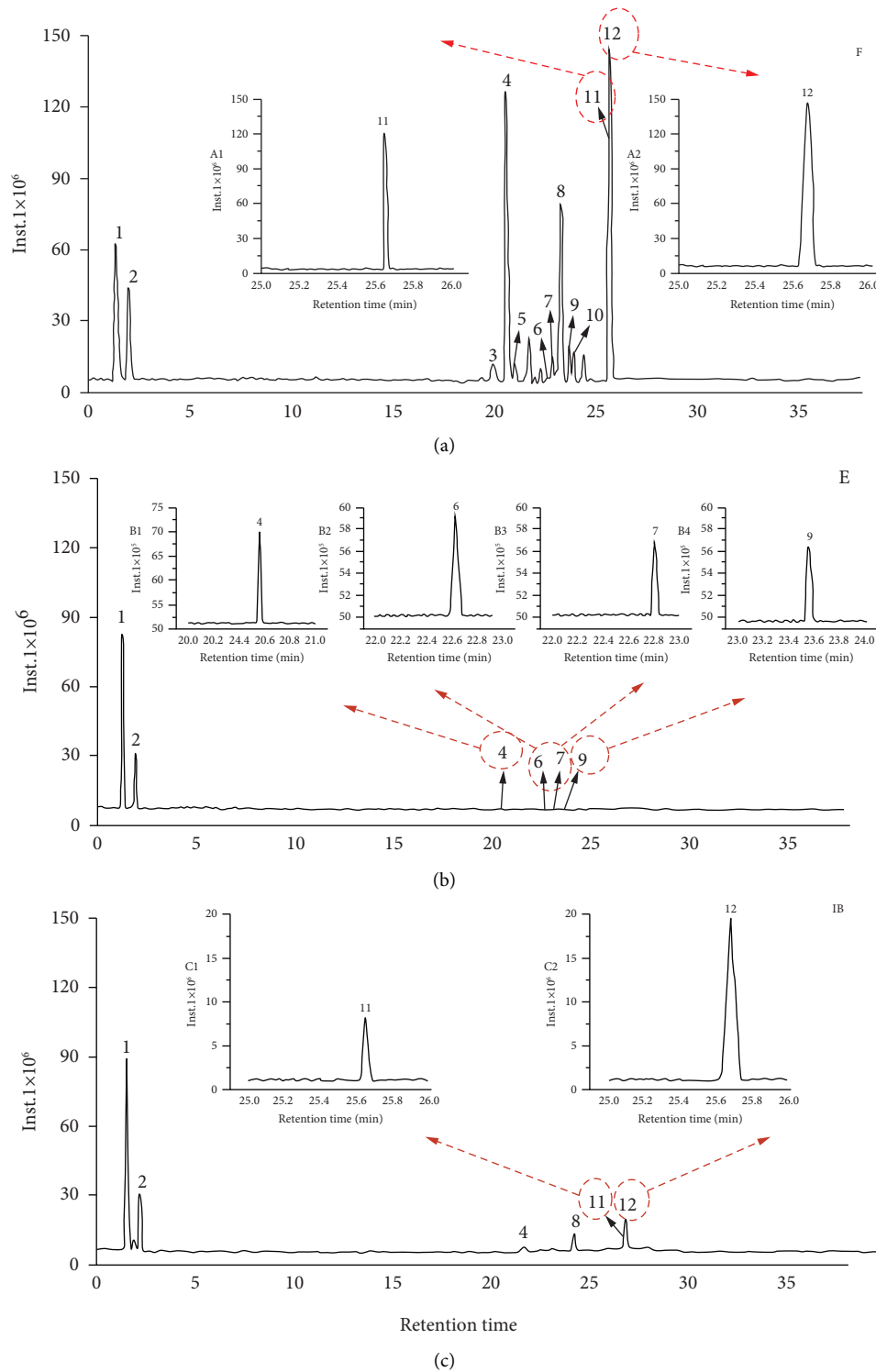

FIGURE 1: Base peak and extracted ion chromatograms of phytochemical compounds in different fractions of *Ginkgo biloba L.* nut shells. F: free fraction (a); E: esterified fraction (b); IB: insoluble-bound fraction (c). Peak identification and their MS data are shown in Table 2.

