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Polyphenolics Composition of the Leaves of *Zanthoxylum bungeanum* Maxim. Grown in Hebei, China, and Their Radical Scavenging Activities

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ABSTRACT: High performance liquid chromatography coupled with tandem mass spectrometry with electrospray ionization and negative ion detection (HPLC-ESI-MS/MS) was used to identify the polyphenols from the leaves of a traditional vegetable and spice, *Zanthoxylum bungeanum* Maxim., in China by comparison of the retention times and mass spectral fragment with standard substances or related literature. A phenolic acid glucoside, two acids, and eight flavonoids were determined including vanillic acid-4-glucoside (22.75 μg/g, dry weight of leaves), quinic acid (58.58 μg/g), chlorogenic acid (2515.96 μg/g), epicatechin (77.80 μg/g), 5-feruloyquinic acid (16.63 μg/g), syringetin-3-glucoside (103.23 μg/g), rutin (89.41 μg/g), hyperoside (886.36 μg/g), quercetin-3-arabinoside (118.75 μg/g), quercitrin (645.82 μg/g), and isorhamnetin-3-glucoside (104.27 μg/g). The obtained polyphenols show good radical scavenging activities in DPPH and superoxide anion. Through the intracellular antioxidation assay, the result shows that the polyphenols could reduce the ROS in HT-29 cells without cell toxicity.

KEYWORDS: *Zanthoxylum bungeanum Maxim., leaves, polyphenols, HPLC-ESI-MS/MS, radical scavenging activity, intracellular antioxidation*

INTRODUCTION

Zanthoxylum bungeanum Maxim. is an aromatic tree and shrub plant belonging to the genus *Zanthoxylum* of the family Rutaceae and native to eastern China that is now mainly distributed in Hebei, Shanxi, Sichuan, Gansu, and Shandong provinces of China and some Southeast Asian countries. The reddish brown ripe drying pericarp of *Z. bungeanum* is widely used for its flavor as a peppery spice in the Chinese kitchen and medicative characters. It can stimulate saliva production and increase appetite. The leaves of *Z. bungeanum* also are a traditional vegetable and seasoning in China. There are several papers published about the components and antioxidant activity of the essential oil of the pericarps of *Z. bungeanum*.[1−3]

However, only a few people pay attention to the chemical composition of the leaves of *Z. bungeanum*. Deng and co-workers determined mainly nutritious and nutritional ingredients of the leaves of *Z. bungeanum* including proteins, carbohydrate, celluloses, fats, carotene, vitamins, and amino acids.[4] Iseli et al. and Yang found hydroxy-α-sanshool and hydroxy-β-sanshool were the major constituents of the alkylamide fraction.[5,6] Fan and co-workers found that the leaves contain flavonoids, and these compounds show better reductive abilities.[7] Because of this the flavonoids in the human diet and food could offer beneficial physiological effects on human health such as reduced risk of cancer and cardiovascular disease due to their antioxidant and free radical scavenging activities.[8] It is very necessary to identify and quantify polyphenols such as flavonoids in this edible plant.

To the best of our knowledge, studies on individual polyphenols in the leaves of *Z. bungeanum* have not been reported yet, especially the chemical structure of each polyphenol. For identification and quantification of polyphenols, HPLC-MS was considered as the most popular and used technique; high-sensitivity tandem mass spectrometry especially can provide mass spectra of intact molecular ions and fragment ions.[9,10] Therefore, the unknown compounds, even if no reference compounds are available, can be also identified categorically using this technique. The aim of this work is to identify and quantify the polyphenolic components of the leaves of *Z. bungeanum* by use of the HPLC-ESI-MS/MS technique and to determine their radical scavenging activities.

MATERIALS AND METHODS

Apparatus. The HPLC-ESI-MS/MS system was composed of an Agilent 1200 series HPLC system with a DAD detector and an Agilent 6410 triple-quadrupole tandem MS/MS using electrospray ionization (Agilent Technologies, Inc., Santa Clara, CA, USA). Model Sineo MS-I microwave extraction equipment (Sineo Microwave Chemical Technology Co. Ltd., Shanghai, China) was used to extract the polyphenols.

