OXIDATIVE POTENTIAL AND NANOANTIOXIDANT ACTIVITY OF FLAVONOIDS AND PHENOLIC ACIDS IN SOPHORA FLAVESCENS

1. Introduction

Sophora japonica is the unopened flower bud of the leguminous plant Sophora japonica L. It has been used for medicinal purposes in Chinese medicine for more than two thousand years. The “Shen Nong’s Materia Medica” also listed it as the top grade. Huai mi contains a variety of flavonoids, such as rutin, quercetin, etc., among which rutin chemical name is 5,7,3′,4′-tetrahydroxy-3-rutin. Studies have found that it has the effects of cooling blood to stop bleeding, clearing liver, and purging fire [1]. Scholars at home and abroad have carried out research on the chemical components and pharmacological effects of Sophora japonica. More than 30 flavonoids have been isolated, and phenolic acids, alkaloids, esters, and inorganic substances have been detected. Research progress in the antioxidant activity of traditional Chinese medicines containing yellow and phenolic acids: Modern medicine points out that free radical reactions in the body are closely related to the occurrence, development, and aging process of some diseases, and the body is affected by physical or chemical factors in the external environment and a large number of free radicals are generated due to the influence of the metabolism of the internal ring. When the balance is out of balance, the excess free radicals attack the biological macromolecules that constitute the tissues and can gradually develop the morphology and structure of the tissues and organs. The integrity of function is damaged acutely or chronically. Once the damage exceeds the repair capacity or loses its compensatory function, signs of disease and aging will appear. In view of this, the research on the scavenging of free radicals by antioxidants has received general attention, and the search for natural, low-toxic, and efficient antioxidants has become an inevitable trend. Studies have now found that flavonoids and phenolic acids have obvious antioxidant activity. Because of their wide distribution in traditional Chinese medicine, the antioxidant activity of Chinese medicines containing flavonoids and phenolic acids has attracted much attention. Flavonoids generally refer to a series of compounds formed by connecting two benzene rings through...
three broken atoms [2]. They have C_6-C_3-C_6, the basic core compound. From the structure of flavonoids, they can be divided into the following categories: flavonoids, flavonols, isoﬂavones, chalcones, anthocyanins, etc. and the above-mentioned dihydro derivatives. Phenolic acids generally refer to aromatic compounds with hydroxyl or carboxyl groups attached to the aromatic ring and have a certain degree of acidity. Phenolic acids can be divided into the following categories from the structure: phenol derivatives, benzene ring binary phenols, phloroglucinols, naphthalene ring derivatives, pyrogalols, diphenyl ethers and related compounds, etc. For the free radical chain reaction caused by various physical and chemical factors, flavonoids and phenol compounds have an important inhibitory effect: in the antioxidant reaction, it can simultaneously scavenge free radicals in the chain initiation stage and free radicals in the free radical reaction chain [3]. It blocks the free radical chain reaction, plays a dual role of prevention and chain scission, and shows the unique and powerful antioxidant capacity of flavonoids and phenolic acids in Chinese medicine.