TABLE 2: Characterization of phytochemical compounds in three different fractions of *Ginkgo biloba* L. nut shells.

Peak no.	Compounds	Rt^a (min)	$[M-H]-$ m/z	Molecular formula	MS/MS fragment ions	Fractions (%)^b F	E	IB	References
1	Gluconic acid	1.48	195.0506	$C_6H_{12}O_7$	75.0074 (100), 129.0191 (9.37)	100	233.03	262.55	[30]
2	Malic acid	2.12	133.0132	$C_4H_6O_5$	71.0125 (100),72.9918 (74.75)	100	55.65	62.55	[31]
3	4-Hydroxybenzoic acid	19.90	137.0235	$C_7H_6O_3$	99.0334 (100)	100	ND	ND	Standard
4	Eucomic acid	20.56	239.0562	$C_{11}H_{12}O_6$	107.0493 (100), 133.0649(27.46)	100	4.67	7.94	[32]
5	Caffeic acid	21.00	179.0344	$C_9H_7O_4$	134.0364 (100), 135.0445(63.69)	100	ND	ND	Standard
6	Kaempferol-3-*O*-2″-glucosylrhamnoside	22.69	593.1525	$C_{15}H_{18}O_8$	285.0406(100)	100	42.02	ND	[33]
7	Isorhamnetin-3-*O*-rutinoside	22.82	623.1632	$C_{46}H_{23}O_{11}$	315.0514 (100), 314.0438(40.51)	100	12.03	ND	Standard
8	Ginkgolide C	23.27	439.1252	$C_{20}H_{24}O_{11}$	383.0127(88.75),365.9188(11.54)	100	ND	20.48	[33]
9	Quercetin-3-*O*-2″-(6″-*p*-coumaroyl) glucosylrhamnoside	23.55	755.1846	$C_{36}H_{36}O_{18}$	609.1474(100), 301.0341(22.03)	100	18.32	ND	[33]
10	Bilobalide	23.63	325.0935	$C_{15}H_{18}O_8$	163.1121(100)	100	ND	ND	[34]
11	Ginkgolide A	25.65	407.1353	$C_{20}H_{24}O_9$	245.1550 (11.73)	100	ND	6.54	[34]
12	Ginkgolide B	25.67	423.1204	$C_{20}H_{24}O_{10}$	113.0233(89.76)	100	ND	14.21	[34]