Chemicals. Standard substances (≥98%) including epicatechin, rutin, hyperoside, and quercitrin were purchased from Shunbo Co. (Shanghai, China). Chlorogenic acid was obtained from Detian Biological Co. (Chengdu, China). Quinic acid was obtained from Yifang S&T Co. (Tianjin, China). HT-29 was obtained from the Institute of Biochemistry and Cell Biology (Shanghai, China). D101 macroporous adsorption resin was obtained from Tianjin Nankai Hecheng S&T Co. (Tianjin, China). Methanol was of HPLC grade and purchased from Merck (Darmstadt, Germany). Formic acid was of HPLC grade and purchased from Tedia Co. Inc. (Fairfield, OH, USA). Other chemicals used were of analytical grade. Deionized water was

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purified by a Milli-Q system (Millipore, Bedford, MA, USA) and used throughout.

Plant Materials. The leaves of *Z. bungeanum* were collected from Hebei province, China, in July 2011. The leaves were dried in a vacuum drying oven, ground to about 40 mesh, and stored at room temperature in a desiccator before use.

Extraction of Polyphenols from *Z. bungeanum* Leaves. The polyphenols were extracted using a 65% (v/v) ethanol solution according to a microwave-assisted extraction method. The detailed procedure was as follows: 5 g of ground samples and 150 mL of 65:35 (v/v) ethanol/water solution were added to a special microwave vitreous flask. The polyphenols were extracted for 4 min with a microwave power of 500 W and a temperature of 70 °C. The ethanol extracting solution was filtered and extracted using isopyknic petroleum ether three times to remove the fat-soluble components. Finally, the ethanol extracting solution was concentrated in a rotary evaporation apparatus to remove ethanol and then freeze-dried. Thereby, the crude polyphenolic lyophilized extract from the leaves of *Z. bungeanum* were obtained.

Purification of Crude Polyphenols. The crude polyphenols were purified by D101 macroporous adsorption resin. The best conditions for purification were as follows: adsorption liquid pH 4, 2 BV/h adsorption flow, elution agent (ethanol) concentration of 80% (v/v) with 2 BV/h washing flow. The purification effect was then estimated by HPLC. The chromatographic conditions used were as follows: The column was a Zorbax SB-C_{18} (250 mm × 4.6 mm i.d., 5 μm particle size). The injection volume was 10 μL. The detector wavelength was set at 328 nm. The mobile phase was 5:95 (v/v) formic acid/methanol solution (phase A) and 5:95 (v/v) formic acid/water (phase B) at a flow rate of 0.9 mL/min. The linear gradient of phase A was changed after injection as follows: 30−35% from 0 to 8 min; 35−45% from 8 to 10 min; 45−50% from 10 to 27 min; 50−75% from 27 to 30 min; and, finally, isocratic elution with 75% phase A until 35 min.

The solution of 50 μg/mL purified polyphenols was prepared by dissolving an appropriate amount of the purified flavonoids in methanol, filtered through a 0.22 μm microporous syringe filter, and then stored at 4 °C in a refrigerator for HPLC and HPLC-ESI-MS/MS analyses.

HPLC-ESI-MS/MS Analysis. All analyses were performed using a Zorbax SB-C_{18} column (250 mm × 4.6 mm i.d., 5 μm) at 30 °C with a binary phase at a flow rate of 0.3 mL/min. The mobile phase was 0.1:99.9 (v/v) formic acid/methanol solutions (phase A) and 0.1:99.9 (v/v) formic acid/water (phase B). The gradient of phase A was increased from 50 to 72% from 0 to 10 min and was isocratic with 72% phase A to the end. The injection volumes of both the purified polyphenol solution and the mixed standard solution were 5 μL. In the solution of mixed standard substances, the concentration of quinic acid was 6 μg/mL, and other standard substances were 10 μg/mL. The working conditions for the ionization source were as follows: 4 kV capillary voltage, 350 °C source temperature, and 10 L/min gas flow rate. Nitrogen (>99% purity) was used as nebulizing and collision gas.