Phenolics are an important class of phytochemicals (also known as plant secondary metabolites), which are widely found in human diets such as vegetables, fruits, and grains, and have monohydric phenol or polyphenol base structures. At present, more than 8000 kinds of phenolic substances have been discovered, including more than 4000 kinds of flavonoids, and the number of the two types is still increasing. A total polyphenol and flavonoid content, phenolic content, and antioxidant activity were analyzed by HPLC method. Myricetin and resveratrol were also detected in most fruit beer. Among phenolic acids, The enrichment of new chlorogenic acid in most phenolic acids to coumaric acid and caffeic acid relative to traditional beer was determined. Nardini et al. showed that adding fruit during fermentation greatly improved the antioxidant activity of beer and significantly improved the distribution of its phenolic substances in quality and quantity [4]. Chu and others found that the antioxidant activity of germinated Chinese wild rice initially decreased and then increased. The maximum difference is in antioxidant activity between 36 h (G36) and 120 h germination (G120) stage. Levels of p-hydroxybenzoic acid, p-hydroxybenzaldehyde, vanillin, p-coumaric acid, ferulic acid, and epigallocatechin increased significantly during 120 h germination (G120) stage. NEW chlorogenic acid in most phenolic acids to coumaric acid and caffeic acid relative to traditional beer was determined. Nardini et al. showed that adding fruit during fermentation greatly improved the antioxidant activity of beer and significantly improved the distribution of its phenolic substances in quality and quantity [4]. Chu and others found that the antioxidant activity of germinated Chinese wild rice initially decreased and then increased. The maximum difference is in antioxidant activity between 36 h (G36) and 120 h germination (G120) stage. Levels of p-hydroxybenzoic acid, p-hydroxybenzaldehyde, vanillin, p-coumaric acid, ferulic acid, and epigallocatechin increased significantly during germination. Gene expression of four phenylalanine amonnia-lyase G120, Ne4-coumaric acid-CoA ligase, a cinnamoyl-CoA reductase, two cinnamol dehydrogenase, a chalcone synthase and a chalcone synthase, and a chalcone isom2 erase are obviously higher than G36, and promoted the accumulation of phenols. The biochemical mechanism of antioxidant activity and phenolic distribution in wild rice germination in China was elucidated [5]. Zhang et al. studied the herbaceous markers and their antioxidant activity in the methanol extract of citronella and established raw materials and quality evaluation methods to determine their flower products by HPLC method. As a result, the methanol extract of 11 continuous flowers contained high total phenol and flavonoids and had potential antioxidant activity based on idling ability of DPPH radical scavenging test. According to the similarity of chromatographic fingerprint and principal component analysis, 21 phenolic herbaceous labeled ding sections were selected, through UV, HPLC-DAD-ESI-QTOE-MS/MS, and NMR analysis further elucidating their structures. The identified compounds included 2 phenylpropanoids, 11 C-glycosyflavones, and 6 O-glycosylflurals as indicators for quantitative evaluation of flower quality and authenticity [6]. Dong et al. extracted flavonoids from *Sophora flavescens* by heating and refluxing with ethanol and determined the extraction rate under different extraction conditions to obtain the optimal extraction conditions and then added the extracts to lard and rapeseed oil, regularly. The peroxide value and acid value were determined and compared with the natural antioxidant tea polyphenols, in order to provide a basis for the progress and development of new food natural antioxidant substances [7].

On the basis of this research, the oxidation potential and nano-antioxidant activity of flavonoids and phenolic acids in *Sophora flavescens* were proposed in this paper. The contents of total phenolic acids and flavonoids in rutin extracts from different parts of *Sophora flavescens* were determined, and their antioxidant activities were compared, and the basis of antioxidant active substances of *Sophora flavescens* was discussed. The relative content and nano-antioxidant activity of total flavonoids and phenolic acids in the ethyl acetate phase extraction part were significantly stronger than those in the total extract and other extraction parts, and the antioxidant capacity was effectively related to the dose.

### 2. Experiment Preparation

#### 2.1. Materials and Instruments

Sophora rice, absolute ethanol, petroleum ether, ethyl acetate, n-butanol, hydrochloric acid, ferrous sulfate, sodium dihydrogen phosphate, disodium hydrogen phosphate, potassium ferricyanide, trichloroacetic acid, ferric chloride, hydrogen peroxide, salicylic acid, and ferrous sulfate are all analytically pure in Beijing Chemical Plant; rutin, ferulic acid, and ascorbic acid from Shanghai Jinshi Biotechnology Co., Ltd., have a purity of 98%; 2,2′-hydrazine-bis(3-ethyl Benzothiazolin-6-sulfonic acid), diammonium salt (ABTS®), 1,1-diphenyl-2-trinitrobenzene (DPPH) are from Nanjing Aodoformi Biotechnology Co., Ltd., Purity >98%;