^{a}Rt: retention time; ^{b}ND: no detected; the peak areas of each compound in *E* and IB fractions were normalized with that in the F fraction (100%).
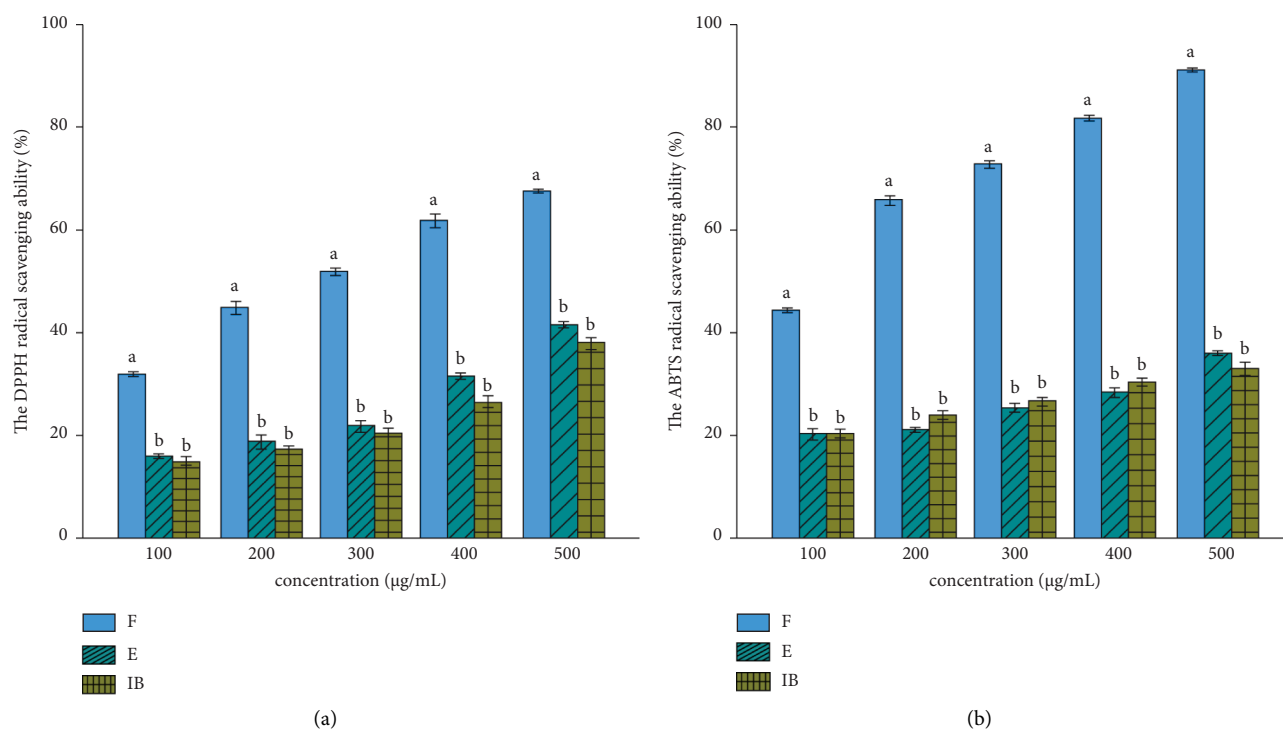

FIGURE 2: DPPH radical (a) and ABTS radical cation (b) scavenging activities of three fractions of *Ginkgo biloba L.* nut shells. All values are expressed as mean ± S.D. *n* = 3. Different letters at the same concentration indicate significant differences at the $p < 0.05$ level. F: free fraction; E: esterified fraction; IB: insoluble-bound fraction.

respectively. The content of eucomic acid in the E and IB fractions accounted for approximately 4.67% and 7.94% of that in the F fraction, respectively. The relative content of isorhamnetin-3-O-rutinoside, which was not detected in the IB fraction, in the E fraction accounted for about 12.03% of that in the F fraction. For four ginkgolides, only ginkgolide B and ginkgolide C were detected in the IB and accounted for about 20.48% and 14.21% of that in the F fraction, respectively.

3.3. Antioxidant Capacity

3.3.1. DPPH Radical Scavenging Activity. The DPPH radical scavenging abilities of the three different fractions of *G. biloba* nut shells are shown in Figure 2(a). All fractions exhibited good DPPH radical scavenging capacities in a dose-dependent manner. Among three fractions, the F fraction possessed the strongest DPPH radical scavenging activity at each tested concentration ($p < 0.05$), while the E

and IB fractions had similar scavenging activity without a significant difference at each tested concentration ($p > 0.05$). The half-maximal inhibitory concentration (IC_{50}) values of the F, E, and IB fractions were 273.57 ± 20.56, 573.18 ± 43.99, and $635.44 \pm 11.68 \, \mu g/mL$, respectively. Pearson's correlation analysis between the phenolic contents and DPPH radical scavenging activities indicated that the phenolic contents of the three fractions were closely related to their DPPH radical scavenging ratios ($r = 0.84$, $p < 0.05$). Vc, used as the positive control in this study, possessed the lowest IC_{50} value of $6.43 \pm 0.17 \, \mu g/mL$. This result was inconsistent with that of the previous study that the insoluble-bound fraction of palm oil possessed the strongest antioxidant activity [35]. This phenomenon occurs due to their different phytochemical compositions.