Qualitative analysis of the unknown compounds (including peaks 4, 5, 6, 9, and 11) in the purified polyphenol extract was performed by HPLC-ESI-MS/MS. According to the mass spectral fragmentation pathways of polyphenol and by comparison with the related literature, the purpose of identification of the unknown compound structure was achieved.

Quantitative analysis on standard substances and the purified polyphenol extract was carried out by HPLC-ESI-MS/MS in multiple-reaction monitoring (MRM) mode monitoring the product ions selected from MS^2 spectra to obtain a high specificity and sensitivity. An external standard method was used for quinic acid, chlorogenic acid, epicatechin, rutin, hyperoside, and quercitrin and an internal standard method for vanillic acid-4-glucoside, 5-feruloyquinic acid, quercetin-3-arabinoside, syringetin-3-glucoside, and isorhamnetin-3-glucoside.

Validation of Assay. The linear calibration curves contained six different concentrations of each reference compound by a series of appropriate dilution with mobile phase. All calibration curves were constructed by plotting the peak areas of the standard substances versus the corresponding concentrations of the injected standard solutions. The limit of detection (LOD) was calculated as 3 times the ratio of signal to noise (S/N ratio), that is, the lowest mass of a standard substance that can be detected, whereas the limit of quantification (LOQ) was calculated as 10 times the S/N ratio, that is, the lowest mass of a standard substance that can be accurately and precisely quantified. For each target component, the LOD and LOQ were determined by serial dilution of standard solution until the S/N ratio for each standard substance reached 3 and 10, respectively.

The recovery test was used to evaluate the accuracy of the proposed method. Accuracy was determined by adding different concentrations of the mixed standard solutions (Table 1) into the known amounts of the purified polyphenol extract solution. Then the polyphenols in the resultant samples were analyzed with the proposed method. The recovery was calculated as follows:

Table 1. Concentrations of the Added Standard Solutions

compound	added amounts (μg/mL)		
quinic acid	0.1	1.5	3
chlorogenic acid	2.0	4.0	8.0
epicatechin	0.5	2.5	5
rutin	0.1	8.0	15.0
hyperoside	1.0	4.0	8.0
quercitrin	2.0	6.0	10.0

$$\text{recovery}\,(\%) = \left(\frac{\text{total detected amount} - \text{original amount}}{\text{added amount}}\right) \times 100$$

DPPH Radical Scavenging Activity. The DPPH free radical scavenging activity was carried out according to the method of Kong with some modifications.[11] The solution of 50 μg/mL purified polyphenols (0.5 mL) at different concentrations reacted with 3.5 mL DPPH ethanol solution (1×10^{-4} mol/L) at room temperature in the dark for 30 min. The absorbance was measured at 517 nm. The percentage of DPPH consumed was calculated from the equation

$$S\,(\%) = \left(1 - \frac{A - A_0}{A_1}\right) \times 100$$

where A was the absorbance of the reaction mixture of the purified polyphenols with DPPH solution, A_1 was the absorbance of the purified polyphenols without DPPH, and A_0 was the initial absorbance of DPPH without the purified polyphenols. The efficient concentration of samples that inhibits 50% of the DPPH radicals (IC_{50}) was calculated and expressed as micrograms per milliliter. All indices were measured three times.

Superoxide Anion Radical Scavenging Activity. Superoxide anion radical scavenging activity was evaluated according to previous work with slight modifications.[12] Aliquots of 2.2 mL of pH 8.2 Tris-HCl buffer solution, 0.1 mL of doubly distilled water, and 0.3 mL of 3.0 mmol/L pyrogallol were added into a 1 cm quartz cell. The excitation spectra from 250 to 475 nm were measured at an emission wavelength of 509 nm with a 10.0 nm slit width. The emission spectra of these mixture solutions from 475 to 700 nm were also monitored at the excitation wavelength of 444 nm.