#### 2.2. Experimental Method

##### 2.2.1. Sample Processing

Take 10 kg of dry powder, use ethanol with a material-to-liquid ratio (1:12) of 70% under 400 W power for 40 min, extraction temperature 40°C, extract 3 times, recover ethanol under reduced pressure, and freeze-dry it to obtain a total extract of 1.26 kg. Take 1 kg of the total extract, dissolve it in 5 L of distilled water, extract 3 times with an equal volume of petroleum ether, take 15 L of the upper petroleum ether solution, and recover the petroleum ether under reduced pressure to obtain the petroleum ether phase (241 g) [7, 8]; the remaining lower aqueous solution water bath evaporates the petroleum ether. Extract 3 times with an equal volume of ethyl acetate; take the upper extract (15 L) to recover the ethyl acetate under reduced pressure to obtain the ethyl acetate phase (31 g), the...
same operation to obtain the n-butanol phase (200 g); the remaining aqueous layer is freeze-dried to obtain water phase (517 g), dried to constant weight, and set aside.

2.2.2. Determination of Total Phenolic Acid and Total Flavonoids. The content of total flavonoids in different extraction parts of Huai Mi was determined by sodium nitrite-aluminum nitrate color method.

Regarding the drawing of the standard curve of rutin for flavonoid content: accurately weigh 2.5 mg of the rutin standard that has been dried to a constant weight, and mix it with 95% ethanol to make 0, 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL standard solution. Take 1 mL of the standard solution of different concentrations in a 10 mL volumetric flask, add 0.3 mL of 5% sodium nitrite solution by mass, mix well, and place at room temperature for 6 minutes, add 0.3 mL of 10% aluminum nitrate by mass, mix well, and store at room temperature for 15 minutes, use 95% ethanol as a blank control solution, and measure the absorbance at 510 nm wavelength. Draw the standard curve with the mass concentration of rutin as the abscissa and the absorbance value as the ordinate, and establish the regression equation.

The total phenolic acid content in each extract was determined by the Folin-Ciocalteu method.

Regarding the drawing of the standard curve of ferulic acid for the content of phenolic acid: accurately weigh 2.5 mg of ferulic acid standard and make up a standard solution of 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL. Take 3 mL of standard solution, add 1 mL of Folin-Ciocalteu color reagent, mix well, and store at room temperature for 1 hour, and the absorbance was measured at 760 nm wavelength. Draw a standard curve with the mass concentration of ferulic acid as the abscissa and the absorbance value as the ordinate, and establish the regression equation [9, 10]. Take the test solution, measure the absorbance values of the samples of different extraction parts of the ethanol extract of Sophora japonica according to the above method, and obtain the total phenolic acid content of each sample through the regression equation.

2.2.3. Determination of Antioxidant Activity. (1) Determination of reducing power. Precisely measure 1 mL of ascorbic acid with a mass concentration of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL and the test solution for different extraction sites, and add 2.5 mL of pH 6.6 phosphate buffer solution and 1% potassium ferricyanide in turn 2.5 mL, shake well, put in water bath at 50°C for 20 minutes, add 2.5 mL of 10% trichloroacetic acid, centrifuge at 4500 r/min for 10 minutes after mixing, take 5 mL of supernatant, add 5 mL of distilled water, and finally add 1 mL of 0.1% ferric chloride. After 10 minutes, the absorbance value was measured at 700 nm wavelength. Repeat 3 times for each sample.

(2) Determination of scavenging ability of light free radicals (·OH). Precisely measure 1 mL of the test solution of different concentrations of ascorbic acid and different extracts.