3.3.2. ABTS Radical Cation Scavenging Activity.

The ABTS radical cation scavenging abilities of three different fractions of *G. biloba* nut shells are shown in Figure 2(b). The overall trend of scavenging capacities was similar to that of DPPH radical. All three fractions also exhibited good ABTS radical cation scavenging capacities in a dose-dependent manner. The F fraction had the strongest ABTS radical cation scavenging activity ($p < 0.05$), followed by the E fraction and IB fraction, and the latter two fractions had no significant difference with each other ($p > 0.05$). The IC_{50} values of the F, E, and IB fractions were 273.57 ± 20.56, 573.18 ± 43.99, and $635.44 \pm 11.68 \, \mu g/mL$, respectively. Pearson's correlation analysis between the phenolic contents and ABTS radical cation scavenging activities also indicated that the phenolic contents of the three fractions significantly contributed to their ABTS radical cation scavenging ratios ($r = 0.97$, $p < 0.05$). The IC_{50} value of Vc, used as a positive control, was $8.68 \pm 0.25 \, \mu g/mL$ in the current work. A previous study also showed that three different fractions of *Rhus chinensis* Mill. fruits exhibited good scavenging activities against ABTS radical cation, and the free fraction was the strongest [18], which was consistent with the current work.

3.3.3. Inhibitory Effect on Intracellular ROS Generation.

Overproduction of intracellular ROS will lead to tissue or organ damage in the organism and cause many chronic diseases [36]. H_2O_2, which could pass through the cell membrane and generate free radicals, was normally used as an ROS generator. A previous study has confirmed that H_2O_2 could induce an increase in the ROS level to trigger oxidative stress in EA.hy926 cells [23]. Therefore, the H_2O_2-induced oxidative damaged cell model in EA.hy926 cells was used in this study, and the inhibitory effects of different fractions of *G. biloba* nut shells on intracellular ROS generation are shown in Figure 3. The intracellular ROS level of the H_2O_2 group was more than 1.5 times that of the control group (CK), indicating that the H_2O_2-induced oxidative model was successfully established in EA.hy926 cells by $0.8 \, mM$ of H_2O_2. Compared with the H_2O_2 group, all samples could significantly inhibit intracellular ROS production ($p < 0.05$). Among the three samples, the F fraction displayed the strongest inhibition on intracellular ROS generation at $50.0 \, \mu g/mL$ ($p < 0.05$), which was similar with Vc at $10.0 \, \mu g/mL$ ($p > 0.05$). The E fraction and the IB fraction exhibited a similar inhibition with no significant difference ($p > 0.05$). Previous studies have also reported that both phenolic compounds of grapefruit peels and *Astragalus* polysaccharide had good inhibitory effects on intracellular ROS generation in EA.hy926 cells [37, 38].

3.4. α-Glucosidase Inhibitory Activities.

α-Glucosidase, one of the most vital carbohydrate digestive enzymes, catalyzes the hydrolysis of dietary starch into simple sugars for absorption, resulting in the increase of the postprandial blood glucose level. Inhibiting the activities of α-glucosidase could control the postprandial blood glucose level effectively, which is one of the important mechanisms of the clinical drug, acarbose [39]. Results of inhibitory effects of the different fractions of *G. biloba* nut shells on α-glucosidase are shown in Figure 4. The inhibitory effects of three different fractions on α-glucosidase are all in a dose-dependent manner. Among the three fractions, the F fraction possessed the strongest inhibitory capacity with an IC_{50} value of $434.03 \pm 22.57 \, \mu g/mL$ ($p < 0.05$). The IC_{50} values of the *E* and IB fractions are 629.87 ± 15.37 and $644.76 \pm 12.62 \, \mu g/mL$, respectively. In addition, Pearson's correlation analysis showed a positive correlation between the TPCs and the inhibition rate of α-glucosidase ($r = 0.583$, $p < 0.05$), indicating that the phenolic compounds in *G. biloba* nut shells may be the main bioactive compounds for inhibiting the α-glucosidase activity. Previous literature also found that phenolic compounds may be the main contributor to the inhibitory activity of α-glucosidase [40, 41]. For example, a high correlation between the phenolic contents and α-glucosidase inhibitory ratios was observed in *Prinsepia utilis* Royle fruits [40]. Another study also observed a strong correlation between the total phenolic content and α-glucosidase inhibitory activity of the extracts of *G. biloba* leaf [41]. Acarbose, the clinical drug as a α-glucosidase inhibitor, was used as the positive control in this study and possessed the lowest IC_{50} value of $0.21 \pm 0.02 \, \mu g/mL$.