Aliquots of 2.2 mL of pH 8.2 Tris-HCl buffer solution, 0.1 mL of different concentration solutions of the purified polyphenols, and 0.3 mL of 3.0 mmol/L pyrogallol were added into a 1 cm quartz cell. The fluorescence intensities of the mixture were measured at 1 min intervals from 3 to 7 min at emission and excitation wavelengths of 509 and 444 nm. The oxidation rate of pyrogallol was denoted V_s. When 0.1 mL of doubly distilled water was added instead of the solution of the purified polyphenols, the oxidation rate of pyrogallol was detected as V_0. All indices were measured three times. Then the scavenging percentage of $O_2^{\bullet -}$ was calculated as follows:

$$P\,(\%) = \frac{V_0 - V_s}{V_0} \times 100$$

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Evaluation of Cell Viability in HT-29 Cells. The cell viability was measured using the method of Hamid[13] with some modifications. HT-29 cells were seeded at a density of 1×10^5/well on a 96-well plate in 100 μL of growth medium and incubated at 37 °C under 5% CO_2. After 24 h, the medium was removed and wells were washed with PBS twice. Then the cells were treated with the purified polyphenols in serial concentrations for another 24 h, and then 5 mg/mL 3-(4,5-dimethylthiazol-2)-2,5-diphenylterazolium bromide (MTT) was added. After incubation for 4 h, an appropriate amount of dimethyl sulfoxide (DMSO) was added to dissolve the crystal, and the absorbance was measured at 570 nm.

Intracellular Antioxidation Assay. The intracellular antioxidation was performed according to the method of Hu[14] and Schantz[15] with some modifications. Briefly, HT-29 cells were seeded at a density of 1×10^5/well on a 96-well plate in 100 μL of growth medium. After seeding for 24 h, the medium was removed and wells were washed with PBS. Triplicate wells were treated for 30 min with 100 μL of different concentrations of the purified polyphenols plus 10 μM DCFH-DA. When wells were washed by PBS, 100 μM H_2O_2 was applied to the wells in 100 μL. The fluorescence intensities were measured at 5 min intervals over a 1 h period at emission and excitation wavelengths of 525 and 488 nm, respectively. Intracellular antioxidant activity was expressed as relative fluorescence units.

Statistical Analysis. Statistical analysis was performed using the general linear model procedure from Statistics Analysis System (SAS) (version 9.2, SAS Institute Inc., Cary, NC, USA). All determinations were based on three replicate samples, and the results for content are shown as mean values. Differences between the means of sample were analyzed by the least significant differences test at a probability level of 0.05.

RESULTS AND DISCUSSION

HPLC Analysis. Under the above-mentioned extraction conditions, the yield of crude polyphenols was 63.7 mg/g dry weight. Furthermore, the purified crude polyphenols by D101 macroporous adsorption resin were estimated by HPLC. The content of polyphenols in the purified crude polyphenols ranged from 26.1 to 58.6%. The chromatogram of the purified crude polyphenols is presented in Figure 1A. In addition, the mixture of six standard substances (epicatechin, rutin, hyperoside, quercitrin, chlorogenic acid, and quinic acid) was also separated under such conditions. By comparing the retention times of six standard substances with that of each component in the purified crude polyphenols, it can be preliminarily concluded that there is correspondence to six compounds in the purified crude polyphenols.

Qualitative Analysis. The total ion chromatogram of purified polyphenols from *Z. bungeanum* leaves is shown in Figure 1B, and the one of the standard substances is presented in Figure 1C. Each of six standard substances has the same retention time, molecular ion, and MS^2 spectrum with relative component of the sample solutions under the same analytical conditions. Other peaks that have no standards in Figure 1B were identified by MS/MS.

Peak 4 revealed an $[M - H]^-$ ion at m/z 329, and the MS^2 spectrum showed a characteristic vanillic acid molecular ion at m/z 167, thus indicating that peak 4 was vanillic acid hexoside. According to previous study,[16] peak 4 could be identified as vanillic acid 4-glucoside.