The scavenging ability of each test solution to light free radicals was determined by the Fenton reaction according to the following steps: take 1 mL of 6 mmol/L ferrous sulfate, 1 mL of 6 mmol/L salicylic acid, and 1 mL of test solution of different concentrations, and finally, add 8.8 The reaction was started with 1 mL of mmol/L H2O2 and reacted at 37°C for 30 minutes. Using distilled water as a reference, the absorbance of each concentration was measured at 510 nm. Calculate the ·OH clearance rate of different extraction parts of Sophora solani alcohol extract by:

\[
\text{Clearance rate} \times 100\% = \left( \frac{A_0 - (A_1 - A_{ab})}{A_0} \right) \times 100\% \tag{1}
\]

where \(A_0\) is distilled water instead of the absorbance of the test product; \(A_1\) is the absorbance after adding the test product solution; \(A_{ab}\) is without the color reagent (H2O2), the background absorbance of the test solution.

(3) Measurement of DPPH free radical scavenging ability. Precisely measure 100 μL of the test solution of different concentrations of ascorbic acid and different extracts of Sophora japonica in a 96-well plate, add 100 μL DPPH solution (concentration 0.2 mmol/L) methanol solution, keep it in the dark for 30 min, and measure its absorbance at 517 nm. Calculate the scavenging rate of DPPH free radicals according to formula (2):

\[
\text{Clearance rate} \times 100\% = \left( \frac{A_0 - (A_1 - A_{ab})}{A_0} \right) \times 100\% \tag{2}
\]

where \(A_0\) is without the test substance DPPH methanol absorbance value; \(A_1\) is adding the test substance and DPPH methanol absorbance value; \(A_{ab}\) is the background absorption value of the test substance.

(4) Measurement of ABTS + free radical scavenging ability. First configure solution A: accurately weigh 0.0384 g of ABTS distilled water into a 10 mL volumetric flask; solution B: accurately weigh 0.0134 g of potassium persulfate distilled water into a 10 mL volumetric flask. Mix A solution and B solution 1:1, avoid light for 12–16 h to obtain ABTS· stock solution, add pH 7.4 PBS solution to dilute to absorbance value 0.7–0.8 before use, and set aside. Precisely measure 40 μL of the test solution of different concentrations of ascorbic acid and different extraction parts of Sophora japonica extract in a 96-well plate, add the diluted ABTS· stock solution 160 μL, and measure the absorbance value at 734 nm wavelength. Take 40 μL of the test solution solvent and 96-well plate, add 160 μL diluted ABTS· stock solution, and measure the absorbance value, as a blank control. The scavenging rate of ABTS· free radicals is shown in formula 3:

\[
\text{Clearance rate} \times 100\% = \left( 1 - \frac{A_1}{A_2} \right) \times 100\% \tag{3}
\]

among them, \(A_1\) is the absorbance of the test solution and ABTS•; \(A_2\) is the absorbance of the test solution and ABTS•.

2.3. Statistical Analysis. The experimental data are measured 3 times in parallel, and the data are averaged ± the standard deviation is expressed, using Duncan’s multiple range test in
SPSS software to analyze the significance of differences between samples ($p < 0.05$).

3. Results and Analysis

3.1. Establishment of the Standard Curve of Rutin and Ferulic Acid. As shown in Figure 1, the standard curve is drawn with the concentration as the abscissa and the absorbance value as the ordinate, and the regression equation of rutin is obtained: $y = 12.91x + 0.0124$, $R^2 = 0.9994$. In the formula: $Y$ is the absorbance of rutin, $x$ is the mass concentration of rutin (mg/mL); the regression equation of ferulic acid: $y = 23.876x + 0.0087$, $R^2 = 0.9991$. In the formula: $Y$ is the absorbance of ferulic acid, and $x$ is the mass concentration of ferulic acid (mg/mL).

3.2. The Relative Content of Total Phenolic Acid and Total Flavonoids in Different Extraction Parts of Huai Mi Ethanol Extract. The relative content of total flavonoids and total phenolic acid in the 70% ethanol total extract and the extracts of each part of Huai Mi is very different (see Figure 2) (61.55 ± 0.11), (19.10 ± 10.23) mg/g; the relative content of total flavonoids in the petroleum ether phase is the smallest, only (5.70 ± 10.19) mg/g, the relative content of total phenolic acid in the water phase is the smallest, which is (4.07 ± 10.94) mg/g [11]. The experiment showed that ethyl acetate enriched the phenolic acids and flavonoids in the ethanol extract of Sophora japonica with the highest content, indicating that, through stepwise extraction, the phenolic acids and flavonoids in the extract of Sophora japonica get preliminary separation and purification.