3.5. Pancreatic Lipase Inhibitory Activities of the Three Fractions.

Pancreatic lipase, the most important enzyme that hydrolyzes dietary fat, makes triglycerides degradation into diglycerides, monoglycerides, glycerols, and fatty acids [42]. Inhibiting the activities of pancreatic lipase could effectively reduce the absorption of dietary fat, thereby reducing the incidence of some cardiovascular diseases, such as hyperlipidemia. The inhibition results of three fractions of *G. biloba* nut shells against pancreatic lipase are presented in Figure 4. Figure 4 shows that three different fractions of *G. biloba* nut shells possess strong inhibitory effects against pancreatic lipase, and inhibitory capacities gradually upgraded with the increased concentrations. The inhibitory effect of the F fraction was much stronger than that of the E fraction and IB fraction ($p < 0.05$). The inhibitory effects of E and IB fractions did not have significant difference with each other at each tested concentration ($p > 0.05$). At the
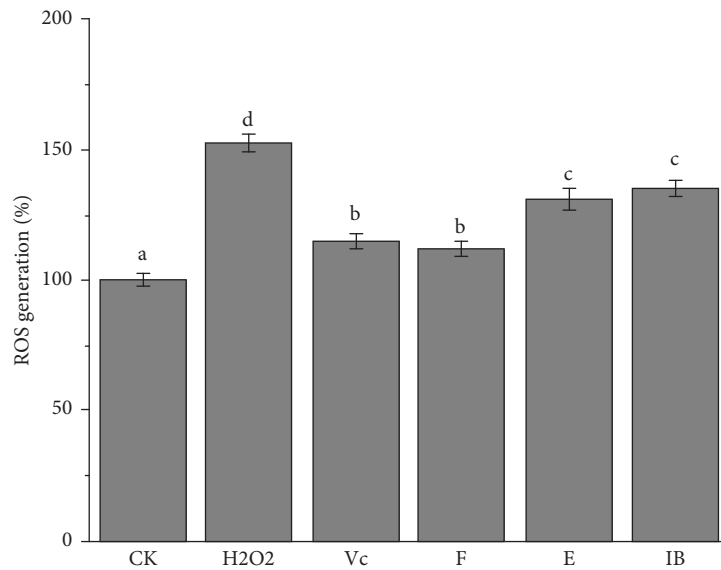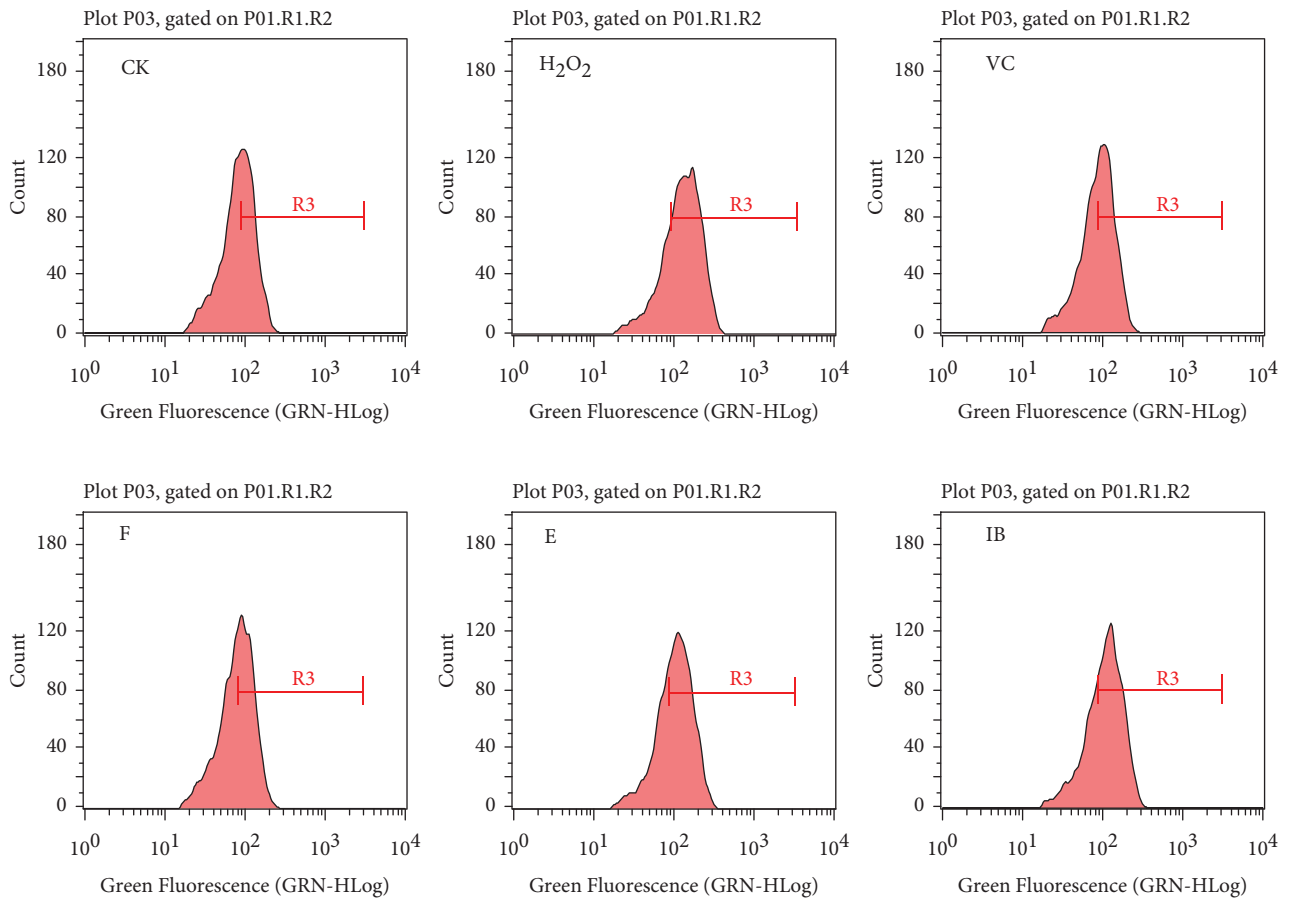
FIGURE 3: Inhibitory effects of three fractions of *Ginkgo biloba L.* nut shells on intracellular ROS production of H_2O_2-induced EA.hy926. All values are expressed as mean $\pm$ *S*.D. ((n) = 3). Different letters indicate significant differences at the $p < 0.05$ level. CK: control group; H_2O_2: H_2O_2-induced group; Vc: positive group; F: free fraction; E: esterified fraction; IB: insoluble-bound fraction.
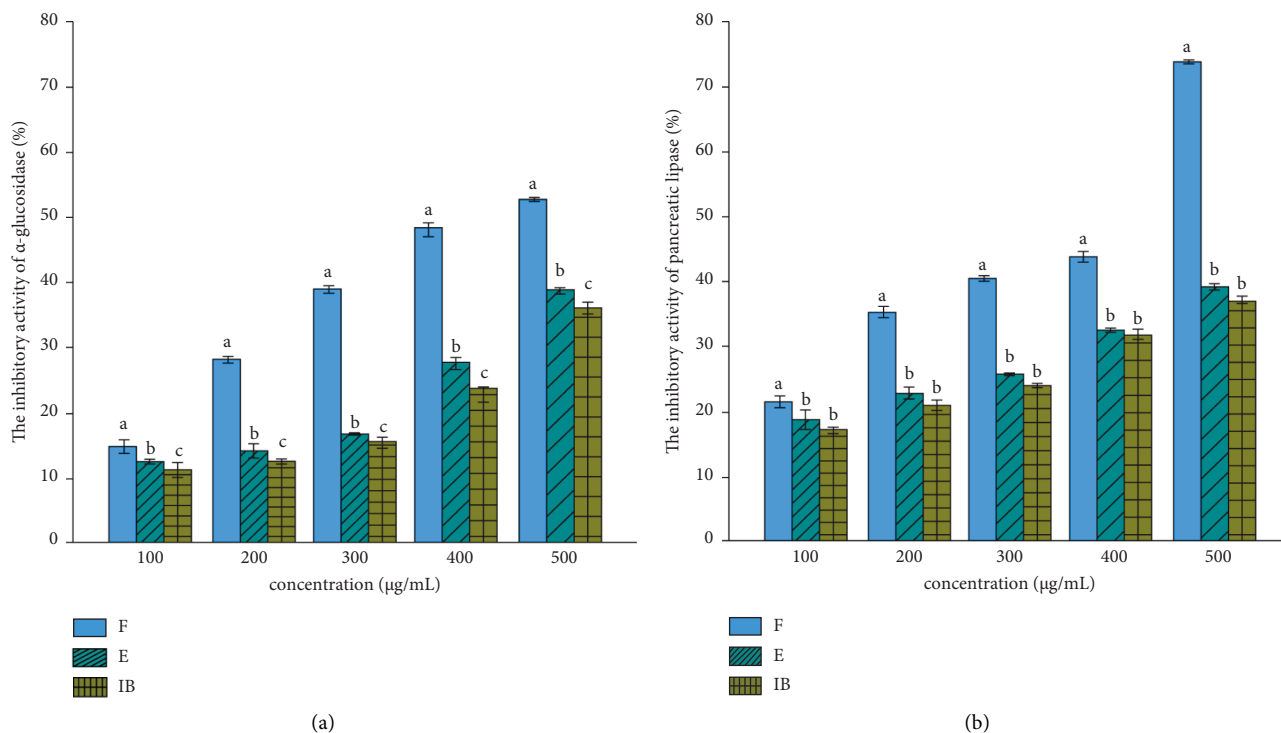
(a)