Peak 5 showed the molecular ion $[M - H]^-$ at m/z 367. MS/MS analysis showed the presence of two fragment ions at m/z 191 and 173. The fragment ion at m/z 191 was attributed to the loss of a ferulic acid group. For a neutral loss of a molecule of water, another fragment ion was at m/z 173. Thus, the main fragment ion is m/z 191, and peak 6 should identified as 5-feruloylquinic acid.[17−21]

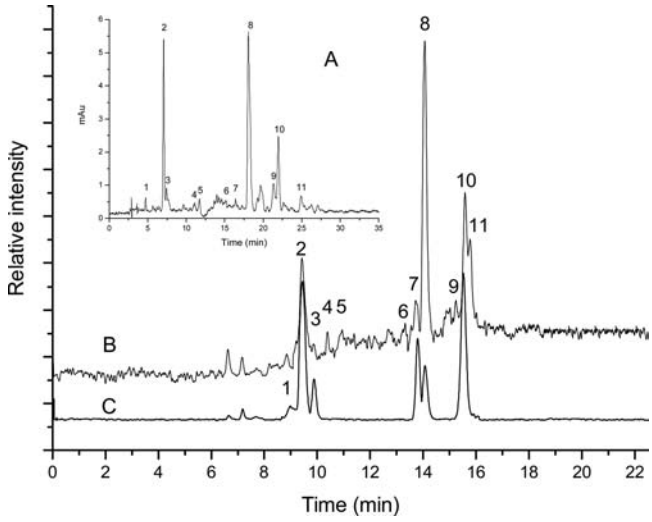

Figure 1. RP-HPLC chromatogram of the purified flavonoid extract from *Z. bungeanum* leaves and total ion chromatograms of flavonoids: (A) RP-HPLC chromatogram of the purified flavonoid extract; (B) total ion chromatogram of the purified flavonoid extract from *Z. bungeanum* leaves; (C) total ion chromatogram of mixture of standard substances. Peaks: (1) quinic acid, (2) chlorogenic acid, (3) epicatechin, (4) vanillic acid-4-glucoside, (5) feruloylquinic acid, (6) syringetin-3-glucoside, (7) rutin, (8) hyperoside, (9) quercetin-3-arabinoside, (10) quercitrin, and (11) isorhamnetin-3-glucoside.

Peak 6 had the molecular ion $[M - H]^-$ at m/z 507. The second-order MS measurement showed a daughter ion at m/z 345, thus proving the loss of a glucose unit. According to the previous studies,[22−26] peak 6 could be identified as syringetin-3-glucoside.

The molecular ion of peak 9 was at m/z 433 in its negative MS spectrum, and a base peak at m/z 301 appeared in the MS^2 spectrum, which suggested the presence of a pentose. According to Xiong et al.,[27] most of the flavonoids in the pericarps of *Z. bungeanum* were quercetin aglycone. By comparison with the literature,[28,29] peak 9 could be identified as quercetin-3-arabinoside.

Peak 11 had the molecular ion $[M - H]^-$ at m/z 477. The MS^2 measurement showed a daughter ion at m/z 315, thus proving the loss of a glucose moiety. By comparison with the standard substance of isorhamnetin, m/z 285 and 151 were the daughter ions of isorhamnetin. According to the previous studies,[23,30−34] peak 11 was identified as isorhamnetin-3-glucoside.

In summary, not only the major flavonoids were determined by HPLC-ESI-MS/MS, but also quinic acid, chlorogenic acid, and vanillic acid 4-glucoside were identified. The results showed the polyphenols of *Z. bungeanum* leaves were composed by quinic acid, chlorogenic acid, quercitrin, quercetin-3-arabinoside, syringetin-3-glucoside, epicatechin, rutin, hyperoside, isorhamnetin-3-glucoside, vanillic acid 4-glucoside, and 5-feruloylquinic acid. Due to the similar polarity, purified by D101 macroporous resin, these compounds can be identified through the same analysis conditions.