3.3. Determination of Reducing Power. It can be seen from Figure 3 that, with ascorbic acid as a positive control, the 70% ethanol extract of Sophora japonica and the extracts of various parts have different degrees of reducing power, and there is a dose-effect relationship within the experimental concentration range. As the concentration increases, the reducing power shows upward trend. The reducing ability of each part is from strong to weak: ethyl acetate phase > total extract > ether phase > n-butanol phase > water phase. It can be seen that the reducing ability is related to total flavonoids and phenolic acids, and the reducing ability of the total extract may be related to the higher content of flavonoids. The reducing ability of petroleum ether phase may be a certain chemical component with strong reducing ability in this part, maybe related to phenolic acids. At the same concentration, the reduction ability of the ethyl acetate phase was significantly stronger than that of the other phases in the alcohol extract of Sophora sophora. When the concentration reached 1.0 mg/mL, the removal rate was the highest, which was 0.8065. It can be seen that the active ingredients with strong reducing ability are concentrated in the ethyl acetate phase, and the effective parts with stronger reducing ability than the total extract can be obtained through segmented extraction.

3.4. Scavenging Ability of Light Free Radicals (·OH). It can be seen from Figure 4 and Table 1 that, with ascorbic acid as a positive control, the alcohol extracts of Sophora sophora and the extracts of various parts showed different degrees of scavenging effects on light free radicals (·OH) and showed a certain dose-effect relationship. The increase in sample concentration increases the removal ability. In the experimental concentration range, the scavenging ability of different extraction sites on light free radicals (·OH) varies with concentration. The ethyl acetate extraction site has the strongest scavenging ability on light free radicals (·OH), and the IC$_{50}$ is ((0.321 ± 0.0026) mg/mL, the total extract and n-butanol have equivalent scavenging abilities to light free radicals (·OH), IC$_{50}$ are (1.020 ± 0.0134) mg/mL and (0.95410.0067) mg/mL.

The IC$_{50}$ of petroleum ether phase was (1.7040.0015) mg/mL, and the scavenging effect was weakest. Combined with
Figure 2, free radical (-OH) scavenging ability may be related to total flavonoids content in various parts. The total flavonoids of ethyl acetate phase were much higher than those of other phases. At a mass concentration of 1.0 mg/mL, *Sophora* ethyl acetate had a relatively light free radical (-OH) scavenging rate of 66.89, 54.37% of the total extract, 50.92% of the n-butanol phase, much higher than 47.75% of the aqueous phase, and 37.04% of the petroleum ether phase.

Furthermore, for the activity of scavenging light free radical (-OH) from *Sophora japonica* the components are concentrated in the ethyl acetate phase. The active antioxidant components can be obtained by extraction.

3.5. Ability to Clear ABTS+. It can be seen from Figure 5 and Table 1 that, within the experimental concentration
3.6. Ability to Remove DPPH. As shown in Figure 6 and Table 1, with ascorbic acid as the positive control, *Sophora japonica* extract and extracts from various parts have a clearing effect on DPPH. With dose-effect relationship, the scavenging ability of different polar parts on DPPH is different at the same concentration. The scavenging capacity of the ethyl acetate phase of Huai Mi is higher than the other phases, and the IC$_{50}$ is (0.169 ± 0.0014) mg/mL, the total extract and n-butanol phase removal ability is relatively weak, IC$_{50}$ were (0.352 ± 0.0007) mg/mL and (0.460 ± 0.0021) mg/mL, petroleum ether has the weakest removal ability, and IC$_{50}$ is ((1.296 ± 0.0064) mg/mL.