(b)

FIGURE 4: The inhibitory activities of the different fractions of *Ginkgo biloba L.* nut shells towards α-glucosidase (a) and pancreatic lipase (b). All values are expressed as mean $\pm$ S.D. ((n) = 3). Different letters indicate significant differences at the $p < 0.05$ level. F: free fraction; E: esterified fraction; IB: insoluble-bound fraction.

maximum concentration in the experiment, the pancreatic lipase inhibition rate of the F fraction was more than 70%. IC_{50} values of F, E, and IB fractions were 386.62 ± 18.91, 621.69 ± 18.28, and $668.04 \pm 28.04 \, \mu g/ml$, respectively. Moreover, Pearson analysis was used to measure the correlation coefficient between the TPCs and lipase inhibitory abilities of three fractions, and the result exhibited a good positive correlation between the two parameters ($r = 0.94$, $p < 0.05$), suggesting that phenolic compounds may play a crucial role in pancreatic lipase inhibition. Many previous studies have shown that plants with high content of polyphenols have good lipase inhibitory effects, such as Chinese sumac (*Rhus chinensis* Mill.) fruits [18] and *Prinsepia utilis* Royle fruits [34], which were consistent with the finding of this study. Orlistat, a positive control, had the lowest IC_{50} value of $35.47 \pm 1.26 \, \mu g/mL$.

4. Conclusions

This study analyzed phytochemical compounds in different fractions of *G. biloba* nut shells and their antioxidant and digestive enzymes inhibitory activities. The TPCs and TFCs of the F fraction were significantly higher than those of the E and IB fractions. Twelve different phytochemical compounds were identified by LC-MS. The three fractions had good DPPH radical and ABTS radical cation scavenging activities, and the F fraction exhibited the strongest activity, followed by the E and IB fractions. The F fraction also showed the strongest effect on inhibiting the generation of intracellular ROS. Moreover, results displayed that the three

fractions had good inhibitory effects towards α-glucosidase and pancreatic lipase, and the F fraction had the strongest inhibitory capacities. A positive correlation between TPCs and digestive enzyme inhibitions implied that the phenolic compounds in *G. biloba* nut shells might be the main bioactive compounds. These findings may provide insights and bases for the further application of *G. biloba* nut shells in clinical medicine or the nutraceutical industry.

Data Availability

All data included in this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Luo Liu and Yiyi Shang contributed equally to this work.

Acknowledgments

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