Quantitative Analysis. Six standard substance solutions at different concentrations were analyzed using the above-mentioned conditions of qualitative analysis of polyphenols with HPLC-ESI-MS/MS. The calibration curves and the contents of each of six polyphenols in *Z. bungeanum* leaves are presented in Table 2. In addition, on the basis of

Table 2. Retention Time and Fragment Ions of Each Compound in Sample Solution and Standards

peak	compound	$[M]^-$ (m/z)	RT (min)		MS^2 (m/z)		calibration curve	linear range (μg/mL)	r	content[a] (μg/g)	recovery[a] (%)	LOD (ng/mL)	LOQ (ng/g)
			sample	standard	sample	standard							
1	quinic acid	191	9.425	9.489	84.8, 93.1	85.0, 92.9	$y = 5.8858 \times 10^3 x + 1980$	0.05–4.0	0.9993	58.58 ± 2.09	91.34 ± 3.14	6.27	25.1
2	chlorogenic acid	353	9.433	9.516	191.2, 85.1	191.1	$y = 2.8917 \times 10^3 x + 370.3$	2.0–10.0	0.9998	2515.96 ± 100.28	96.65 ± 3.23	15.9	58.5
3	epicatechin	289	9.914	9.897	122.7, 109.0	122.9, 108.8	$y = 2.8887 \times 10^3 x - 1073$	0.20–6.0	0.9999	77.80 ± 2.46	99.87 ± 3.10	14.9	54.5
4	vanillic acid 4-glucoside	329	10.387		167.0					22.75 ± 2.46[b]			
5	feruloylquinic acid	367	10.920		191.4, 172.9					16.63 ± 1.19[b]			
6	syringetin-3-*O*-glucoside	507	13.317		345.1					103.23 ± 2.29[b]			
7	rutin	609	13.798	13.800	300.2	300.0	$y = 4.8322 \times 10^6 x + 62303$	0.5–20.0	0.9982	89.41 ± 2.07	95.54 ± 2.21	1.19	4.36
8	hyperoside	463	14.057	14.074	300.0, 271.2	300.1	$y = 2.3350 \times 10^6 x + 29971$	0.10–10.0	0.9984	886.36 ± 21.06	101.5 ± 2.33	2.39	10.4
9	quercetin-3-arabinoside	433	15.248		301.1					118.75 ± 11.06			
10	quercitrin	447	15.518	15.546	300.1, 271.1	300.1, 270.8	$y = 1.6512 \times 10^4 x + 18376$	1.0–12.0	0.9978	645.82 ± 10.32	96.21 ± 1.54	3.45	16.1
11	isorhamnetin-3-glucoside	477	15.751		315.1, 284.8, 151.0					104.27 ± 6.57			

[a]Mean ± SD. [b]Internal standard method.

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comparison of the peak areas of compounds **4**, **5**, **6**, **9**, and **11** with one of the next standard substances, the contents of these polyphenols in *Z. bungeanum* leaves were obtained and are also shown in Table 2. It can be seen that major polyphenolic components in *Z. bungeanum* leaves grown in Hebei, China, are chlorogenic acid (2515.96 μg/g), hyperoside (886.36 μg/g), quercitrin (645.82 μg/g), quercetin-3-arabinoside (118.75 μg/g), isorhamnetin-3-glucoside (104.27 μg/g), and syringetin-3-glucoside (103.23 μg/g).

Validation of Assay. As seen in Table 2, the linear regression results indicated good linear correlation by the correlation coefficients between 0.9978 and 0.9999 for all of the substances within the appropriate concentration ranges. The LOD and LOQ values of all standard substances are also shown in Table 2. It could be seen that the LOQ values for all standard substances were very low, which indicated the proposed method was sufficiently sensitive.

The recoveries of these standard substances were in the range of 91.34−101.5% with relative standard deviation (RSD) values between 0.15 and 1.87%. It is well-known that the RSD was taken as a measure of precision, which indicated good reliability and accuracy of the developed method.

DPPH Radical Scavenging Activity. Antioxidants can scavenge the radicals by hydrogen donation, which causes a decrease of DPPH absorbance at 517 nm. Figure 2A shows the results that were plotted as a scavenging activity function of flavonoids from *Z. bungeanum* leaves. The DPPH radical scavenging activity increased dramatically with the concentration from 2.5 to 5.0 μg/mL of polyphenols; when the concentration of polyphenols was >5.0 μg/mL, the DPPH radical scavenging activity increased slightly. The IC_{50} value was 4.81 μg/mL, and a low concentration was adequate to inhibit the DPPH radicals on the basis of the existence of the polyphenolic structure.