Based on Figure 2, it is speculated that the scavenging ability of the alcohol extract of *Sophora japonica* and extracts of various parts on DPPH is related to the content of flavonoids. When the concentration is 1.0 mg/mL, the clearance rate of the ethyl acetate phase of Huai Mi is 91.52%, and that of the petroleum ether phase is 47.40%.

### Table 1: Comparison of OH radical, ABTS$^+$, DPPH, and scavenging ability of different extraction parts of *Sophora japonica* ethanol extract.

| Extraction site         | OH IC$_{50}$ (mg/mL) | ABTS$^+$ IC$_{50}$ (mg/mL) | DPPH IC$_{50}$ (mg/mL) |
|-------------------------|----------------------|-----------------------------|------------------------|
| Total extract           | 1.020 ± 0.0134$^c$   | 0.753 ± 0.0020$^b$         | 0.352 ± 0.0007$^b$    |
| Petroleum ether phase   | 1.704 ± 0.0015$^c$   | 1.675 ± 0.0083$^c$         | 1.296 ± 0.0064$^c$    |
| Ethyl acetate phase     | 0.321 ± 0.0026$^a$   | 1.213 ± 0.0010$^a$         | 0.169 ± 0.0014$^a$    |
| Normal butanol phase    | 0.954 ± 0.0067$^b$   | 1.665 ± 0.0140$^c$         | 0.460 ± 0.0021$^c$    |
| Water phase             | 1.539 ± 0.0055$^d$   | 4.171 ± 0.1099$^d$         | 0.826 ± 0.0042$^d$    |

3.7. The Half-Scaevenging Rate IC of Different Extraction Parts of Huai Mi Ethanol Extract on -OH Radical, ABTS$^+$, and DPPH 50. From Table 1, the half-scaevenging rate IC$_{50}$ of different extraction parts of the ethanol extract of *Sophora japonica* on -OH radicals, ABTS$^+$, and DPPH shows that the antioxidant components are concentrated in the ethyl acetate phase. Combined with the relative content of flavonoids and phenolic acids in each extract phase in Figure 2, it can be seen that flavonoids are the main effective components of *Sophora japonica* antioxidation. Through systematic extraction method, the effective components of *Sophora japonica* are enriched and preliminary separation is achieved.
4. Conclusion

In this paper, the oxidation potential of the flavonoids and phenolic acid components of Sophora sophora was evaluated and their antioxidant activity was studied. The total phenolic acid and flavonoids content of different extraction parts of the rutin extract of Sophora Sophora were determined and the antioxidant activity was compared. To explore the basis of antioxidant active substances in Sophora japonica after ultrasonic extraction and concentration of Sophora japonica with 70% ethanol, organic solvent extraction was used to obtain four extracts of petroleum ether, ethyl acetate, n-butanol, and aqeous phase; the total flavonoids and phenolic acid content of the ethyl acetate extract and other extraction parts, and its antioxidant capacity was dose-effect relationship. Experiments have found that the flavonoids and phenolic acid content of the ethyl acetate extract was significantly higher than other extracts. Its reducing power, light free radicals, DPPH, ABTS\^*, and scavenging rate are also significantly higher than n-butanol, petroleum ether, and aqueous extracts. The ethyl acetate extract has an effect on hydroxyl radicals and ABTS\(^+\). Clearing ability IC\(_{50}\) values are (0.321 ± 0.0026), (0.213 ± 0.0010), and (0.169 ± 0.0014) mg/mL. Finally, it was found that the effective components of Sophora japonica were preliminarily separated after the solvent extraction of each phase. The relative content and antioxidant activity of total flavonoids and phenolic acids in the ethyl acetate phase extraction part were significantly stronger than the total extract and other extraction parts, and its antioxidant capacity was dose-effect relationship. Experiments have found that the flavonoids and phenolic acids in Sophora japonica are closely related to its antioxidant capacity.

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.

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