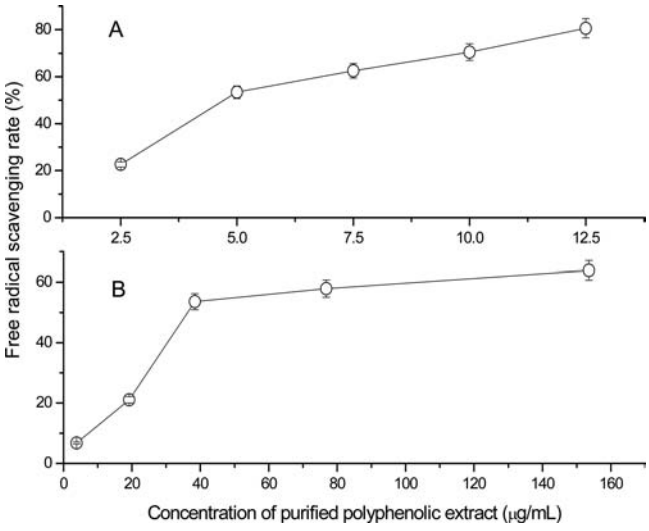

Figure 2. Free radical scavenging activities: (A) DPPH radical scavenging activity of the total polyphenols; (B) superoxide anion radical scavenging activity of the total polyphenols. Data are reported as the mean ± SD of three replicates. $P < 0.05$.

Superoxide Anion Radical Scavenging Activity. The excitation and emission spectra of pyrogallol autoxidation at emission and excitation wavelengths of 509 and 444 nm, respectively. There was a good linear relationship between the fluorescence intensity and the reaction time. When the concentrations of polyphenols from *Z. bungeanum* leaves were 3.84, 19.20, and 38.40 μg/mL, based on the above-mentioned method, scavenging percentages of superoxide anion were 6.77, 21.06, and 53.56%, respectively (Figure 2B). The IC_{50} value was 37.20 μg/mL, and the polyphenols from *Z. bungeanum* leaves demonstrated a good scavenging capacity for superoxide anion.

Cytotoxicity of the Polyphenolic Compounds toward HT-29 Cells. The cytotoxicity of the polyphenolic compounds was assessed using HT-29 cells to evaluate the intracellular action of antioxidation. The results show that the polyphenols extracted from leaves of *Z. bungeanum* had no cytotoxic effects, even at a concentration of 1 mg/mL (Figure 3). In addition, a marked difference could not be found between the different concentrations of polyphenolic compounds and the control.

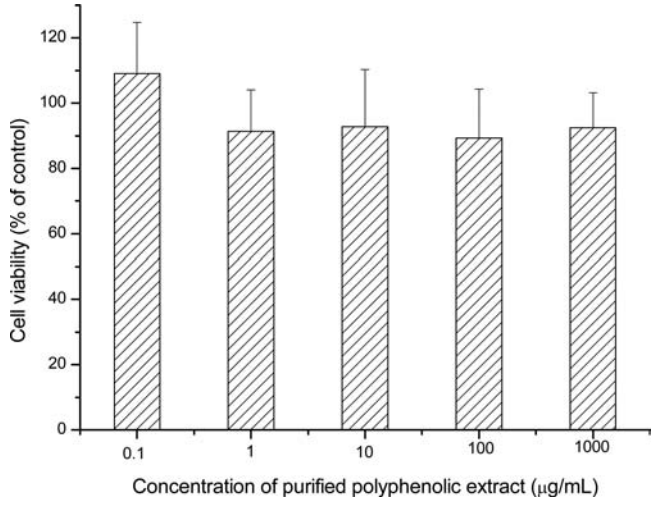

Figure 3. Effects of the polyphenolic compounds from leaves of *Z. bungeanum* on cell viability in HT-29 cells. Data are reported as the mean ± SD of three replicates.

Intracellular Antioxidation Activity. To evaluate the direct radical scavenging effects of the polyphenolic compounds in cells, DCFH-DA was used as the substrate to measure intracellular reactive oxygen species (ROS) production. DCFH-DA can freely penetrate into cells and hydrolyze to DCFH by intracellular esterases. The cellular ROS could oxidize the nonfluorescent dye to fluorescent DCF, so the cellular ROS can directly be reflected by the fluorescence intensity. As shown in Figure 4, the average fluorescence in the control, which was treated with 100 μM H_2O_2, was almost 8 times the blank after 1 h. Compared with the control, polyphenolic compounds could decrease the average fluorescence. This indicates that the polyphenolic compounds had markedly intracellular radical scavenging activities in HT-29 cells and a 1 h IC_{50} value of 207 μg/mL. The greatest antioxidant activity was due to the structure of the flavonoids containing a 3′,4′-*O*-dihydroxyl group in the B-ring, a 2,3-double bond combined with a 4-keto group in the C-ring, and a 3-hydroxyl group.[35]

In conclusion, the qualitative and quantitative analyses of the polyphenolic constituents from *Z. bungeanum* leaves were first reported. A sensitive and accurate method was established, and the total polyphenols showed strong radical scavenging activities. The result of this study offers a theoretical basis for a further study on the bioactive compounds from *Z. bungeanum* leaves.

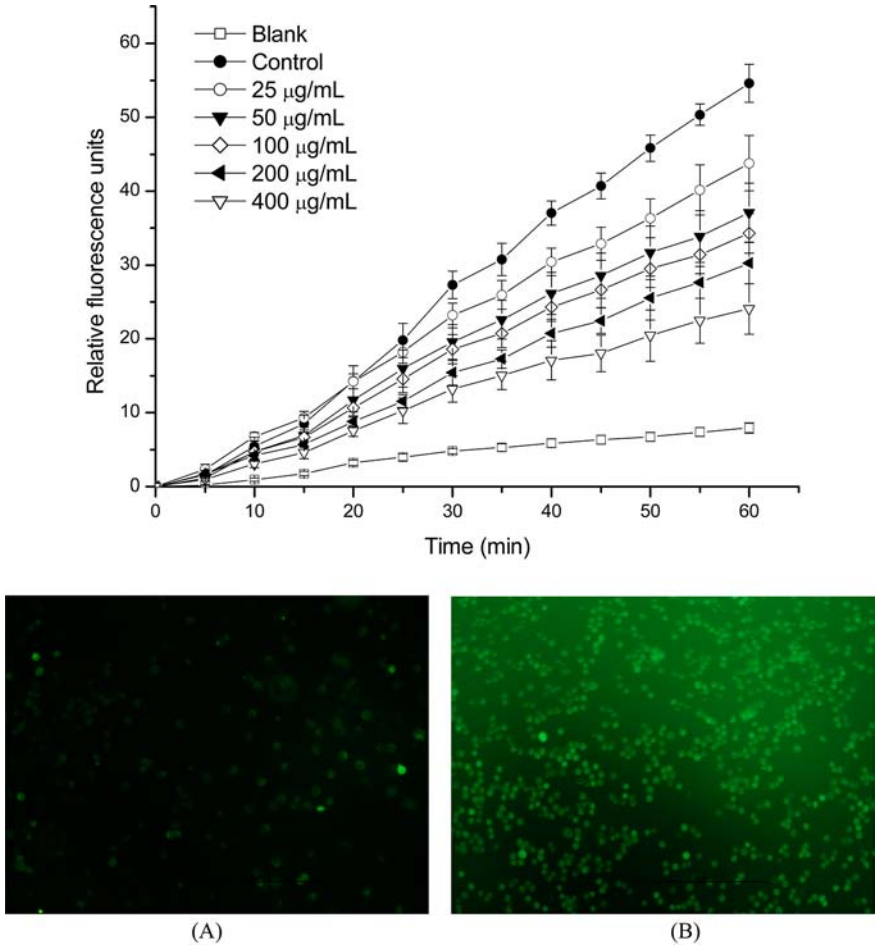

Figure 4. Intracellular radical scavenging activities of the polyphenolic compounds from leaves of *Z. bungeanum* in HT-29 cells: (A) blank was the fluorescence intensity of the cells without H_2O_2 treatment; (B) control was the fluorescence intensity of the cells with 100 μM H_2O_2 treatment. Data are reported as the mean ± SD of three replicates.

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Notes

The authors declare no competing financial interest.

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